Identification of a Functional Peroxisome Proliferator-responsive Element in the Murine Fatty Acid Transport Protein Gene*

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Fatty acid transport protein (FATP), a plasma membrane protein implicated in controlling adipocyte transmembrane fatty acid flux, is up-regulated as a consequence of adipocyte differentiation and down-regulated by insulin. Based upon the sequence of the FATP gene upstream region (Hui, T. Y., Frohnert, B. I., Smith, A. J., Schaffer, J. A., and Bernlohr, D. A. (1998) J. Biol. Chem. 273, 27420–27429) a putative peroxisome proliferator-activated receptor response element (PPRE) is present from −458 to −474. To determine whether the FATP PPRE was functional, and responded to lipid activators, transient transfection of FATP-luciferase reporter constructs into CV-1 and 3T3-L1 cells was carried out. In CV-1 cells, FATP-luciferase activity was up-regulated 4- and 5.5-fold, respectively, by PPARα and PPARγ in the presence of their respective activators in a PPRE-dependent mechanism. PPARδ, however, was unable to mediate transcriptional activation under any condition. In 3T3-L1 cells, the PPRE conferred a small but significant increase in expression in preadipocytes, as well as a more robust up-regulation of FATP expression in adipocytes. Furthermore, the PPRE conferred the ability for luciferase expression to be up-regulated by activators of both PPARγ and retinoid X receptor α (RXRα) in a synergistic manner. PPARα and PPARδ activators did not up-regulate FATP expression in 3T3-L1 adipocytes, however, suggesting that these two subtypes do not play a significant role in differentiation-dependent activation in fat cells. Electromobility shift assays showed that all three PPAR subtypes were able to bind specifically to the PPRE as heterodimers with RXRα. Nuclear extracts from 3T3-L1 adipocytes also showed a specific gel-shift complex with the FATP PPRE. To correlate the expression of FATP to its physiological function, treatment of 3T3-L1 adipocytes with PPARγ and RXRα activators resulted in an increased uptake of oleate. Moreover, linoleic acid, a physiological ligand, up-regulated FATP expression 2-fold in a PPRE-dependent manner. These results demonstrate that the FATP gene possesses a functional PPRE and is up-regulated by activators of PPARα and PPARγ, thereby linking the activity of the protein to the expression of its gene. Moreover, these results have implications for the mechanism by which certain PPARγ activators such as the antidiabetic thiazolidinedione drugs affect adipose lipid metabolism.

Obesity, defined as an excessive accumulation of body fat, has become an increasingly common health concern in industrialized societies. Excessive adiposity has been linked to the pathogenesis of many diseases, including type 2 diabetes mellitus, coronary artery disease, and hypertension. The increased awareness of the detrimental effects of obesity contributes to the search for a greater understanding of the molecular mechanisms controlling the accumulation of adipose tissue and its metabolism.

A central issue in the function of fat tissue is the method by which adipocytes take up and release fatty acids. This process has been the source of considerable debate (1–3). Because free fatty acids are hydrophobic, they freely crossed membranes by passive diffusion. However, studies in adipocytes, hepatocytes, jejunal enterocytes, skeletal muscle, and heart myocytes support a saturable, protein-mediated mechanism for fatty acid transport (4–8). Thus far, five putative mammalian fatty acid transporters have been identified: fatty acid-binding protein (plasma membrane) (9), 56-kDa renal fatty acid-binding protein (10), caveolin (11), fatty acid translocase (12), and fatty acid transport protein (FATP)1 (13). FATP is an integral plasma membrane protein with four to six predicted membrane-spanning regions with the highest levels found in skeletal muscle, heart, and fat with lower levels in brain, kidney, lung, and liver. Although FATP mRNA is present at low levels in 3T3-L1 preadipocytes, it is up-regulated 5–7-fold as a consequence of adipose conversion (13, 14). This increase is consistent with the increase in oleic acid uptake shown during preadipocyte differentiation (15).

The differentiation of adipose precursor cells into adipocytes has been shown to be mediated by three groups of transcription factors: peroxisome proliferator-activated receptor γ (PPARγ 1 and 2) the CCAATT enhancer-binding proteins (C/EBP), and the sterol-response element binding proteins (SREBP or ADD1) (16). The importance of PPARs for the development and maintenance of the adipocyte phenotype can be more directly shown by the existence of peroxisome proliferator response elements (PPREs) in the promoters of several genes whose protein products are critical for lipid metabolism and the development of the adipocyte phenotype such as lipoprotein lipase, phosphoenolpyruvate carboxykinase, acyl-CoA synthetase, malic enzyme, and adipocyte lipid-binding protein (ALBP or aP2) (16–21).

PPARs constitute a subfamily of the steroid hormone receptor superfamily. PPARα is predominantly expressed in liver, heart, kidney, and adipose tissue, whereas PPARδ (also known as NUC1 or FAAR) shows a similar expression, with the ex-

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1 The abbreviations used are: FATP, fatty acid transport protein; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; PPRE, peroxisome proliferator response element; PPAR, peroxisome proliferator-activated receptor; Me3SO, dimethyl sulfoxide; BSA, bovine serum albumin; RXR, retinoid X receptor.
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Exception of low levels in liver (22–25). Two isoforms of mouse PPARα have been cloned, γ1 (22, 24, 26) and γ2 (19), which are transcribed from the same gene and alternatively spliced (27, 28). PPAR-γ is expressed in liver, heart, and kidney, similar to PPARα, whereas PPAR-γ2 is primarily expressed in adipose tissue (29). The two PPAR isoforms do not appear to differ significantly in ligand binding affinities, ability to bind DNA response elements, or ability to activate transcription. The critical difference identified, thus far, is the distribution of expression.

FATP expression has been shown to be up-regulated in mouse liver by PPARα activators and in white adipose tissues by activators of PPARγ (30, 31). This evidence, together with the differentiation-dependent regulation of FATP in 3T3-L1 adipocytes, led us to investigate the upstream region of the recently cloned FATP gene (32) for a possible PPRE and to examine the role of the various PPAR subtypes in FATP expression. In this report we detail the regulation of the FATP gene, the identification of a functional PPRE, and its up-regulation by PPARα and PPARγ agonists.

**EXPERIMENTAL PROCEDURES**

**Materials—**Troglitazone and Delta Selective C were gifts from Alan Saltiel (Parke-Davis) and David E. Moller (Merck). WY14643 was purchased from Cayman Chemical. Linoleic acid and 9-cis-retinoic acid were purchased from Biomol. Expression plasmids prPPARα, pS5-FAA (PPARγ), pS5-PPAR-γ2, and pRS-hRXRα were provided by Drs. Donald Jump, Paul A. Grimaldi, Bruce M. Spiegelman, David J. Mangelsdorf, and Ronald M. Evans, respectively.

**Plasmid Constructs—**Polymerase chain reaction was used to introduce Nhel sites into the upstream sequence of FATP. Reporter constructs pNH11 (−971/+84), pNH13 (−556/+84), and pNH15 (−160/+971/NheI) were constructed by ligating varying lengths of FATP upstream sequence into theNhel and HinflIII sites of the pBluescript luciferase expression vector (Promega). Construct pNH13A1P was generated by single-stranded mutagenesis of construct pNH13 using the Mutan-Gen in vitro mutagenesis kit (Bio-Rad, Hercules, CA) and the oligonucleotide: 5′-CTGGAAACATCTCCTGAGTACTTCCTCCTCTCCC-3′. Sequence fidelity and the introduced mutation were verified by sequencing. The PPAR-γ2 expression construct, pAH215, was made by cutting pBS-PPAR-γ2 with HindIII and XhoI and ligating into pcDNA3.1 (Promega). The expression construct for RXRα, pAH232, was subcloned from pRS-hRXRα into the EcoRI site of pcDNA3.1. Cell Culture and Transfection—CV-1 cells were cultured in DMEM with 10% fetal bovine serum (FBS) until the day prior to transfection experiments, when the cells were plated in phenol red-free DMEM and 10% charcoal-treated FBS. CV-1 cells were transiently transfected using the calcium-phosphate method as described previously (33, 34). For all transient transfection experiments, when the cells were plated in phenol red-free DMEM and 10% fetal bovine serum (FBS) until the day prior to transfection the cells were cultured in DMEM containing 10% calf serum and maintained in DMEM with 2 mM glucose. Oleate uptake was measured at 25 °C in a 20 μM, 1:1 oleate:BSA mixture, which contained trace [3H]oleate (approximately 12,000 dpm/nmol). At various time points, uptake was terminated by aspirating the medium. Components of 3T3-L1 adipocytes were washed two times with phosphate-buffered saline, lysed, and luciferase activities were determined using the Dual Luciferase Reporter Assay System (Promega) according to the manufacturer’s protocol. Firefly luciferase activities were normalized to sea pansy luciferase activities to adjust for transfection efficiency. Activities were also normalized to the promoterless reporter construct, pGL3-Basic.

**In Vitro Transcription/Translation—**cDNAs for PPARα, mPPARγ2, mPPARγ, and hRXRα were transcribed and translated in vitro from the plasmids prPPARα, pH215, pS5-GA and pH232, respectively. The TNT Coupled Reticulocyte Lysate System (Promega) was used according to the manufacturer’s instructions. The following expression plasmids were used prPPARα, pS5-GA (PPARγ), pH215, and pH232. Translation products were verified by SDS-polyacrylamide gel electrophoresis.

**Gene Electrosensitivity Shift Assays—**Nuclear extracts were prepared from 9 days 3T3-L1 adipocytes essentially as described previously (36). To study the binding of nuclear hormone receptors to the putative PPRE, a double-stranded oligonucleotide, PPREwt, containing nucleotides −482 to −453 of the FATP upstream sequence was 32P-labeled with polynucleotide kinase (Promega). A 15-μl reaction containing 0.5 ng of PPRE probe and 9 μg of nuclear extract or 0.5–1 μl of in vitro translation reaction was incubated for 20 min at 25 °C and 15 min at 4 °C in a buffer containing 20 mM HEPES (pH 8), 60 mM KCl, 1 mM dithiothreitol, 10% glycerol, and 2 μg poly(dI-dC). The DNA-protein complexes were resolved from the free probe by electrophoresis at 4 °C on a 4% polyacrylamide gel in 0.5 X TBE buffer (1 X TBE = 9 mM Tris, 90 mM borate, 20 mM EDTA) (pH 8). Double-stranded oligonucleotide complexes composed of the following sequences were used for competition of the nuclear receptor: PPREwt, 5′-GATCTAGAGGGAAAGTGGGCGGAAGGCGAGAATG-3′; PPREmut, 5′-GATCTAGAGGGAAAGTGGGCGGAAGGCGAGAATG-3′; PPRE-CAGGAGA-3′. Mutated bases are shown in lowercase letters.

**Fatty Acid Uptake—**3T3-L1 adipocytes were treated for 4 days with either 20 μM troglitazone and 1 μM 9-cis-retinoic acid or their carrier, Me2SO. The adipocytes were then assayed for uptake of oleate essentially as described previously (37). Briefly, cells were first preincubated for 2–4 h in serum-free DMEM and then for 10–30 min at 25 °C in Krebs-Ringer phosphate solution with 2 mM glucose. Oleate uptake was measured at 25 °C by incubating cells in a 100 μM, 1:1 oleate:BSA mixture, which contained trace [3H]oleate (approximately 12,000 dpm/nmol). At various time points, uptake was terminated by aspirating the medium. Components of 3T3-L1 adipocytes were washed two times with phosphate-buffered saline, lysed, and luciferase activities were determined using the Dual Luciferase Reporter Assay System (Promega) according to the manufacturer’s protocol. Firefly luciferase activities were normalized to sea pansy luciferase activities to adjust for transfection efficiency. Activities were also normalized to the promoterless reporter construct, pGL3-Basic.
the cells and washing three times with ice-cold phosphate-buffered saline containing 0.1% albumin and 200 $\mu$M phloretin. Cell lysate was then quantitated for radioactivity using a Beckman 3801 liquid scintillation counter.

RESULTS

The Mouse Fatty Acid Transport Protein Gene Contains a Functional PPRE—Previous studies of FATP regulation in mice indicated that transcription was activated in both liver and white adipose tissue by treatment of mice with activators of PPAR\(\alpha\) or PPAR\(\gamma\), respectively (30, 31). Furthermore, FATP expression was observed to be up-regulated during adipose differentiation, a process known to be mediated in part by PPAR\(\gamma\) (14). The upstream sequence of the recently cloned FATP gene (32) was therefore examined for a possible PPRE. A putative PPRE was identified, which is similar to the consensus sequence proposed by Palmer et al. (38) (Fig. 1, Table I).

In order to determine whether the putative PPRE identified in the FATP upstream sequence was able to mediate transcriptional activation, portions of the FATP 5'-flanking sequence were tested for their ability to mediate PPAR-activated transcription of a reporter gene. Four luciferase reporter constructs were made, each containing portions of the FATP 5'-flanking region linked to a promoterless firefly luciferase gene. Two of these plasmids, pNH11 and pNH13, contained the putative PPRE sequence, while the other two, pNH13\(\Delta P\) and pNH15 did not (Fig. 2). In the first set of experiments, two of these constructs, pNH13 and pNH13\(\Delta P\), as well as the promoterless pGL3-Basic control construct, were transfected into 3T3-L1 preadipocytes. Following transfection, the cells were maintained for 24 h in serum-free media and treated with 100 $\mu$M linoleic acid or its

![Fig. 2. Construct map of the luciferase reporter constructs used in transfection assays. Constructs contain various portions of FATP upstream sequence, as indicated. The putative PPRE is denoted by a shaded box.](http://www.jbc.org/)

![Fig. 3. Activation of FATP expression in 3T3-L1 preadipocytes by linoleic acid. 3T3-L1 preadipocytes were transfected with the reporter constructs pNH13 and pNH13\(\Delta P\), as well as the control construct, pGL3-Basic. The cells were then treated for 24 h in serum-free medium with either vehicle (EtOH) or 100 $\mu$M linoleic acid. Asterisks indicate statistical difference from activity of the control-treated construct (\(*\) 5\(p\), 0.005).](http://www.jbc.org/)

### TABLE I

| Gene                        | Species | Element | Sequence                  | Protein function                        | Ref. |
|-----------------------------|---------|---------|---------------------------|-----------------------------------------|------|
| HMG-CoA synthase            | Rat     | HMG    | AACT GGGCCA A AGGTCT     | Liver ketogenesis/sterol synthesis      | 48   |
| Acyl-CoA synthase           | Rat     | ACS(CI)| TTTG AGGGCA T CAGTCA     | Fatty acid activation                    | 20   |
| Acyl-CoA oxidase            | Rat     | ACOA   | GACC AGGGCA A AGGTCA     | Peroxisomal \(\beta\)-oxidation          | 52, 53|
| Bifunctional enzyme         | Human   | hACOX  | TAGA AGGCTA C TTGGCA     | Peroxisomal \(\beta\)-oxidation          | 47   |
| Malic enzyme                | Rat     | MEp    | TTCT GGGTCA A AGTTGA     | Peroxisomal \(\beta\)-oxidation          | 54   |
| Cytochrome P450 A1          | Rat     | CYP4A1 | AACT AGGGTA A AGTTCG     | Fatty acid synthesis                     | 21   |
| Cytochrome P450 A6          | Rabbit  | CYP4A6| AACT AGGGCA A AGGTCA     | \(\omega\)-Oxidation                      | 46   |
| PEPCK                       | Rat     | PCK1   | CCCC AGGGCA A AGGTCA     | Glycoerogenesis and gluconeogenesis      | 55   |
| ALBP/aP2                    | Mouse   | ARE6   | CTCT GGGTGA A ATGTCG     | Fatty acid binding                       | 19   |
| L-FABP                      | Rat     | FABP   | ATAT AGGCCA T AGTTCG     | Fatty acid binding                       | 56   |
| Uncoupling protein 1        | Mouse   | URE1   | AGTG TGGTCA A GGGTCA     | Thermogenesis                            | 50   |
| Lipoprotein lipase          | Rat     | LPL    | AACA GGGGGA A AGGGCA     | Triglyceride clearance                   | 18   |
| Apolipoprotein CIII         | Human   | APOCIIIB| GCCC TGGCCA A AGGTCA     | Triglyceride clearance                   | 57   |
| Muscle-type carnitine       | Human   | MCPT 1 | ATGT AGGGAA A AGGTCA     | Fatty acid transport                     | 49   |
| Palmitoyltransferase        |         |        |                           |                                         |      |
| Fatty acid transport Protein|         |        |                           |                                         |      |
| Consensus                   |         |        | AACT AGGTCA A AGGTCA     |                                         |      |

**Fig. 2. Construct map of the luciferase reporter constructs used in transfection assays.** Constructs contain various portions of FATP upstream sequence, as indicated. The putative PPRE is denoted by a shaded box.

**Fig. 3. Activation of FATP expression in 3T3-L1 preadipocytes by linoleic acid.** 3T3-L1 preadipocytes were transfected with the reporter constructs pNH13 and pNH13\(\Delta P\), as well as the control construct, pGL3-Basic. The cells were then treated for 24 h in serum-free medium with either vehicle (EtOH) or 100 $\mu$M linoleic acid. Asterisks indicate statistical difference from activity of the control-treated construct (** 5\(p\), 0.005).
carrier, ethanol. This experiment was performed in the absence of serum, since serum albumin binds fatty acids, leaving low levels of available fatty acid activator. Linoleic acid treatment activated transcription of the PPRE-containing construct approximately 2-fold over control-treated cells (Fig. 3), but did not affect transcription of the PPRE-deletion construct. Similar results were seen in transfection of 3T3-L1 adipocytes (data not shown). These experiments indicated that the putative PPRE identified in the FATP upstream sequence was indeed functional.

To further characterize the responsiveness of this PPRE to the various PPAR subtypes, all four reporter constructs were transfected into CV-1 cells and assayed for luciferase activity in the presence and absence of various PPARs and their activators. The activators used were troglitazone, a thiazolidinedione (PPARγ activator); WY14643, a fibrate drug (PPARα activator); and the PPARδ activator, Delta Selective C. As shown in Fig. 4A, cells transfected with PPARγ and treated with troglitazone demonstrated a 5.5-fold increase in transcription of the PPRE-containing reporter constructs. PPARγ alone was able to activate transcription approximately 3-fold. PPARα was also

**Fig. 4. The putative PPRE confers selective responsiveness to PPAR-mediated activation.** Reporter constructs were cotransfected with or without an expression vector for PPARγ (A), PPARα (B), or PPARδ (C) and treated for 48 h with activator or vehicle (Me2SO (DMSO)). Activators were 20 μM Troglitazone, 10 μM WY14643, or 40 nM Delta Selective C, respectively. Normalized luciferase activities are shown as mean ± S.E. (n = 4) and are expressed as -fold induction relative to the activity in the absence of expression vectors and activators. Asterisks indicate statistical difference from activity of the reporter construct alone (* = p < 0.05, ** = p < 0.005).
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**Fig. 5.** Activation of FATP expression by endogenous PPARs in preadipocytes and adipocytes. 3T3-L1 preadipocytes and adipocytes were transfected with the reporter constructs pNH13 and pNH13ΔP as well as the control construct, pGL3-Basic. The cells were then treated with either vehicle or PPAR activators: 20 μM troglitazone, 10 μM WY14643, or 40 nM Delta Selective C. Luciferase activities were normalized to pGL3-Basic and are shown as mean ± S.E. (*p < 0.05, **p < 0.005).

**Fig. 6.** Synergistic activation of FATP expression in 3T3-L1 adipocytes by PPAR and RXRα activators. 3T3-L1 preadipocytes and adipocytes were transfected with the reporter constructs pNH13 and pNH13ΔP as well as the control construct, pGL3-Basic. These cells were then treated with the PPARγ activator, troglitazone (20 μM), and the RXRα activator, 9-cis-retinoic acid (1 μM). Luciferase activities were normalized to pGL3-Basic and are shown as mean ± S.E. (*p < 0.05, **p < 0.005).

able to activate transcription in the PPRE-containing constructs, albeit to a lesser extent; transcription was increased 4- and 2-fold in the presence and absence of activator, respectively (Fig. 4B). Finally, the PPARα subtype was unable to positively regulate transcription of any of the reporter constructs, regardless of activator treatment (Fig. 4C). Deletion of the PPRE rendered the promoter unresponsive to any PPAR or agonist combination.

**PPRE Involved in Differentiation-dependent Regulation of FATP**—In order to determine the role of the FATP PPRE in the process of its gene regulation during the process of adipose differentiation, the luciferase reporter constructs were introduced into both 3T3-L1 preadipocytes as well as mature adipocytes. These experiments relied upon endogenous PPARs to mediate transcriptional activation. Cells were also treated with activators of the various PPAR subtypes to determine whether transcription could be further increased. In preadipocytes, which contain low levels of PPARγ as well as PPARα, the PPRE containing construct, pNH13, was expressed at about 1.6-fold the level of the PPRE-deletion construct, pNH13ΔP, when both were treated with Me2SO. Treatment with troglitazone had a small, but not statistically significant, effect on pNH13 expression. In adipocytes, the PPRE-containing construct was expressed at levels 5-fold higher than the PPRE-deletion construct (Fig. 5). Furthermore, troglitazone treatment resulted in a further 3-fold increase in expression. Neither WY14643 nor Delta Selective C caused any significant change in luciferase expression in either preadipocytes or adipocytes.

**Synergistic Activation by PPARγ and RXRα Activators**—Iassmann et al. (39) showed that the RXR ligand, 9-cis-retinoic acid enhances PPAR action. To determine whether RXRα activation affected PPARγ-dependent transactivation, 3T3-L1 adipocytes were treated with either troglitazone, 9-cis-retinoic acid, or both. Retinoic acid did not significantly affect transcription by itself; however, when added to cells in conjunction with troglitazone, it was able to produce an almost 2-fold increase in activity above that produced by troglitazone alone (Fig. 6). This result demonstrates the synergistic activation of the FATP gene in response to activation of both PPARγ and RXRα.

**PPARs and RXRα Bind as Heterodimers to the FATP PPRE**—In order to determine whether PPARs bind to the PPRE as heterodimers with RXRα, gel mobility shift assays were performed with a double-stranded oligonucleotide containing the FATP PPRE (Fig. 7A). The double-stranded probe, PPREwt, was end-labeled with [32P]ATP and incubated with in vitro translated protein as well as 3T3-L1 nuclear extract. As shown in Fig. 7B, neither PPARs nor RXRα alone could bind to the PPRE; however, all three PPAR subtypes were able to bind as heterodimers with RXRα to the probe. Furthermore, nuclear proteins from 3T3-L1 adipocytes were able to form an in vitro complex with the PPRE (Fig. 7C). In order to test the specificity of the protein-DNA interactions, an excess of unlabeled oligonucleotide (PPREwt) was added to the reactions. The unlabeled oligonucleotide was able to compete for binding of all three PPAR-RXRα-DNA complexes, as well as the nuclear protein-DNA complex. The introduction of 3-base pair substitutions (Fig. 7A) produced an oligonucleotide, PPREmut, which was no longer able to significantly compete for protein binding.

**PPARγ and RXRα Activators Cause an Increase in Oleate Uptake**—Finally, in order to correlate PPAR-mediated transcriptional activation with putative in vitro protein function, fatty acid uptake was analyzed in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated for 4 days with either troglitazone and retinoic acid or their carrier Me2SO. Cells were then incubated with [3H]oleate-BSA mixture (1:1), washed, lysed at various time points, and assayed for radioactivity. As shown in Fig. 8, treatment with the activators of PPARγ and RXRα resulted in a significant increase in oleate uptake. This result is consistent with the up-regulation of FATP transcription by troglitazone and 9-cis-retinoic acid, as shown by the previously described transfection studies, and correlates well with the increase in FATP mRNA expression upon treatment of 3T3-L1 adipocytes.
with the PPARγ agonist BRL49653, as shown by Martin et al. (30).

**DISCUSSION**

The critical role of PPARs in the regulation of lipid metabolism has become increasingly apparent. Many genes whose products take part in some aspect of fatty acid catabolism, synthesis, or trafficking have been shown to contain functional PPREs (Table I). FATP, which has been argued to play a role fatty acid uptake, is a likely candidate for regulation by this group of nuclear hormone receptors. Indeed, two previous studies have shown that treatment of various cell types with PPAR activators leads to an increase in FATP mRNA levels (30, 31).

In this paper we have identified a sequence in the 5' region of the murine FATP gene, which is very similar (16/17) to the consensus sequence for previously identified PPREs (see Table I).

By deletion analysis and mutation of this putative PPRE, we have demonstrated that this PPRE is necessary for the PPAR-mediated up-regulation of FATP expression. Transfection into 3T3-L1 preadipocytes showed that FATP transcription can indeed be activated by the naturally occurring compound, linoleic acid. This fatty acid has been shown previously to be able to activate transcription via both the α and γ PPAR subtypes (40–42). Further transfection experiments into CV-1 cells demonstrated that FATP transcription was activated by both PPARα and PPARγ. Both subtypes activate transcription upon treatment with synthetic activator; however, the receptors are also able to activate transcription in the absence of exogenous activator. This could be explained either by the presence of an endogenous activator, such as a fatty acid or its metabolite, or by a ligand-independent activity of these subtypes (43).

In addition to showing the functionality of the FATP PPRE, our experiments demonstrate a differential activation of gene expression by the various PPAR subtypes. Although both PPARα and PPARγ are able to activate transcription, our studies show that PPARγ mediates a greater response both in the presence and absence of a synthetic activator. PPARδ, in contrast, did not significantly activate transcription, either alone or upon treatment with activator. This difference has been demonstrated previously in other systems (44) and has been hypothesized to reflect the differing roles of the PPAR subtypes in the regulation of fatty acid metabolism. PPARα has been best characterized in the liver, where it up-regulates many genes involved in the catabolism of fatty acids. PPARγ is chiefly active in the adipose tissue, where it contributes to lipid accumulation and the development of the adipose phenotype. In contrast to the other two subtypes, the role of PPARδ in whole-body fatty acid metabolism has not been well defined.

We and others have reported the up-regulation of FATP during adipocyte conversion (13, 14). We hypothesized that this differentiation-dependent regulation of FATP is mediated by PPARγ and is dependent on the presence of the FATP PPRE. This was investigated in a further series of transfections, which

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**Fig. 7.** PPARs and RXRα bind as heterodimers to the FATP PPRE. A, sequences of oligonucleotides used in gel-shift studies. PPRE sequence is underlined; mutated bases are in lowercase. B, the double-stranded probe, PPREwt, was end-labeled with 32P and incubated with in vitro translated PPARs and RXRα. The competitors PPREwt and PPREmut were used in 20- and 100-fold molar excess. Protein-DNA complexes were analyzed by electrophoretic mobility shift assay. C, labeled PPREwt probe was incubated with 3T3-L1 adipocyte nuclear extracts with or without competitor. Competitors PPREwt, PPREmut, and NS (a nonspecific competitor oligonucleotide) were added in 20-, 50-, and 200-fold molar excess.
FIG. 8. Oleate uptake by 3T3-L1 adipocytes treated with troglitazone and retinoic acid. 3T3-L1 adipocytes were treated for 4 days with either 20 μM troglitazone and 1 μM 9-cis-retinoic acid or their carrier MeSO (DMSO). Cells were then incubated with 1:1 oleate:BSA mixture in Krebs-Ringer phosphate buffer which contained trace [3H]oleate. Cells were washed and lysed at various time points and cell lysate was assayed for radioactivity. Asterisks indicate statistical difference between uptake of treated and nontreated cells (* = p < 0.05).

Comparison of transcriptional activity in both 3T3-L1 preadipocytes and adipocytes, in both the presence and absence of synthetic activators. The PPRE conferred a 1.6-fold activation of FATP transcription in preadipocytes. At this point in the differentiation process, PPARγ is present in low levels relative to fully differentiated adipocytes. Exogenous activators of the three PPAR subtypes did not significantly increase transcription above that of untreated cells. In adipocytes, the PPRE conferred a 5-fold increase in FATP expression, and this activation was further stimulated by the PPARγ activator, troglitazone. Neither the activator of PPARα nor PPARβ was able to increase FATP expression over untreated cells. This can be explained by the lack of significant PPARβ expression in adipocytes. PPARα, while present in adipocytes, has been demonstrated in the previously described studies to be unable to activate FATP expression.

Several previous studies have shown a synergistic effect of the IXXR activators, 9-cis-retinoic acid on PPAR-activated expression (18, 19, 21, 45–48). This convergence of the PPAR and RXR signaling pathways was also demonstrated in transfection studies of FATP reporter constructs in 3T3-L1 adipocytes. Although the IXXR activator alone was unable to increase expression of FATP in 3T3-L1 adipocytes, it was able to enhance the troglitazone-mediated activation of expression.

The ability of the various PPAR subtypes to bind to the FATP PPRE in vitro was examined by electromobility shift assay. As has been shown in other systems, neither the PPARs nor RXR were able to bind to the PPRE as homodimers (20, 21, 45–50); however, all three PPAR subtypes were able to bind as heterodimers. These protein-DNA complexes were sequence-specific, as shown by competition analysis, and were dependent upon the presence of an intact PPRE. Mutation of 3 base pairs of the PPRE abolished the formation of protein complexes on this element. It is interesting to note that PPARα, while unable to activate transcription, was able to form a heterodimer complex with the PPRE. This indicates that binding of the receptor heterodimers to an element is not equivalent with transcriptional activity.

In order to correlate FATP regulation by PPARs with its putative function, oleate uptake was measured in cells that were treated with troglitazone and 9-cis-retinoic acid. Uptake was significantly increased in activator-treated cells when compared with control-treated cells. This leads to an interesting model for positive feedback regulation of FATP. Increased FATP expression has been shown to result in increased fatty acid uptake (13). Fatty acids, in turn, are activators of PPARγ and PPARα, which are able up-regulate the expression of FATP.

We have reported previously the regulation of FATP by insulin via an insulin-responsive element (PELE3) in the upstream region of the FATP gene (−1353 to −1347). The down-regulation of FATP by insulin, an anabolic hormone, seems counterintuitive, since fatty acid uptake would be expected to rise in response to insulin stimulation. It is important to note, however, that regulation of FATP at the transcriptional level is unlikely to be the result of the transient postprandial insulin peak, but rather a more chronic hyperinsulinemia, such as in type 2 diabetes mellitus. Furthermore, the majority of type 2 diabetics are obese, a condition associated with a down-regulation of PPARγ expression in adipose tissue (51). The combination of these two factors may contribute to the elevation in serum free fatty acid levels observed in type 2 diabetics. This leads to a possible mechanism for the antidiabetic effects of the drug, troglitazone; by reversing the effects of hyperinsulinemia and obesity on FATP regulation, troglitazone may enable adipose tissue improve fatty acid uptake. Further studies on the function of FATP and its regulation in the diabetic state may lead to insight into both normal and deranged fatty acid metabolism.

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