A Polymorphic Autoregulatory Hormone Response Element in the Human Estrogen-related Receptor α (ERRα) Promoter Dictates Peroxisome Proliferator-activated Receptor γ Coactivator-1α Control of ERRα Expression*

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The orphan nuclear estrogen-related receptor α (ERRα) and transcriptional cofactor peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) are involved in the regulation of energy metabolism. Recently, extensive cross-talk between PGC-1α and ERRα has been demonstrated. The presence of PGC-1α is associated with an elevated expression of ERRα, and the two proteins can influence the transcriptional activities of one another. Using a candidate gene approach to detect proteins that can influence the transcriptional activities of ERRα, we have identified a 29-bp sequence (ESRRA23) containing two nuclear receptor recognition half-site motifs that is present in 1–4 copies within the promoter of the human ERRα gene encoding ERRα. The ESRRA23 sequence contains a functional ERR response element that is specifically bound by ERRα, and chromatin immunoprecipitation shows that endogenous ERRα occupies its own promoter in vivo. Strikingly, introduction of PGC-1α in HeLa cells by transient transfection induces the activity of the ESRRA promoter in a manner that is dependent on the presence of the ESRRA23 element and on its dosage. Coexpression of ERRα and PGC-1α results in a synergistic activation of the ESRRA promoter. In experiments using ERRα null fibroblasts, the ability of PGC-1α to stimulate the ESRRA promoter is considerably reduced but can be restored by addition of ERRα. Taken together, these results demonstrate that an interdependent ERRα/PGC-1α-based transcriptional pathway targets the ESRRA23 element to dictate the level of ERRα expression. This study further suggests that this regulatory polymorphism may provide differential responses to ERRα/PGC-1α-mediated metabolic cues in the human population.

Nuclear hormone receptors are transcription factors that control essential developmental and physiological pathways (1). Although the transcriptional activity of nuclear receptors is primarily regulated by specific ligands, several members of the superfamily of nuclear receptors have no known natural ligands and are therefore referred to as orphan receptors (2). Estrogen-related receptor α (ERRα; NR3B1) was the first orphan nuclear receptor to be identified on the basis of its similarity with estrogen receptor α (ERα; NR3A1) (3). Phylogenic tree reconstruction confirmed that ERRα belongs to the sub-group of receptors for steroid hormones (4), and ERRα was subsequently shown to share both structural and functional attributes with the ERs including binding to synthetic estrogenic ligands (reviewed in Ref. 5). ERRα also recognizes estrogen response elements (EREs), but characterization of its DNA binding properties demonstrated a preference for sites composed of a single half-site preceded by three nucleotides with the consensus sequence TNAAGGTTCA, referred to as an ERR (6–10). The transcriptional activity of ERRα is independent of exogenously added ligand, and its relative potency as a transcriptional activator appears to be cell context- and promoter-dependent (3, 8, 11–15). ERRα has also been described as a potent repressor of the SV40 late promoter (6) and to interfere with the functions of glucocorticoid, retinoic acid, and peroxisome proliferator-activated receptors (8, 16, 17). Although the exact physiological role of ERRα has not been defined precisely, increasing evidence suggest that ERRα plays an important role in regulating cellular energy balance. ERRα is predominantly expressed in tissues demonstrating a high capacity for fatty acid β-oxidation (3, 8, 18), and has been shown to regulate the medium-chain acyl coenzyme A dehydrogenase gene (8, 9). More recently, ERRα null mice have been shown to display reduced body weight and peripheral fat deposition and be resistant to high fat diet-induced obesity (19). In agreement with the observed phenotype, gene microarray experiments demonstrated alteration in the expression of genes involved in adipogenesis, mitochondrial biogenesis, and energy metabolism, including cytochrome c, medium-chain acyl-coenzyme A dehydrogenase, acetyl-coenzyme A synthetase 2, and fatty-acid synthase (19).
The transcriptional activity of nuclear receptors is dependent on specific interactions with coregulatory proteins (20). The recent identification and functional characterization of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), PGC-1-β, and PGC-1-related protein revealed the existence of a family of coactivators that possess the unique characteristic of relaying diverse physiological signals to transcription factors that regulate gene networks controlling energy balance (reviewed in Refs. 21 and 22). In particular, PGC-1α has been shown to regulate thermogenesis in brown fat (23), mitochondrial biogenesis and respiration in skeletal muscle (24), and gluconeogenesis in the liver (25–27). PGC-1α may also contribute to the control of energy metabolism as overexpression of this gene in transgenic mice induces a high energy expenditure and antagonizes obesity (28). Although PGC-1α was originally identified as a transcriptional coactivator specific for peroxisome proliferator-activated receptor γ (23), subsequent studies have demonstrated that PGC-1α influences the activity of numerous transcription factors, including a wide array of nuclear receptors (22). Recently, two groups, using similar yeast two-hybrid approaches, independently identified ERRα as a novel PGC-1α-binding protein (18, 29). Huss et al. (29) demonstrated that PGC-1α enhances ERRα transcriptional activity on the medium-chain acyl-coenzyme A dehydrogenase promoter. In contrast, Ichida et al. (18) described ERRα as a repressor of PGC-1α activity on the PEPCk promoter. ERRα transcriptional activity has also been shown to be stimulated by PGC-1α and -β in transient transfection assays using synthetic promoters (30). Interestingly, ERRα and PGC-1α show similar expression profiles in adult tissues, including induction of expression of both genes by exposure to cold (18, 30). Consistent with this observation, PGC-1α has been shown to induce the expression of ERRα (30). However, the molecular mechanisms underlying this phenomenon remain to be elucidated.

In this study, we first used a candidate gene approach to detect variants within genes encoding nuclear hormone receptors likely to play a role in physiology and be associated with detect variants within genes encoding nuclear hormone receptors in French Canadian women for a polymorphic repeat in the ESRRA promoter. A remarkable sequence includes a functional ERRE that is located upstream of the promoter that can be found in 1–3 copies in human chromosomes. Remarkably, this sequence includes a functional ERRα that is responsive to the presence of PGC-1α. Our results demonstrate the existence of an autoregulatory mechanism by which ERRα can control its own expression and further suggest the existence of an interdependent PGC-1α/ERRα pathway involved in the control of energy balance.

**EXPERIMENTAL PROCEDURES**

**Identification of a Polymorphic Repeat in the ESRRA Promoter**—The promoter 5′-CTTTGGTGTCGCTGACTG-3′ and 5′-GCCTGGCGAG-CCAAAGA-3′ were used to produce a 1054-bp fragment from exon 1. PCR was performed according to standard protocols with Taq polymerase from Qiagen. PCR products were purified with a QiAquick PCR purification kit (Qiagen) and quantified by gel electrophoresis with standardized amounts of DNA. Automated sequencing of PCR products was performed with fluorescently labeled deoxyxide terminator using the BigDye terminator cycle-sequencing kit on a ABI 377 DNA-Sequencer (Applied Biosystems).

**Genotyping**—Genomic DNA was purified from 200 μl of peripheral blood leukocytes with whole DNA DNA purification kits for 96-well plates from Qiagen. The collected DNA was further diluted five times with a solution of 10 mM Tris-HCl, pH 7.5, containing a 50 mM ethylenediaminetetraacetic acid solution of an inert fluorescent dye, DROX (Molecular Probes, C-1309). The final concentration of ROX was 45 μM in each DNA sample, and the mean DNA concentration was 5 ng/μl. For each PCR, 5 μl of reaction mixture was used regardless of the DNA concentration. Genotyping for the ESRRα323 minimalsite in the ESRRα promoter was performed using the following primers: 5′-CGTGGCCTCGCTCTTC-3′ and 5′-CTGACCTGGCTGCGAC-3′. PCR was performed in a 96-well microplate (Axygen) with 5 μl (25 ng) of genomic DNA and 20 μl of PCR premix containing 2.5 μl of 10× buffer (Qiagen), 200 μM concentration of each dNTP, 7.5 pmol of each primer, 1× Q-solution (Qiagen), 2% Me2SO, and 1 unit of HotStart Taq DNA polymerase. PCR setup in the microplates was performed with a Qiagen Biobot 3000 or an ABI 377 cycle-sequencer with 12-channels. The plates were then covered with a silicone mat (Axymat from Axogen) and properly sealed using a roller (MJ Research). PCR was performed on a MJ PTC-200 (MJ Research), 95°C for 15 min, 30 cycles of 45 s at 95°C, 45 s at 58°C, 45 s at 72°C, and a final extension at 72°C for 7 min. PCR products were run on a 2% agarose gel in TBE 1× buffer (0.089 M Tris, 0.089 M boric acid, 2 mM EDTA) for 2 h at 10 V/cm. The reaction produces ampiclons of 198 bp with two repeats, 221 bp with three repeats, 244 bp with four repeats, and 175 bp with only one repeat. The gels were photographed, and the genotype was assigned by two independent readers. 180 samples were run in duplicate with a concordance rate of 99%.

**Cloning of the ESRRα Promoter**—Genomic DNA from blood samples or lymphoblastoid cell lines containing two or three repeats was prepared with the QiAamp kit (Qiagen) and used as the template in a PCR to amplify the promoter region of the human ESRRα gene. The primers used had the sequences 5′-GGGATGACACTGAGTTGCTGCTAC-3′ (forward) and 5′-CCAGATGTCATCCGCTCTCCTC-3′ (reverse) and produced a product of 181 bp. This fragment was digested with SpeI and HindIII and subcloned into the luciferase reporter plasmid pGL3 (Promega, Madison, WI). All of the selected clones were sequenced with fluorescently labeled deoxyxide terminators using the BigDye terminator cycle-sequencing kit on a ABI 377 DNA-Sequencer.

**Plasmids and Cell Transfections**—The ERRα and DNA was cloned into the expression vector pCMX. Plasmids expressing the ERRα-VP16 fusion protein were constructed by subcloning PCR-amplified ERRα DNA into pCMX-VP16 downstream of the VP16 activation domain. The DNA binding mutant of ERRα was generated by substituting the glutamic acid and alanine residues of the ERRα box for glycine residues. ERRα does not bind DNA as examined by EMSA in vitro but locates to the nucleus when transfected in mammalian cells (2). Expression vector for human ERRα was described (31). The pCDNA3.1 HA-hpc-1α vector was described previously (32) and obtained from A. Kralif (La Jolla, CA). The luciferase reporter plasmid ESRRα23-TKLuc and ESRRα23 (3)-TKLuc contained 1 and 3 copies, respectively, of the ESRRα323 response element (see Fig. 1A) cloned into pTKLuc. A fragment containing ESRRα323 luciferase reporter 1.2 kb upstream of the transcriptional start site was subcloned into the luciferase reporter plasmid pGL3 to give pGL3ESRRα. To construct the ESRRα promoter luciferase reporter gene, sequences 5′ and 3′ adjacent to the ESRRα323 elements and putative ERRα binding site were amplified by PCR and subcloned sequentially into pGL3. HEK293 cells were obtained from American Type Culture Collection and maintained in Dulbecco’s modified Eagle medium (Invitrogen) with 10% fetal bovine serum. Mouse embryonic fibroblasts (MEFs) were isolated from 13.5-day-old wild-type and ESRRα null embryos (19). The embryos were minced with a razor blade, and the cells were dissociated by trypsin. The cells were cultured in Dulbecco’s modified Eagle medium and supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Twenty-four hours prior to transfection, cells were seeded in 12-well plates and grown in phenol red-free Dulbecco’s modified Eagle medium containing charcoal-treated fetal bovine serum. One-hundred ng of expression vector for nuclear receptors, 0.4 μg of expression vector for the PGC-1α, and 1 μg of pCMX-VP16 reporter, and 0.3 μg of CMX β-galactosidase plasmids were introduced into cells using LipofectAMINE (Invitrogen) or FuGENE 6 transfection reagent (Roche Applied Science). When using LipofectAMINE, cells were maintained in the presence of liposomes for 16 h and cultured for an additional 24 h. Cells were harvested in potassium phosphate buffer containing 1% Triton X-100. Luciferase activity was determined using Steady-Glo (Promega), and values were read with the Vector 2 in the luminescence mode. The transfection was normalized to the β-galacto-
Identification of a Polymorphic Hormone Response Element in the ESRRA Promoter—Our search for functional coding and regulatory polymorphisms in genes encoding members of the nuclear receptor superfamily led us to identify a 23-bp element (Fig. 1A) located at position −682 in the ESRRA promoter that is present in 1–4 copies in human chromosomes. Sequence analysis of the 23-bp element, herein referred to as ESRRA23, revealed the presence of two nuclear receptor half-site recognition motifs (Fig. 1A). The upstream half-site is preceded by the three nucleotides TGA thus generating a consensus ERα binding site, also referred to as an ERRE (8). An additional putative ERRE, TCAAGGTGCA, can also be found in the promoter region 1 bp downstream of the ESRRA23 element (Fig. 1B). The ESRRA23 element and the few base pairs flanking it, including the putative ERRE downstream of the repeated element, are absolutely conserved between human and mouse genomic sequences (Fig. 1C). However, the ESRRA23 element is present in a single copy in the mouse genome. The observed ESRRA allelic frequencies among 5490 human chromosomes (2745 individuals) were \(1\) = 0.06, \(2\) = 93.15, \(3\) = 6.3 and \(4\) = 0.36 (Table I).

Functional Characterization of the Polymorphic ESRRA23 Element—Given the observation that the polymorphic sequence contained a putative ERRE, we first tested whether the ESRRA23 motif could serve as an autoregulatory element for ERα. Electromobility shift assays using \textit{in vitro} translated proteins and a set of oligonucleotide probes derived from the ESRRA23 element (Fig. 2A) showed that ERα binds ESRRA23 with high specificity (Fig. 2B). Nucleotide changes within the upstream AGGTCA motif (ESRRA23m1) abolished ERα binding, whereas similar mutations in the downstream CGGTCA half-site (ESRRA23m2) had little effect on recognition of the element by ERα (Fig. 2B). The ERβ and \(\gamma\) isoforms also bound the ESRRA23 element with similar affinity (data not shown). It has been shown recently (35) that treatment with the synthetic estrogen diethylstilbestrol can enhance the expression of ERα. However, the related ER\(\beta\) did not significantly bind the ESRRA23 element or the downstream half-site in this assay, but did recognize a control ER (Fig. 2B and data not shown). Previous studies (8, 10, 15, 36) have shown that the ER\(\alpha\)s can bind to their response element as either monomers or homodimers. The presence of an intermediate retarded complex in a binding experiment using a mixture of wild-type and amino-terminal truncated receptors clearly demonstrates that ER\(\alpha\) preferentially binds ESRRA23 as a homodimer (Fig. 2C, \textit{arrow}). We next examined whether the ESRRA23 element could act as a functional ERRE \textit{in vivo} when linked to a heterologous promoter. As expected, the generally transcriptionally silent ERα failed to generate a significant response when assayed in HeLa cells (Fig. 2D). Therefore, the ESRRA23 element was ligated to a luciferase expression vector, and the resulting construct was transfected into HeLa cells. As shown in Fig. 2E, the ESRRA23 element functioned as an ERRE, driving expression of luciferase from the reporter construct in a dose-dependent manner. These results suggest that the ESRRA23 element is a functional ERRE that can act as an autoregulatory element for ERα.

**Table I**

| Genotype | 1 | 2 | 3 | 4 | 5 | 6 |
|----------|---|---|---|---|---|---|
| No.      | 2 | 1 | 231 | 334 | 16 | 8 |
| Percent  | 0.074 | 0.097 | 86.74 | 12.17 | 0.58 | 0.29 |

**Observed ESRRA allelic frequencies among 5490 human chromosomes**

No. 1 = 0.06, \(2\) = 93.15, \(3\) = 6.3 and \(4\) = 0.36 (Table I).
test whether ERRα recognizes the ESRRA23 element in vivo, we used a mammalian one-hybrid system in which ERRα is linked to the potent transcriptional activation domain of the viral VP16 protein. Indeed, the constitutively active ERRα-VP16 chimera induced strong transcriptional responses (10–57-fold) in an element-dosage manner (Fig. 2E) demonstrating that ERRα can recognize the ESRRA23 element in vivo. We next studied the functional consequence of the ESRRA23 regulatory variant on the ESRRA promoter itself. Human ESRRA promoters containing either 2 or 3 copies of the ESRRA23 element representing the most commonly observed genotypes were cloned upstream of the luciferase reporter gene (Fig. 3A).

We also engineered a mutant ESRRA promoter construct in which all copies of the ESRRA23 element as well as the non-polymorphic putative downstream ERRE were removed (Fig. 3A, ΔESRRA). ERRα induced a small but significant transcriptional response of 1.5- or 2-fold on the ESRRA promoter containing 2 or 3 copies of ESRRA23, respectively (Fig. 3B). The effect is specific as removal of the elements abolishes the ERRα-induced transcriptional response. As observed with the synthetic ESRRA23-thymidine kinase (TK) promoters, the ERRα-VP16 chimera elicited strong responses from the reporter gene driven by the ESRRA promoters containing either 2 or 3 copies of ESRRA23 being 7- and 14-fold, respectively (Fig. 3C). The mammalian one-hybrid assay thus confirms that ERRα can directly interact with the ESRRA promoter. The specificity of the transcriptional effect was demonstrated in that the strength of the response was directly related to the copy number of ESRRA23 and that ablation of the ESRRA23 completely abolished ERRα-VP16-induced luciferase activity. Lastly, we used a chromatin immunoprecipitation assay to test whether endogenous ERRα interacts with the ESRRA promoter in the context of the native chromatin. The human breast cancer cell line MCF-7 was shown previously to express endogenous ERRα and therefore was used for this assay (15). As shown in Fig. 3D, an antibody raised against human ERRα immunoprecipitates a DNA fragment that includes the ESRRA23 elements. Quantitative PCR showed a 25-fold enrichment of the promoter fragment over the control fragment located 4 kb upstream of the ESRRA23 element. Taken together, these experiments clearly show that ERRα recognizes its own promoter via the polymorphic ESRRA23 element.

The ESRRA23 Element Dictates PGC-1α Control of ERRα Expression—As introduced above, recent studies (18, 29, 30) have shown that the coactivator PGC-1α can regulate both the expression and transcriptional activity of ERRα. However, the molecular mechanism underlying the action of PGC-1α on ERRα expression has not yet been elucidated. As shown in Fig. 4A, the introduction of PGC-1α alone by transient transfection in HeLa cells has a significant effect on ESRRA promoter activity, leading to a 4- and 6.5-fold induction in luciferase activity generated by the ESRRA promoter reporter constructs containing 2 and 3 copies of the ESRRA23 element, respectively. The increased ESRRA promoter activity induced by PGC-1α is not only ESRRA23 dosage-dependent but is mediated through ESRRA23 and possibly with a contribution by the flanking ERRα, given that deletion of the region encoding these elements from the ESRRA promoter resulted in a complete loss of the stimulatory activity (Fig. 4A). The direct involvement of the ESRRA23 element in the PGC-1α response was further demonstrated by the observation that PGC-1α can activate TK-luciferase reporter genes containing 3 copies of the...
The polymorphic ESRRα23 element is a functional ERRα response element in the context of the ESRRα promoter. A, schematic representation of the allelic ESRRα promoter luciferase reporter constructs containing 2 (2X) or 3 (3X) copies of the ESRRα23 element or a synthetic mutant in which ESRRα23 is absent (Δ). B and C, effect of the presence of ERRα on ESRRα promoter activity. HEK293 cells were cotransfected with the three ESRRα-based reporter constructs and wild-type ERRαs (B) or the chimeric ERRα-VP16 construct (C). Results are expressed as the fold induction over control vector in the absence of a receptor. D, binding of ERRα to the ESRRα promoter as determined by chromatin immunoprecipitation assay. PCRs containing primer pairs amplifying a region of the ESRRα promoter containing the ESRRα23 element (ESRRα23) or the nonspecific region (−4 kb control) were performed following immunoprecipitation of the DNA/protein complexes with the ERRα antibody (α-ERRα).

ESRRα23 element but not the parent vector (Fig. 4B). The presence of a single copy of the ESRRα23 element was not sufficient to confer PGC-1α responsiveness to the TK promoter in HEK293 cells. However, PGC-1α activity is much more potent in COS-1 cells on both the ESRRα and ESRRα23-TK promoters suggesting that cell context may be important for the PGC-1α response (data not shown).

ERRα-dependent PGC-1α Activity—PGC-1α has been described recently (29, 30) as a potent coactivator of ERRα and the related ERRγ isoform. We therefore investigated the interaction between ERRα and PGC-1α on the polymorphic ESRRα promoter in HEK293 cells. As observed in Fig. 5, coexpression of ERRα and PGC-1α in HEK293 cells results in a synergistic activation of the ESRRα promoter. This set of experiments also demonstrates that both the independent and combined transcriptional activities of ERRα and PGC-1α are not observed in the absence of either the region containing the ESRRα23 elements or a functional ERRα DNA binding domain. The response to ERRα and PGC-1α is also element dosage-dependent, as the promoter containing 3 copies of the ESRRα23 element displays higher activity than the promoter containing 2 copies of the element in the presence of these regulatory factors (Fig. 5).

We next tested whether the presence of ERRα was absolutely essential to the activity of PGC-1α on the ESRRα23 element. Mouse embryonic fibroblasts (MEFs) were derived from both wild-type and ERRα null mice (19) and transfected with PGC-1α and ERRα, alone or in combination with the ESRRα promoter reporter construct containing 3 copies of the ESRRα23 element. ERRα is not transcriptionally active in MEFs, but the constitutively active ERRα-VP16 chimera displays identical activity in MEFs derived from both strains, indicating that ERRα recognizes the ESRRα promoter in a similar manner in both cell types (Fig. 6). As observed previously in HEK293 cells, the introduction of PGC-1α in wild-type MEFs leads to a significant (3-fold) induction of ESRRα promoter activity. However, PGC-1α transcriptional activity is considerably reduced in ERRα null MEFs, but this activity can be completely restored by the introduction of exogenous ERRα (Fig. 6). The response to PGC-1α was not observed when the ΔESRRα construct was used as a reporter in this assay (data not shown). Taken together, these results demonstrate a central role for ERRα in PGC-1α-induced activation of the ESRRα promoter via the ESRRα23 element.
regulate ERRα expression (30) and by which ERRα can control its own expression in a positive fashion. Our results also clearly establish a direct correlation between the number of ESRR23 repeat elements present and the response of the ESRRα promoter to ERRα and PGC-1α proteins alone or in combination. To our knowledge, this is the first example of a natural regulatory polymorphism consisting of a sequence, present in one copy or in tandem-repeated elements two, three, or four times, containing a functional hormone response element for a nuclear receptor-coactivator complex.

The expression patterns of ERRα and PGC-1α and their response to specific physiological stimuli such as cold and starvation are nearly identical (18, 30). Furthermore, in agreement with these observations, PGC-1α has been shown to induce ERRα expression (30). Our functional characterization of the ESRR23 element clearly demonstrates it to be the direct target of PGC-1α action. However, PGC-1α is a coactivator protein that does not bind ESRR23 and thus requires interaction with a transcription factor that has the requisite docking site on the target promoter. One clear candidate is ERRα itself. ERRα binds to the ESRR23 element and activates transcription from it in the presence of PGC-1α and other coactivators such as GRIP-1 (data not shown). The role of ERRα as a PGC-1α DNA binding partner is further corroborated by the transient transfection experiments performed in HeLa cells and MEFs. A strong activation of the ESRRα promoter in HeLa cells is only observed in the presence of both factors, and transcriptional activation is not detected with an ERRα mutant unable to bind DNA. Using MEFs obtained from ERRα null mice, we have also shown that PGC-1α activity is considerably reduced in those cells, indicating an important and direct role for ERRα in PGC-1α action at the ESRRα promoter. We have also observed that PGC-1α retains some transcriptional activity in the ERRα null MEFs suggesting that factors other than ERRα can transduce PGC-1α activity in these cells. Indeed, PGC-1α has been shown to interact with and stimulate the activity of a large number of transcription factors, including many nuclear receptors (21). The likely candidates are nuclear receptors, because the region encoding the ESRR23 elements contains several nuclear receptor binding sites that do not bind DNA. Using MEFs obtained from ERRα null mice, we have also shown that PGC-1α activity is considerably reduced in those cells, indicating an important and direct role for ERRα in PGC-1α action at the ESRRα promoter. We have also observed that PGC-1α retains some transcriptional activity in the ERRα null MEFs suggesting that factors other than ERRα can transduce PGC-1α activity in these cells. Indeed, PGC-1α has been shown to interact with and stimulate the activity of a large number of transcription factors, including many nuclear receptors (21). The likely candidates are nuclear receptors, because the region encoding the ESRR23 elements contains several nuclear receptor binding sites that do not bind DNA. Using MEFs obtained from ERRα null mice, we have also shown that PGC-1α activity is considerably reduced in those cells, indicating an important and direct role for ERRα in PGC-1α action at the ESRRα promoter. We have also observed that PGC-1α retains some transcriptional activity in the ERRα null MEFs suggesting that factors other than ERRα can transduce PGC-1α activity in these cells. Indeed, PGC-1α has been shown to interact with and stimulate the activity of a large number of transcription factors, including many nuclear receptors (21). The likely candidates are nuclear receptors, because the region encoding the ESRR23 elements contains several nuclear receptor binding sites that do not bind DNA. Using MEFs obtained from ERRα null mice, we have also shown that PGC-1α activity is considerably reduced in those cells, indicating an important and direct role for ERRα in PGC-1α action at the ESRRα promoter. We have also observed that PGC-1α retains some transcriptional activity in the ERRα null MEFs suggesting that factors other than ERRα can transduce PGC-1α activity in these cells. Indeed, PGC-1α has been shown to interact with and stimulate the activity of a large number of transcription factors, including many nuclear receptors (21). The likely candidates are nuclear receptors, because the region encoding the ESRR23 elements contains several nuclear receptor binding sites that do not bind DNA. Using MEFs obtained from ERRα null mice, we have also shown that PGC-1α activity is considerably reduced in those cells, indicating an important and direct role for ERRα in PGC-1α action at the ESRRα promoter. We have also observed that PGC-1α retains some transcriptional activity in the ERRα null MEFs suggesting that factors other than ERRα can transduce PGC-1α activity in these cells. Indeed, PGC-1α has been shown to interact with and stimulat...
pathways on a polymorphic hormone response element controlling ERRα expression.

In conclusion, this study clearly demonstrates that the identification of regulatory variants in the human genome can reveal physiologically relevant interactions between the distinct components of complex transcriptional pathways. It would be of interest to pursue further genetic studies to investigate whether the ESRRA23 polymorphism is linked to a particular phenotype or susceptibility to metabolic diseases in the human population, including obesity and diabetes.

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