RIP140 Expression Is Stimulated by Estrogen-related Receptor α during Adipogenesis*

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RIP140 is a co-repressor for nuclear receptors that regulates energy expenditure in adipose tissue by suppressing the expression of clusters of metabolic genes involved in glucose and lipid metabolism. The gene encoding RIP140/Nrip1 contains only one coding exon but has multiple promoters and 5′ non-coding exons that are subject to alternative splicing. In adipocytes we have defined a promoter, referred to as P2, that is preferentially utilized and activated during adipogenesis. Expression studies and chromatin immunoprecipitation experiments indicate that estrogen-related receptor α (ERRα), the level of which increases during adipogenesis in parallel with RIP140, stimulates transcription from the P2 promoter. Further analysis indicates that ERRα is capable of activating RIP140 gene transcription by two mechanisms, directly by binding to an estrogen receptor element/ERR element at −650/−633 and indirectly through Sp1 binding sites in the proximal promoter. Thus, the up-regulation of RIP140 by ERRα during adipogenesis may provide an inhibitory feedback mechanism to control the expression of many nuclear receptor target genes.

Adipogenesis is regulated by the coordinated expression of transcription factors that control the formation and function of white adipose tissue as a fat storage depot. The identification of many of these transcription factors has relied upon the analysis of preadipocyte cell lines, notably 3T3-L1, that can be induced to undergo differentiation upon exposure to a number of hormone signals, namely glucocorticoid, insulin, and inducers of cAMP. This leads to the activation of a cascade of transcription factors including CCAAT enhancer-binding protein β, CCAAT enhancer-binding protein δ, and SREBP1c that then induce the expression of peroxisome proliferator-activated receptor γ and CCAAT enhancer-binding protein α and the formation of adipocytes (1–5). Many other transcription factors, such as Kruppel-like factor 5 and E2F1, also facilitate adipogenesis by promoting expression of peroxisome proliferator-activated receptor γ, whereas some, such as E2F4 and GATA2/3 that are inhibitory, are down-regulated during adipogenesis (6–9). The importance of these transcription factors has been confirmed by the analysis of mouse gene knockouts, but CCAAT enhancer-binding protein α and peroxisome proliferator-activated receptor γ2 appear to play a crucial role, with the latter often regarded as a master regulator of adipogenesis (10–12).

The function of adipose tissue in the control of fat storage and utilization also depends on the transcriptional control of networks of genes. Among the many transcription factors that control metabolic gene networks are nuclear receptors including peroxisome proliferator-activated receptors, thyroid hormone receptors, and estrogen-related receptors (ERRs) (11–17). The ability of nuclear receptors to regulate the expression of genes involved in triglyceride synthesis or fatty acid oxidation is mediated by coactivators and corepressors. Among these are the PGC-1 coactivators that seem to play key roles in activating metabolic gene networks (18–20). Although the importance of gene activation is well established, it is evident that gene repression is also a regulatory mechanism in adipogenesis (21). Recent studies have determined that gene repression is also a crucial factor in adipocyte function. In particular, the RIP140 corepressor is essential for triglyceride storage in adipose tissue, with RIP140 null mice being lean and resistant to high fat diet-induced obesity (22). The depletion of fat stores in white adipose tissue is accomplished by an increase in expression of clusters of genes involved in fatty acid oxidation, glycolysis, tricarboxylic acid cycle, oxidative phosphorylation, and glucose utilization (22–24). As a consequence, RIP140 plays an important role in energy expenditure and glucose tolerance in mice.

Previous work has demonstrated that RIP140, which is encoded by a single exon (25, 26), is expressed in specific cell types in many tissues. Its expression is subject to regulation by peptide hormones, retinoids, and steroid hormones (26–29), but potential promoter(s) and regulatory elements are poorly characterized. The possibility of additional exons and remote regulatory elements became evident when estrogen-regulated RIP140 expression was investigated in breast cancer cells (29–34). Genome-wide screens have identified two EREs −100 and 200 kb upstream of the coding region, with the distal element

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1 The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. S1.

2 The abbreviations used are: ERR, estrogen-related receptor; ERE, estrogen receptor element; ERRE, ERR element; RIP140, repressor-interacting protein 140; Sp1, specificity factor 1; SREBP, sterol response element-binding protein; PGC-1, peroxisome proliferator-activated receptor γ coactivator; RACE, rapid amplification of cDNA ends; siRNA, small interfering RNA; ChIP, chromatin immunoprecipitation.
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RIP140 is highly expressed in white and brown adipose tissue relative to other tissues, and expression profiling and real-time PCR analysis indicate that it increases progressively in 3T3-L1 preadipocytes when they are induced to undergo adipogenesis (22, 37). This increase is consistent with the role of RIP140 in controlling the function of mature adipocytes rather than the process of adipogenesis. In this study, we have determined the exon-intron organization of the RIP140 gene and found that it is subject to alternate splicing from multiple promoters, one of which is specifically utilized in adipocytes. We focused on the mechanism by which RIP140 expression is up-regulated following adipogenesis and report a dual role for the orphan nuclear receptor ERRα in regulating RIP140 expression.

EXPERIMENTAL PROCEDURES

5′RACE—RNA from the human breast cancer cell line ZR-75, mouse 3T3-L1 adipocytes, and mouse ovarian tissue was extracted using TRIzol (Invitrogen) as described by the manufacturer. 5′RACE using the RNA described above and the RLM-RACE kit (Ambion) was carried out according to the manufacturer’s guidelines. PCR products were cloned using pCRRII-TOPO vector (Invitrogen) and sequenced using standard methods by the AB1 3100 sequencer.

Real-time PCR—RNA was extracted using TRIzol as described above, and 1 μg was reverse transcribed using the Superscript First Strand synthesis kit (Invitrogen). Real-time PCR was carried out with the Opticon 2 (GRI) detection system. Primers (Thermoelectron) and FAM-labeled Taqman probes (Qiagen) were designed using Primer Express software (PerkinElmer Life Sciences). The ribosomal coding gene L19 was used to normalize cDNA levels, and the bacterial artificial chromosome RP23-71H12 (bacpac.chori.org/) containing the whole mouse RIP140 gene was used to generate a standard curve for absolute quantification of mRNA.

In Situ Hybridization—Exons 1a and 1b were amplified using specific primers and cloned into pCRII-TOPO vector. RNA probes were transcribed using a Megascript SP6 or T7 kit (Ambion). 3T3-L1 cells, grown on plastic chamber slides, were fixed in neutral buffered formalin and hybridized to digoxigenin-labeled probes corresponding to RIP140 exons 1a and 1b as described previously (38).

Plasmid Construction—Each RIP140 promoter region was cloned into the pGL3 basic vector (Promega). To generate P1, a 3.5-kb region, located directly upstream of exon 1a, was amplified from mouse genomic DNA using 5′-CCAATTGAGCTCTCCTGAGCTTCTCCTGTCT-3′ (forward primer, F) and 5′-AATTGAGCTGGAACCTGTCTCTCTCTC-3′ (reverse primer, R) and cloned using SacI and Xhol. For P2, a 791 to +16 nucleotide region relative to exon 1b was amplified using 5′-CCAATTCTCGAGCAAGACGAGAAGAGAAACC-3′ (F) and 5′-AATTGAGGCTCCTCTGTCTGCAATTCTGAGGACTG-3′ (R) and cloned using Xhol and HindIII. The P1/P2 construct was amplified using the forward P1 primer and a modified reverse P2 primer (Xhol site instead of HindIII), and the fragment was cloned using SacI and Xhol. The P2 construct containing point mutations in the ERR binding sites was generated using the QuickChange site-directed mutagenesis kit (Stratagene). All P2 deletion mutants were constructed from PCR products amplified from the P2 wild-type construct. Reporter construct sequences were confirmed using standard methods by the AB1 3100 sequencer.

Transient Transfection Assay—Transient transfection assays were carried out using Cos1 and 3T3-L1 cells. Cos1 cells were seeded in 96-well plates in 5% dextran charcoal-stripped fetal bovine calf serum. After 24 h, when cells reached between 50–80% confluence, 20 ng of either wild-type or mutant P2 constructs were transfected into each well with 5 ng of pCMV- Renilla (Promega) using FuGENE (Roche Applied Science). 24 h post-transfection the ERαs were added as indicated in the figure legends. 48 h post-transfection, cell extracts were prepared for analysis using the luminescence reporter gene assay system (PerkinElmer), and luciferase activity was measured using the Victor2 1420 Multilabel counter (PerkinElmer). 3T3-L1 cells were seeded in 6-well plates in medium containing 10% newborn calf serum in order to reach confluence 24 h later. Immediately after seeding cells, each well was transfected with 20 μg of either P1, P2, or P1/2 and 500 ng of pCMV-Renilla. 24 h post-transfection, medium was replaced with 10% fetal bovine serum containing an adipocyte differentiation mixture (170 nM insulin, 250 nM dexamethasone, 500 μM 3-isobutyl-1-methylxanthine, 2.5 nM rosiglitazone). 48 h after the differentiation mixture was added, it was replaced with medium containing 10% fetal bovine serum, rosiglitazone (2.5 nM), and insulin (170 nM) and maintained like this for a further 24 h, when cell extracts were prepared for analysis as described above. Data shown are representative of four independent experiments.

Western Blot Analysis—A modified method of Rittenhouse and Marcus was used for protein analysis (39). Protein concentrations were determined by BCA kit (Pierce), and equal amounts of whole cell extracts (50 μg) were separated on a 10% SDS-polyacrylamide gel before electrotransfer at 120 V onto a polyvinylidene difluoride membrane (Hybond P; Amersham Biosciences, Inc.). Nonspecific binding sites were blocked by 1 h of incubation with 5% dried skimmed milk in Tris-buffered saline (TBS), 130 mM NaCl, 20 mM Tris, pH 7.6) containing 1% Tween (TBS-T). Primary antibodies were incubated at the indicated dilutions in TBS-T containing 2.5% dried skimmed milk overnight at 4 °C as follows: ERRα (LS-A5402; 1 in 1000; Life-span), RIP140 (mouse monoclonal antibody generated against residues 301–478; 1 in 500), RNA polymerase II (Sc-899; 1 in 20) and luciferase (sc-299; 1 in 1000; Life-span). Membranes were washed for five 15-min periods in TBS-T and probed with HRP-conjugated secondary antibodies (1 in 1000; Life-span). The immunoreactive bands were visualized using chemiluminescence reagent (Pierce) and detected using Kodak X-OMAT film (Eastman Kodak). Band intensities were quantitated using Kodak 1D software.
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1000; Santa Cruz), SREBP-1 (Sc-8984X; 1 in 5000; Santa Cruz), E2F-1 (Sc-22820; 1 in 5000; Santa Cruz), and Sp1 (1 in 5000; Sigma). All secondary antibodies were incubated in TBS-T at room temperature, 1 h, 1 in 5000. For detection of ERRα, RNA polymerase II, SREBP-1, Sp1, and E2F1, the blots were incubated with horseradish peroxidase-conjugated polyclonal goat anti-rabbit IgG (DAKO P0448). For detection of RIP140, the blot was incubated with horseradish peroxidase-conjugated polyclonal goat anti-mouse IgG (DAKO PO447). Protein bands were visualized by enhanced chemiluminescence (ECL plus Western blotting detection; Amersham Biosciences, Inc.).

Electrophoretic Mobility Shift Assays—ERRα was in vitro translated from pCMX-ERRα using the TNT T7 polymerase Quick-Coupled Transcription/Translation System (Promega). Electrophoretic mobility shift assay studies were carried out as described previously (40) with the following modifications: 1 μg of in vitro translated ERRα was incubated with 100 ng of a T4-polynucleotide kinase end-labeled double-stranded oligonucleotide probe corresponding to the RIP140 ERE/ERRE. For supershift analysis, 1 μg of ERRα antibody was added to the reaction. DNA-protein complexes were separated and visualized as described previously.

ChIP—Cells were incubated in 10% formaldehyde for 15 min at 37 °C. The cells were then lysed, sonicated, and chromatin immunoprecipitated with 5 μg of ERRα antibody as described previously (23). DNA fragments were purified, and PCR amplification was performed using P2 promoter-specific oligonucleotide primers.

siRNA—To deplete cells of Sp1, Sp1-targeting small interfering RNA (siRNA) from Dharmacon was used, target sequence: 5′-AAAGCCGUUCAUGAGGAGUAG-3′. Cos1 cells were transfected in a 24-well plate with either 100 pmol siSp1 or siControl (5′-UUCUCGAAGGUGUCAGU-3′) and 100 ng of P2 luciferase reporter gene, 100 ng of pCMX-ERRα, and 20 ng of pcDNA3-PGC-1α using Lipofectamine 2000 (Invitrogen) as described previously (41). Medium was refreshed 5 and 24 h after transfection, and cell extracts were prepared for analysis using the luminescence reporter gene assay system as described above.

Computer-assisted Sequence Analysis—Computer-assisted sequence analysis of the DNA sequence contained in P2 was carried out using the TRANSFAC motif library found at //motif.genome.jp (42) in order to identify putative transcription factor binding sites. All primer and probe sequences not listed are available on request.

RESULTS

Transcription start sites from the RIP140 gene were determined in 5′ RACE using RNA extracted from a number of cell types, including differentiated mouse 3T3-L1 adipocytes, mouse ovarian tissue, and human ZR-75 breast cancer cells. Several distinct tissue- and cell-specific RNA transcripts were identified that differ at their 5′ ends, but they all share a single common coding exon that we had identified previously (25, 26). Comparison of the transcripts indicates that exons 1a, 1b, 2, 3, and 5 are conserved in the mouse and human genome but revealed the existence of additional novel exons, namely 1c, 4a, and 4b that are not conserved (Fig. 1, A and B). Exons 1a, 1b, and 1c were found at the 5′ ends of distinct transcripts (Fig. 1, A and B and supplemental data), suggesting that the mouse gene encoding RIP140 may contain at least three promoters.

From our analysis we established that RIP140 mRNA from 3T3-L1 adipocytes was transcribed from four exons, namely 1a or 1b, 2, 3, and 5 (Fig. 1A). The alternately spliced transcripts contain exon 1a or 1b but never both, suggesting that the Nrip1 gene might be transcribed from two promoters in adipocytes that we named P1 and P2. We determined the relative expression levels of the two RNA transcripts during adipogenesis using real-time PCR. Analysis of exon 5 expression showed an increase as 3T3-L1 cells undergo differentiation (Fig. 1C) in accordance with published data (22, 37), which is accompanied by a parallel increase in exon 1b, but not exon 1a, expression. Likewise, in situ hybridization showed a marked increase in transcripts derived from exon 1b, but not exon 1a, following differentiation (Fig. 1D). Thus we conclude that the Nrip1 gene is transcribed predominantly from the P2 promoter upstream of the exon 1b in adipocytes.

We next investigated the relative promoter activities of DNA sequences contained in either the 3.5-kb region upstream of exon 1a (P1), the −791/+16 region relative to exon 1b transcription start site (P2), or the DNA sequence comprising both (P1/P2). The fragments were fused to a luciferase reporter gene, transiently transfected in 3T3-L1 cells, and luciferase activity was measured before and after differentiation (Fig. 2A). P1 and P2 exhibited similar basal activities in undifferentiated 3T3-L1 cells, but that of P2 increased to a greater extent following adipocyte differentiation. The presence of both P1 and P2 did not result in a further increase in reporter activity. Therefore, we conclude that the 720-bp DNA sequence between the 1a and 1b exons contains regulatory elements sufficient for the increased transcription of the RIP140 gene during adipogenesis.

We used computer-assisted sequence analysis to identify putative transcription factor binding sites in the P2 promoter. In addition to an ERE, which was shown to function as a bona fide binding site for estrogen receptors in breast cancer cells (29, 31–33), we identified a putative ERRE that overlapped the ERE and binding sites for SREBP and E2F families of transcription factors (Fig. 2B). The ERE/ERRE and SREBP binding elements were conserved particularly well across species (Fig. 2C). We monitored the expression of these factors in 3T3-L1 adipocytes during adipogenesis. Western blot analysis showed that ERRα, SREBP1, and E2F1 were expressed, but we focused on the role of ERRα because its level increases during adipogenesis approximately in parallel with that of RIP140 (Fig. 2D). Moreover, previous gene expression profiling (43) and our RNA analysis (data not shown) suggest that the levels of ERRα greatly exceeded those of ERRβ and ERRγ. In contrast, the levels of E2F1 decline while ERα expression is low and unchanged during adipogenesis (43) (data not shown).

To investigate the ability of ERRα to bind to the P2 promoter we performed electrophoretic mobility shift assays using in vitro translated ERRα and a 32P-labeled oligonucleotide encompassing the sequence from −656 to −629 relative to the transcription start site. In this way, we detected a complex (Fig. 3A, lane 2) whose mobility was retarded upon addition of an...
antibody specific for ERRα (Fig. 3A, lane 3). This complex was not observed when the oligonucleotide was incubated with a mock in vitro translation (Fig. 3A, lanes 4 and 5) or when a mutated version of the ERR binding sites was used (Fig. 3A, lanes 7 and 8). These results show that ERRα can bind to these sites in vitro. We next investigated the binding of ERRα to P2 in intact 3T3-L1 cells during adipogenesis using ChIP assays. The binding of ERRα to the P2 promoter was monitored, using primers that encompass the ERR binding sites identified above, before and after cells were induced to differentiate for 2, 6, and 10 days. ERRα was detectable on the P2 promoter within 2 days of adipocyte differentiation and maintained for at least 10 days (Fig. 3B)

The ability of ERRα to stimulate transcription from the P2 promoter was determined by transient transfection of P2-luciferase reporter genes in Cos1 cells (Fig. 4). The expression of ERRα alone stimulated luciferase activity, and this was further increased in the presence of the coactivator PGC-1α. The coactivator alone had no effect, suggesting that PGC-1α enhances the ability of ERRα to stimulate transcription from the P2 promoter. The contribution of the ERRα binding site at −650/−633 was determined by analyzing the reporter gene activity of a number of deletion mutants and a specific ERE/ERRE point mutant (Fig. 4). Although these mutants were no longer stimulated by PGC-1α, they retained their sensitivity to ERRα, suggesting the presence of other ERR binding site(s) in the proximal promoter region between −246 and +16. The contribution of this region was also supported by our observation that internal deletion mutants lacking nucleotides −220 to −51 exhibited reduced reporter activity in response to ERRα alone, although they retained their sensitivity to PGC-1α acting via the ERR binding sites at −650/−633. However, inspection of the proximal promoter region did not reveal any obvious consensus ERRE sites.

In addition to the ability of ERRα to function as a transcription factor by directly binding to EREs or ERREs, it has been recently reported that ERRs may also function indirectly by means of an interaction with Sp1 bound to its cognate sequence (44). We have investigated these alternative modes of ERRα action by exploiting a number of pharmacological manipulations. First, we investigated the effects of the ERRα inverse agonist, XCT790, which inhibits the ERRα/PGC-1α interaction and reduces ERRα-stimulated transcription (45). We found that the effect of ERRα alone was unaltered in the presence of XCT790 but the ability of PGC-1α to potentiate P2 reporter activity in the presence of ERRα was markedly reduced (Fig. 5A). Interestingly, this reduction in P2 promoter activity was mirrored by a similar reduction in RIP140 mRNA when 3T3-L1 adipocytes were treated with XCT790 (Fig. 5B).

Organization of the mouse (A) and human (B) genes is shown. Exons 1a, 1b, 2, 3, and 5 are conserved between species. A, mRNA transcripts comprising exons 1a or 1b, 2, 3, and 5 were detected in mouse 3T3-L1 adipocytes, and transcripts containing exon 1a or 1b, 2, 3, 4b, and 5 were identified in ZR-75 cells. C, real-time PCR was used to quantitate the relative expression levels of RNA transcripts containing specific exons in preconfluent (PC) and confluent (Con) 3T3-L1 cells and in cells that had been induced to differentiate for 4, 6, 8, and 10 days. D, in situ hybridization analysis was used to monitor exon 1a and 1b expression in 3T3-L1 cells following differentiation into adipocytes.
We investigated whether the ability of ERR\textsubscript{\beta}/H9251 to stimulate P2 promoter activity in the presence of XCT790 might be explained by a mechanism involving the Sp1 transcription factor, given that it is expressed in both undifferentiated and differentiated 3T3-L1 cells (Fig. 5C). First, we examined the effects of Mithramycin A, a compound that selectively binds to GC-rich regions in DNA and prevents factors like Sp1 binding (46). Mithramycin A reduced ERR\textsubscript{\beta}-stimulated transcription from P2 almost to basal level (Fig. 5A), suggesting that Sp1 may play a crucial role in mediating the ability of ERR\textsubscript{\beta} to stimulate P2 transcription. Mithramycin A also partially reduced the ability of ERR\textsubscript{\beta} plus PGC-1\alpha to stimulate P2 transcription, and we assume that the residual P2 promoter activity reflects their action through the ERR binding sites in the distal region of the promoter. Importantly, the combination of XCT790 and Mithramycin A essentially abolished P2 promoter activity. We postulated above that ERR\textsubscript{\beta} may act not only by binding to the ERR binding sites at -650/-633 but also via site(s) located between -246 bp and the transcription start site, despite the absence of a consensus ERE. Interestingly, this region is very GC rich, typical of Sp1 binding sites.

To determine the contribution of Sp1 to ERR\textsubscript{\beta}-stimulated P2 transcription, we examined the effect of depleting cells of Sp1 using siRNA. Transcription from the P2 promoter was markedly reduced in the presence of a specific Sp1 siRNA, suggesting that ERR\textsubscript{\beta} functions, in part, through Sp1 (Fig. 5D). To test whether the residual P2 promoter activity observed in Sp1-depleted cells reflected the action of ERR\textsubscript{\beta} at the -650/-633 ERR binding sites, we examined the effect of mutating these elements. As expected, transcription from this mutant was reduced; moreover, treatment with Sp1 siRNA further reduced promoter activity to a greater extent than that observed with the wild-type promoter. Therefore, we conclude that ERR\textsubscript{\beta} stimulates P2 transcription by binding directly to ERR binding sites at -650/-633 and indirectly through Sp1 binding to a GC-rich region of the proximal promoter.
DISCUSSION

The RIP140 corepressor is widely expressed in tissues, with the highest level found in adipose and ovary (22) (data not shown). In accordance with this observation, mice devoid of RIP140 exhibit overt phenotypic changes in adipose tissue, which accumulates markedly reduced amounts of fat (22), and in the ovary, where ovulation fails to occur (26). Analysis of RNA from a number of mouse and human cell lines and tissues indicates that the RIP140 gene comprises multiple alternately spliced 5' non-translated exons and one coding exon. The overall exon-intron organization of the gene is well conserved, although there are additional non-coding exons in the mouse (exon 1c) and the human (exons 4a and b) that are utilized in a subset of cell types. Transcripts with distinct 5' ends were identified in the different RNA samples, suggesting the presence of several promoters. Alternative start sites of transcription together with differential splicing of 5' non-coding exons lead to a complex repertoire of RIP140 mRNAs with distinct untranslated regions but encoding the same protein. Their relative amounts vary in different cell types, suggesting their expression may be subject to differential regulation. We demonstrate that the P2 promoter upstream of exon 1b is preferentially used in white adipocytes and activated during adipogenesis. Previous work on RIP140 gene expression has focused on its regulation by estrogens in breast cancer cells. A number of studies have demonstrated the presence of functional EREs both proximal and 100 kb distal to the P2 promoter in breast cancer cells (29, 31-33). Consistent with these results, we found that the predominant RIP140 transcript in estrogen-treated ZR-75 breast cancer cells was initiated from the 1b exon, suggesting that the P2 promoter is preferentially utilized in breast cancer cells (data not shown). In adipocytes, RIP140 mRNA is also transcribed from the P2 promoter as a result of an increase in ERR expression during adipogenesis. Activation of the P2 promoter is brought about, in part, by ERR binding to an ERE/ERRE at -650 to -633 and, in part, through a GC-rich region in the proximal promoter in a manner dependent on the Sp1 transcription factor. Thus, the ERE/ERRE in the P2 promoter plays a critical role in RIP140 gene transcription in both breast cancer cells and adipocytes, but in response to distinct nuclear receptors.

ERRs are capable of binding to both EREs found in certain ERR target genes (47-49) and to extended half-sites, so-called ERR-response elements (ERRE), found in other target genes (13, 48, 50). ERR dimers are capable of binding to either EREs or ERREs with a slight preference for an A or a T at the +2 position in the latter, whereas a C at the +2 position, as found in the ERRE located in P2, would favor monomer binding (51). In this and previous studies Giguere and coworkers (51, 52) demonstrated that the coactivator PGC-1 interacts with dimers of DNA-bound ERRα, but not mono-
The activity of RIP140 as a cofactor seems to be modified according to the regulatory elements found in promoters (44). Thus, the activity of RIP140 as a coactivator when these receptors act through Sp1 binding as indicated.

The ability of ERRs to function indirectly through Sp1 binding sites was recently discovered by Cavailles and coworkers (44) to play a role in transcription from the TRα promoters (44). It is noteworthy that RIP140 is not required for adipogenesis itself but regulates adipocyte functions by suppressing the expression of clusters of metabolic genes that are involved in fatty acid oxidation, glucose homeostasis, and energy expenditure. Interestingly, one of the nuclear receptors that is responsible for activating a number of these genes is ERRα (13–15, 17, 24), and many of them are targets for repression by RIP140 (23, 24). Recent work indicates that the ability of RIP140 to regulate glucose uptake by adipocytes and expression of the mitochondrial proteins succinate dehydrogenase complex b and CoxVb requires ERRα (24). We conclude that RIP140, which increases in response to ERRα, suppresses the expression of these genes and acts as a negative regulator of glucose uptake in mice. Because RIP140 gene expression is also increased by ERRα, its transcriptional activity is likely to be subject to feedback inhibition, but the extent to which this occurs may depend on the relative expression levels of PGC-1 and Sp1, given their differential sensitivity to RIP140. It is conceivable that the alternative modes of ERRα action and transcriptional response to RIP140 contribute to the complex differential regulation of gene networks that are necessary to control metabolism.

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