Transcriptional Regulation of the Mouse Uncoupling Protein-2 Gene
DOUBLE E-BOX MOTIF IS REQUIRED FOR PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ-DEPENDENT ACTIVATION*

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Alexander V. Medvedev‡, Sheridan K. Snedden§§§, Serge Raimbault†, Daniel Ricquier, and
Sheila Collins$$$*

From the Departments of !Psychiatry and Behavioral Sciences and $Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710 and §Centre de Recherche sur l’Endocrinologie Moleculaire et le Developpement, Centre National de la Recherche Scientifique, UPR 9078, Meudon, 92190 France

Uncoupling protein-2 (UCP2) is present in many tissues with relevance to fuel metabolism, and its expression is increased in fat and muscle in response to elevated circulating free fatty acids resulting from fasting and high fat feeding. We proposed a role for peroxisome proliferator-activated receptor-γ (PPARγ) as a mediator of these physiological changes in UCP2, because thiazolidinediones also increase expression of UCP2 in these cell types (1). To determine the molecular basis for this regulation, we isolated the 7.3-kilobase promoter region of the mouse UCP2 gene. The −7.3-kilobase/+12-base pair fragment activates transcription of a reporter gene by 50–100-fold. Deletion and point mutation analysis, coupled with gel shift assays, indicate the presence of a 43-base pair enhancer (−86/−44) that is responsible for the majority of both basal and PPARγ-dependent transcriptional activity. The distal (−86/−76) part of the enhancer specifically binds Sp1, Sp2, and Sp3 and is indistinguishable from a consensus Sp1 element in competition experiments. Point mutation in this sequence reduces basal activity by 75%. A second region (−74/−66) is identical to the sterol response element consensus and specifically binds ADD1/SREBP1. However, deletion of this sequence does not affect basal transcriptional activity or the response to PPARγ. The proximal portion of the enhancer contains a direct repeat of two E-Box motifs, which contributes most strongly to basal and PPARγ-dependent transcription of the UCP2 promoter. Deletion of this region results in a 10–20-fold reduction of transcriptional activity and complete loss of PPARγ responsiveness. Point mutations in either E-Box, but not in the spacer region between them, eliminate the stimulatory response to PPARγ. However, gel shift assays show that PPARγ does not bind to this region. Taken together, these data indicate that PPARγ activates the UCP2 gene indirectly by altering the activity or expression of other transcription factors that bind to the UCP2 promoter.

Uncoupling protein-2 (UCP2) was discovered in 1997 as a homologue of the brown fat UCP (UCP1) (2). Like UCP1, which is able to dissipate caloric energy as heat by uncoupling mitochondrial respiration from ATP production, UCP2 can similarly uncouple respiration, at least in vitro (2). This fact, coupled with other significant features, including tissue distribution and genetic location in a strong quantitative trait locus for hyperinsulinemia and leptin levels, made UCP2 an attractive candidate for regulation of resting metabolic rate and as an obesity/diabetes susceptibility gene. However, we recently reported that targeted disruption of the UCP2 gene does not lead to spontaneous or diet-induced obesity and diabetes (3), but changes in sensitivity to glucose-stimulated insulin secretion are observed. Thus, the relationship between energy metabolism and UCP2 remains unclear.

Transcripts and protein expression of UCP2 are found in many tissues with relevance to fuel metabolism such as pancreatic β-cells, white and brown fat, skeletal muscle, and hypothalamus. In response to high fat diets, we have previously reported adipose-specific increases in expression of UCP2 in obesity-resistant mouse strains such as A/J and C57BL/Kalis, which are absent from the obesity-prone C57BL/6J strain of mice (2, 4). Of mechanistic importance, blockade by nicotinic acid of both the fasting-induced rise in free fatty acids, as well as fatty acid transport into mitochondria (5), prevented the fasting-induced increase in UCP2 mRNA in muscle. The importance of changes in fat metabolism for regulation of UCP2 expression was further supported by the observation that fasting-induced increases in circulating free fatty acids led to a stimulation of UCP2 expression in adipose tissue and muscle, whereas subsequent refeeding suppressed UCP2 expression (6–8). However, this down-regulation of UCP2 was not observed when the post-fasting diet contained a high percentage of calories from fat (9).

Although these findings provide compelling evidence for the involvement of fatty acids in regulation of UCP2 expression, the molecular mechanisms remain unknown. However, a PPAR-dependent pathway is one of the possible mechanisms that can...
be considered to mediate the response of UCP2 to fatty acids, because it has been shown that at least some fatty acids can bind PPARγ specifically and may act as natural ligands for this transcription factor (10, 11). It also has been shown that treatment with PPARγ agonists increases UCP2 mRNA levels in vivo and in vitro (1, 12).

PPARγ plays an important role in the control of the expression of many genes involved in energy metabolism by binding specific elements consisting of a direct repeat (DR-1) of a consensus sequence (AGGTCA) separated by one base, although functional peroxisome proliferator-responsive elements (PPREs) can deviate significantly from the consensus (13). The presence of functional PPREs in the regulatory sequences of such genes as the adipocyte fatty acid-binding protein, aP2 (14), phosphoenolpyruvate carboxykinase (15), acyl coenzyme A synthetase (16), and lipoprotein lipase (17) is consistent with the crucial role attributed to PPARγ in lipid metabolism. To further understand the mechanisms regulating transcription of the UCP2 gene, as well as a role of PPARγ in its regulation, we report here cloning and analysis of the transcriptional activity of a 7.3-kb fragment of mouse UCP2 promoter.

EXPERIMENTAL PROCEDURES

Cloning of Mouse UCP2 Promoter and Construction of Deletion and Point Mutants in Luciferase Reporter Plasmids—A mouse genomic library (129 SVJ mouse DNA digested by Sau3A DNA and inserted into lambda FIX II vector, catalog number 946306; Stratagene, Palo Alto, CA) was screened using a 1,304-bp PCR probe encomasing intron 1 and exon II of the mouse UCP2 gene. The PCR probe was generated using sense 5′-TGACTCTGGAGTTTCGTCGGA-3′ and reverse 5′-GACTCTGGAGTTTCGTCGGA-3′ primers and corresponding to intron 1 and exon II. The cloned genomic fragment (made of 7,452 bp) was inserted into the NotI site of pSPORT plasmid (Life Technologies, Inc.) and electroporated into E. coli (strain DH10B). A library of the 129 SVJ mouse gene, the same region was isolated from the A/J, C57BL/6J, and C57Ks/J strains and entirely sequenced. To construct luciferase reporter plasmids, fragments of the A/J mouse UCP2 promoter (GenBank™ accession number AF115319) were cloned into pGL2-Basic (Promega) using restriction fragments or amplified by PCR and cloned using the primer-introduced MluI and NheI restriction sites. Point mutations were made by direct cloning of chemically synthesized oligonucleotides or by PCR. The integrity and fidelity of all promoter-reporter constructs thus made were verified by DNA sequencing.

Cell Culture Transfections and Reporter Gene Assays—C3H10T1/2 (ATCC number CCL-226) and HIB-1B preadipocytes (gift from Dr. Reed Graves) and C2C12 (ATCC number CCL-226) and HIB-1B preadipocytes (gift from Grinnell College, Ames, IA) were cultured in MEM with 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.) at 37 °C in a humidified 5% CO2 atmosphere. For cell culture transfections, plasmid constructs thus made were verified by DNA sequencing. Point Mutants in Luciferase Reporter Plasmids—A mouse genomic library (129 SVJ mouse DNA digested by Sau3A DNA and inserted into lambda FIX II vector, catalog number 946306; Stratagene, Palo Alto, CA) was screened using a 1,304-bp PCR probe encompassing intron 1 and exon II of the mouse UCP2 gene. The PCR probe was generated using sense 5′-TGACTCTGGAGTTTCGTCGGA-3′ and reverse 5′-GACTCTGGAGTTTCGTCGGA-3′ primers and corresponding to intron 1 and exon II. The cloned genomic fragment (made of 7,452 bp) was inserted into the NotI site of pSPORT plasmid (Life Technologies, Inc.) and electroporated into E. coli (strain DH10B). A library of the 129 SVJ mouse gene, the same region was isolated from the A/J, C57BL/6J, and C57Ks/J strains and entirely sequenced. To construct luciferase reporter plasmids, fragments of the A/J mouse UCP2 promoter (GenBank™ accession number AF115319) were cloned into pGL2-Basic (Promega) using restriction fragments or amplified by PCR and cloned using the primer-introduced MluI and NheI restriction sites. Point mutations were made by direct cloning of chemically synthesized oligonucleotides or by PCR. The integrity and fidelity of all promoter-reporter constructs thus made were verified by DNA sequencing.

Isolation and Analysis of RNA—Total cellular RNA was prepared by the Tri reagent method according to the manufacturer’s protocol (Molecular Research Center, Inc.). For Northern blot hybridization, RNA was denatured by the glyoxal procedure, fractionated through 1.2% agarose gels, and blotted onto Biotrans (ICN) nylon membranes (22). Radiolabeled probes were prepared by random primer extension (Prime-It RmT; Stratagene) of the purified DNA fragments in the presence of [α-32P]dCTP to a specific activity > 2 108 dpm/μg DNA. The DNA fragments that were used as probes were obtained from the following sources. A fragment specific for the mouse UCP2 was prepared as described in Ref. 2. A rat cDNA probe for cyclophilin was used as an internal hybridization/quantitation standard. Blots were hybridized and washed as previously described (23, 24). The intensity of hybridization signals was quantified by a phosphorimager (ImageQuant/Storm) and normalized to the values for cyclophilin.

RESULTS

PPARγ Stimulates Expression and Promoter Activity of UCP2—We and others have previously shown that PPARγ agonists are able to increase UCP2 mRNA levels in adipocytes and muscle cells (1, 12, 25). Fig. 1A shows that UCP2 mRNA is present at relatively high levels in HIB-1B brown and D1 white preadipocyte and C2C12 myoblast cell lines. Treatment of these cells for 24 h with the thiazolidinedione BRL49653, a PPARγ ligand, and agonist (26) results in a 2-fold increase of UCP2 mRNA in all cell lines. Note that these results in HIB-1B cells recapitulate our previously reported findings (1).

To analyze the mechanisms responsible for the transcriptional regulation of the mouse UCP2 gene, and to test whether PPARγ affects the activity of the UCP2 promoter, a fragment of the mouse UCP2 promoter region from position −7359 to +12 was isolated, sequenced, and subcloned into a luciferase reporter vector. This DNA fragment spans the entire region between the last exon of UCP3 and the first exon of UCP2 (4). As shown in Fig. 1B, and consistent with the Northern blot data of Fig. 1A, BRL49653 was able to stimulate transcriptional activity of the 7.3-kb fragment of the UCP2 promoter in all cell lines tested. Moreover, cotransfection of the 7.3-kb UCP2 promoter construct with an expression vector containing PPARγ (Fig. 1B) led to a similar 2-fold increase of UCP2 promoter activity. However, simultaneous treatment of these cells with T4 polyacrylamide gel electrophoresis at room temperature in 0.5× Tris-borate EDTA, dried, and exposed to PhosphorImager plates for imaging (Storm880; Molecular Dynamics).

The Region between −86 and −44 Nucleotides of the UCP2
Promoter Is Important for Basal Transcriptional Activity and PPARγ Responsiveness—To identify regulatory sequences that are important for transcriptional control of the UCP2 gene, including the response to PPARγ, we created a series of deletion constructs of the UCP2 promoter as shown in Fig. 2A. The transcriptional activity of these constructs was analyzed by transient transfection in D1, HIB-1B, and C2C12 cells (Fig. 2, B–D). Fig. 2 shows that the largest fragment of the UCP2 promoter (−7.3 kb/−12 bp) is able to increase luciferase activity of the reporter construct by 50–150-fold depending on the cell line, whereas the activity of a minimal promoter (−44/−12 bp) is only 1.5–2-fold above empty vector. Deletion of the region between −7.3 and −5 kb reduces promoter activity by 2–3-fold in HIB-1B and C2C12 (Fig. 2, B and D) but had no effect in D1 cells (Fig. 2C) or in C3H101T1/2 cells (data not shown). Although further deletions between positions −5 kb and −86 bp had no significant effect on activity, deletion of the region between positions −86 and −44 led to a dramatic 25–50-fold depression of activity, indicating that this region is indeed a major contributor to the overall expression of the UCP2 gene in all cell lines. Having established the pattern of basal activity of these deletion fragments, we next examined the activity of these regions in cells treated with BRL49653, as well as cotransfected with PPARγ. As shown in Table I, deletion of the sequence up to −86 bp still retained PPARγ-dependent stimulation of UCP2 promoter activity equivalent to the −7.3-kb fragment. However, deletion of the region between −86 and −44 bp resulted in a total loss of PPARγ-dependent activity, indicating that this region of the UCP2 gene is necessary not only for basal activity but also for regulation by PPARγ.

Identification of the Transcription Elements Located in the −86/−44 Region of the Mouse UCP2 Promoter—Because our data establish that the −86/−44 region of the mouse UCP2 promoter is a major locus of control in cell lines of fat and muscle origin, in this series of experiments we focused on identifying the elements and transcription factors involved in regulation of this region. Examination of the sequence of the −86/−44 region of the UCP2 promoter of mouse revealed that this region has no significant homology with any known PPARγ response element (see Fig. 4). Moreover, as shown in Fig. 3, PPARγ does not bind to the −86/−44 fragment in HIB-1B nuclear extracts, and this region does not compete for PPARγ binding versus a bona fide PPRE from the acyl-CoA oxidase promoter (AGGACAAAGGTC) (28). The inability of this −86/−44 UCP2 region to bind PPARγ indicates that stimulation of the UCP2 promoter by PPARγ must be indirect, utilizing other transcription factors or/and cofactors interacting with this region. As Fig. 4 shows, the −86/−44 region of the mouse UCP2 promoter contains several putative regulatory elements that are also found in active promoter regions of other genes involved in energy metabolism. These elements include Sp1 (−86/CTCCGCTCT/−76), sterol response element (SRE) (−74/
Transcriptional Regulation of UCP2

TABLE I

|       | −7.3 kb | −5.0 kb | −86 bp | −44 bp |
|-------|---------|---------|--------|-------|
| BRL   | 2.40 ± 0.23** (4) | 1.93 ± 0.14** (3) | 2.47 ± 0.19** (3) | 0.75 ± 0.15 (3) |
| PPARγ| 1.89 ± 0.14** (4) | 1.70 ± 0.15* (3) | 1.89 ± 0.05** (4) | 0.94 ± 0.11 (3) |

* p < 0.01; ** p < 0.001; significantly different from basal activity by one-way ANOVA and post-hoc Newman-Keuls test. Values in parentheses indicate number of replicates. HIB-1B cells were transfected as in Fig. 2 with deletion mutant constructs of the UCP2 promoter. PPARγ-dependent activation was tested by treatment with 1 μM BRL49653 for 24 h (BRL) or cotransfection with expression plasmid for PPARγ (PPARγ). Results are shown as fold increase over unstimulated basal activity.

DISCUSSION

PPARγ is best known as a key transcriptional regulator of adipocyte differentiation, stimulating transcription of many genes involved in glucose and lipid metabolism. Adipocyte differentiation is considered to be regulated by the interplay of pyruvate kinase and are recognized by upstream stimulatory factor (USF) family transcription factors (29). As shown in Fig. 7, an oligonucleotide comprising this region of the UCP2 promoter (−71/−44) specifically binds in vitro-translated USF1 and USF2 transcription factors and binds USF1 protein in the nuclear extracts from HIB-1B cells.

Having shown that these three motifs are capable of binding their factors in gel shift experiments, we next proceeded to determine which of these regions might be important for transcriptional activity of the UCP2 enhancer. Therefore, we introduced further deletions, as well as point mutations, in the −86/−44 region of the UCP2 promoter and used these constructs in transient transfection assays. Table II depicts the specific mutations made, and Fig. 8A presents these schematically. When transfected into all cell lines, deletion of the sequence between positions −86 and −76, containing the Sp1 motif, or a point mutation of two bases in this region (−86M1) reduces activity of the reporter gene by 70% (Fig. 8B). Further deletion of five more bases to position −71 disrupts SRE located between −76 and −67, but this mutation did not affect transcriptional activity of the UCP2 promoter. The region between −71 and −44 contains a direct repeat of two imperfect E-Boxes separated by five bases (see Fig. 4). Fig. 8 shows that point mutations in either the upstream or downstream E-Box motifs result in a significant loss of transcriptional activity. Complete deletion of the upstream E-Box to position −58 reduced activity to the level of the minimal UCP2 promoter (−44/+12) indicating that both E-Boxes are necessary for promoter activity. Interestingly, mutations replacing two bases in the spacer region between the E-Boxes do not affect transcriptional activity. However, insertion of three additional bases between E-Boxes again reduces promoter activity, highlighting the importance of the precise positioning of the two parts of the repeat relative to each other.

To determine whether these putative elements were capable of binding transcription factors we performed a series of gel shift assay experiments with in vitro-translated transcription factors or nuclear extracts. The results in Fig. 5A show that the −84/−76 element has transcription factor binding activity similar to an Sp1 consensus element. An oligonucleotide containing this region (−86/GGCTCGGCTCGTCAG/−70) is able to compete efficiently with an Sp1 consensus oligonucleotide (GGCCCGCGGCGGCA) whereas mutation of the core region (−86/GGCTCTACCTGTCCAG/−70) disrupts this binding. Fig. 5B shows that antibodies against Sp1, Sp2, and Sp3 are able to supershift bound proteins, and a mixture of all three antisera resulted in complete supershift of this major binding species.

The transcription factor ADD1/SREBP1 has been shown to have the unique ability to bind two distinct regulatory elements, SRE and E-Box (31). The SRE (consensus sequence, 5′-TCACGCCAC/-66) is known to interact with USF family proteins (29). As described under “Experimental Procedures.” In gel shift assays containing radiolabeled oligonucleotides, competition with unlabeled SREBP1 or SREBP2 oligonucleotides resulted in a significant reduction in shifted bands.

Fig. 3. PPARγ does not bind to the −86/−44 region of the mouse UCP2 promoter. Nuclear extracts were prepared from HIB-1B cells as described under “Experimental Procedures.” In gel shift assays containing [32P]–radiolabeled oligonucleotides containing PPRE of the ACOX gene (GGACCGAGAAGGGTCACG) or the −86/−44 region of the UCP2 promoter (see Fig. 4) were incubated with HIB-1B nuclear extract for 25 min at room temperature. Anti-PPARγ antibody (PPAR-AB) (SC-7196X, Santa Cruz) or 100-fold molar excess of unlabeled competitor oligonucleotides were added to the reaction at the same time as labeled probe. The dark arrow shows the PPARγ-specific band, and the open arrow shows antibody supershift. Lanes 1–4 contain radiolabeled ACOX PPRE, and lanes 5–8 contain the radiolabeled −86/−44 region of the UCP2 promoter.

TCACGCCAC/−66), and a double E-Box-like motif (CACGCCAC) separated by 5 bases found in several genes critical for energy metabolism (reviewed in Ref. 29) and that can also be recognized by SRE-binding proteins (SREBP1) (30).

These data we conclude that stimulation of the UCP2 gene by PPARγ acts through elements in the −86/−44 region of the UCP2 promoter. This is consistent with the previous findings of Srinivasan et al. (26) and Terman et al. (27). The role of PPARγ in the regulation of UCP2 expression is therefore likely to be complex, involving both direct and indirect mechanisms. Further studies are required to elucidate the precise role of PPARγ in the regulation of UCP2 expression in vivo.
Transcriptional Regulation of UCP2

Fig. 4. Comparison of the −86/−44 UCP2 enhancer region with active regions of the genes involved in energy metabolism. The consensus SRE is described in Ref. 30, and specific SRE from 3-hydroxy-3-methylglutaryl-CoA synthase (−256/−248) (42), fatty acid synthase (−71/−62) (43), and low density lipoprotein (LDL) receptor gene (44) are shown. The double E-Box element is reviewed in Ref. 29. E-Box motifs shown are from liver-specific pyruvate kinase (LPK) (−167/−148), rS14 (−1441/−1422) (45), and mS14 (−1444/−1425) (38).

Fig. 5. The region −86/−70 is a functional Sp1 binding element. A, comparison of transcription factor binding activity of the −86/−70 region of the UCP2 promoter (GGCTCCGCTCTGATCGA) and an Sp1 consensus (Sp1 cons) oligonucleotide (GGCCCGCGCGGATCGA) in HIB-1B nuclear extract. Lanes 1–4 contain labeled −86/−70 oligonucleotide. Lanes 5–8 contain the labeled Sp1 consensus oligonucleotide (Promega). All competing oligonucleotides were used in 100-fold molar excess. The oligonucleotide −86/−70M (GGCTCTACGGATCGA) in HIB-1B nuclear extract. Arrows indicate bands specific for Sp1, Sp2 (filled), and Sp3 (striped) transcription factors. The open arrow shows supershift with antibodies.

Fig. 6. Region −78/−62 specifically binds the ADD1/SREBP1 transcription factor. A, schematic presentation of oligonucleotides used in the gel shift assay. B, comparison of the different parts of the −86/−44 region for their ability to bind in vitro-translated ADD1/SREBP1 in gel shift assay. The ADD1/SREBP1 (nuclear form, amino acids 1–403) was in vitro-translated using the TnT® coupled transcription/translation system (Promega). The oligonucleotides corresponding to the indicated regions (A) were incubated with 2 μl of the ADD1/SREBP1ic reaction mix. Lane 1 shows the −86/−44 fragment incubated with in vitro translation reaction mix containing no template. Lane 6 contains antibody against ADD1/SREBP1 (sc-8984X; Santa Cruz). The filled arrow indicates the ADD1/SREBP1-specific band. The open arrow shows anti-SREBP1 antibody supershift.

Fig. 7. Region −71/−44 specifically binds USF1 and USF2 transcription factors. A, USF1 and USF2 were in vitro-translated with the TnT® coupled transcription/translation system (Promega). Radiolabeled oligonucleotide containing the −71/−44 region of the UCP2 promoter was incubated with 2 μl of in vitro-translated transcription factors USF1, USF2, or both for 25 min. Ab, antibody. B, the −86/−44 and −71/−44 oligonucleotides bind USF1 in HIB-1B nuclear extract. Specified oligonucleotides were incubated with HIB-1B nuclear extract as described under “Experimental Procedures.” For supershift analysis antibodies against USF1 (sc-8983X; Santa Cruz) or USF2 (sc-861X; Santa Cruz) were added to the reaction, together with the labeled probe. Arrows indicate USF1- and USF2-specific bands (filled) or antibody supershift (open).

Three families of transcription factors, PPARγ, C/EBPα, and ADD1/SREBP1 (32), and like most tissues (33) it is characterized by a cascade activation of genes that includes other transcription factors and their targets. However, the powerful role of PPARγ to regulate other transcription factors is highlighted by the fact that PPARγ-specific agonists alone can induce adipose differentiation and the expression of C/EBPα even in fibroblasts ectopically expressing PPARγ (34, 35).

Our results demonstrate that PPARγ activation leads to increased UCP2 expression, and we defined the −86/−44 region of the promoter as responsible for stimulation by PPARγ. However, as we show, PPARγ does not bind to this −86/−44 region, suggesting that its role is indirect and likely involves other transcription factors. Parenthetically, PPARγ lacking its DNA-binding domain was unable to stimulate UCP2 promoter activity (data not shown), indicating that at some level DNA binding of PPARγ is required. Instead, the −86/−44 region is composed of three overlapping putative elements, Sp1, SRE, and a double E-Box. Each of these sequences is capable of binding multiple proteins in nuclear extracts, including Sp1 members, SREBP, and the USFs, respectively.

Our point mutation analysis showed that the E-Box motif is required for PPARγ responsiveness. The ability of USF1 and USF2 to bind to the E-Box-containing region (−71/−44) in our experiments makes these transcription factors likely candidates for mediating the effect of PPARγ on the UCP2 gene.
Fig. 8. Sp1 and E-boxes are required for transcriptional activity of the UCP2 promoter. A, schematic representation of mutant constructs of the −86/−44 region of the UCP2 promoter used in transfection experiments. B, transient transfection of the mutant constructs into HIB-1B cells was performed as described under “Experimental Procedures.” Each transfection reaction included 0.7 μg of mutant construct and 0.3 μg of β-actin-CAT as control of transfection efficiency. LUC, luciferase.

Fig. 9. The paired E-boxes, but not the Sp1 site, are required for UCP2 promoter activity in response to PPARγ. Transient transfection of the UCP2 −86/−44 mutant constructs into HIB-1B cells was performed as in Fig. 8 and as described under “Experimental Procedures.” To test the effect of PPARγ on these mutants, 0.3 μg of PPARγ expression vector was included in transfection reaction. Luciferase (LUC) activity of the mutant constructs was normalized to CAT activity of β-actin-CAT. Bars represent three independent experiments performed in triplicate as average ± S.D. * p < 0.001; significantly different from basal activity by one-way analysis of variance and a post-hoc Newman-Keuls test.

However, we cannot rule out a role for SREBP because of the known dual specificity of SREBP to interact with both the SRE and E-Boxes (30). Another possibility that we must consider in future work is that the SRE frequently requires the presence of other active elements such as Sp1 or NF-Y in close proximity. For example, mutation of the Sp1 element, positioned next to the SRE site in the low density lipoprotein receptor promoter, abolished the response of this gene to sterol regulation (36, 37). It has also been suggested that the double E-Box element of the mouse S14 gene is regulated by glucose through as yet unidentified factors that were clearly not USFs (38).

Finally, coactivators must also be considered as candidate mediators of the PPARγ response on the UCP2 gene. Wu et al. (41) showed that over-expression of PGC1 led to increased expression of endogenous UCP2, along with increased oxygen consumption and uncoupled respiration. In the cells we have studied, expression of PGC1 is rapidly increased in response to PPARγ stimulation. Our preliminary studies, together with the data of Monsalve et al. (39), indicate that PGC1 increases expression of the UCP2 promoter. Considering the ability of PGC-1 to interact with a number of other nuclear receptors and coactivators (40, 41) it is possible that PGC1, together with (an)other transcription factor(s), serves to mediate the PPARγ response on the UCP2 promoter. All of these possibilities remain to be explored.

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