

ADD1/SREBP1 activates PPAR γ through the production of endogenous ligand

(fatty acid metabolism/nuclear hormone receptor/adipocyte differentiation)

JAE BUM KIM, HAROLD M. WRIGHT, MARGARET WRIGHT, AND BRUCE M. SPIEGELMAN*

Dana-Farber Cancer Institute and the Department of Cell Biology, Harvard Medical School, Boston, MA 02115

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ABSTRACT Adipose differentiation is an important part of the energy homeostasis system of higher organisms. Recent data have suggested that this process is controlled by an interplay of transcription factors including PPAR γ , the C/EBPs, and ADD1/SREBP1. Although these factors interact functionally to initiate the program of differentiation, there are no data concerning specific mechanisms of interaction. We show here that the expression of ADD1/SREBP1 specifically increases the activity of PPAR γ but not other isoforms, PPAR α , or PPAR δ . This activation occurs through the ligand-binding domain of PPAR γ when it is fused to the DNA-binding domain of Gal4. The stimulation of PPAR γ by ADD1/SREBP1 does not require coexpression in the same cells; supernatants from cultures that express ADD1/SREBP1 augment the transcriptional activity of PPAR γ . Finally, we demonstrate directly that cells expressing ADD1/SREBP1 produce and secrete lipid molecule(s) that bind directly to PPAR γ , displacing the binding of radioactive thiazolidinedione ligands. These data establish that ADD1/SREBP1 can control the production of endogenous ligand(s) for PPAR γ and suggest a mechanism for coordinating the actions of these adipogenic factors.

The adipocyte is a highly specialized cell type that plays an important role in energy homeostasis. The primary role of fat cells is to store triglycerides in periods of energy excess and to release this energy during nutritional deprivation. Adipocyte differentiation is a complex process accompanied by coordinated changes in cell morphology, hormone sensitivity, and gene expression. These changes result from the action of several transcription factors that influence these processes, including PPAR γ , the C/EBPs, and ADD1/SREBP1 (ref. 1 and references therein). These factors do not work completely independently but interact functionally in several important ways.

PPAR γ is a member of the nuclear hormone receptor family that is expressed preferentially in adipose tissue (2). It functions as an obligate heterodimer with the retinoid X receptor (RXR) to bind to target sequences termed DR-1 sites (2). Activation of PPAR γ by ligands is sufficient to stimulate adipose differentiation in many types of fibroblastic cells (2–4). Ligands for this receptor include the antidiabetic thiazolidinedione (TZD) drugs, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J2 (15-deoxy-PGJ2), and certain polyunsaturated fatty acids (5–7). The biological ligands now known bind to PPAR γ with relatively low affinity ($K_d = 2\text{--}50\ \mu\text{M}$), compared with the affinity (typically $K_d < 1\ \text{nM}$) for most bona fide ligands for nuclear receptors. Whether the known PPAR γ ligands represent endogenous activators for this receptor is not clear.

The C/EBP family also appears to be very important in adipogenesis. Ectopic expression of C/EBP β or C/EBP α can promote the differentiation of fibroblastic cells (8–13). There is evidence that C/EBP β does this by increasing the expression of PPAR γ , whereas C/EBP α appears to be able to powerfully synergize with PPAR γ to promote differentiation (8, 14).

Another key transcription factor in adipose development is ADD1/SREBP1 (15). As a member of the basic helix-loop-helix transcription factor family, ADD1/SREBP1 has an unusual ability to bind to two distinct DNA sequences (16, 17): E-boxes (CANNTG) and the sterol regulatory element (SRE), a sequence important in the control of genes involved in cholesterol homeostasis (18). This protein and its close homolog SREBP2 (19) are expressed as large precursors that are proteolytically cleaved from the endoplasmic reticulum and nuclear membrane (20, 21). The cleavage of SREBP2 is controlled by cholesterol level *in vivo*. Although SREBP1 can be controlled by cholesterol levels in cultured cells, this does not seem to occur *in vivo*, leaving the question of its mechanism of activation unresolved (22).

Ectopic expression of ADD1/SREBP1 is sufficient to increase endogenous expression of fatty acid synthetase and lipoprotein lipase in cultured fibroblasts or in the livers of transgenic animals (23–25). In addition, expression of ADD1/SREBP1 promotes adipogenesis when cells are also stimulated with multiple hormonal inducers (23). Coexpression of ADD1/SREBP1 increases the transcriptional activity of PPAR γ , suggesting that this cooperation may be responsible for the effects of ADD1/SREBP1 in differentiation.

We have now investigated in detail the biochemical basis of the cooperation between ADD1/SREBP1 and PPAR γ . We find that ADD1/SREBP1 can promote the production of ligand(s) for PPAR γ . Expression of various mutant alleles of ADD1/SREBP1 indicates that the production of PPAR γ ligand occurs via transcription through E-box motifs.

MATERIALS AND METHODS

Cell Culture. 3T3-L1 preadipocytes were grown in DMEM containing 10% calf serum. Differentiation of 3T3-L1 cells was induced as described previously (11). At confluence, the medium was changed to DMEM supplemented with 10% fetal bovine serum (FBS), 1 μM dexamethasone, 0.5 mM iso-butyl methyl xanthine, and 5 $\mu\text{g}/\text{ml}$ of insulin for 2 days in the absence or presence of 5 μM pioglitazone. Thereafter, the medium was replaced every other day with 5 $\mu\text{g}/\text{ml}$ of insulin and 10% FBS. Cells were fixed with 10% formaldehyde in PBS and stained with Oil Red O (Sigma). Stable cell lines expressing different ADD1 alleles were derived as described previ-

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Abbreviations: PPARE, PPAR response element; RXR, retinoid X receptor; TZD, thiazolidinedione; LBD, ligand-binding domain; DBD, DNA-binding domain.

*To whom reprint requests should be addressed at: Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115. e-mail: Bruce.Spiegelman@dfci.harvard.edu.

ously (23). NIH 3T3 cells were grown in DMEM containing 10% calf serum (HyClone), and Rat1-IR cells were grown in DMEM/F12 (50:50) medium containing 10% FBS as previously described (26).

Transient Transfection and Enzyme Assays. NIH 3T3 cells were cultured as described above and transfected 1 day before confluence by the calcium phosphate method as previously described (16). Construction of the 520-bp aP2 enhancer linked to a chloramphenicol acetyltransferase (CAT) reporter was described previously (27). The ADD1 expression vector (ADD1-403) (2 μ g) was cotransfected with each CAT reporter plasmid (2 μ g) and/or combinations of PPAR α /RXR α , PPAR δ /RXR α , or PPAR γ /RXR α expression vector (1 μ g/1 μ g). The luciferase reporters such as UAS_G X4-TK-Luc and PPAR response element (PPRE) X3-TK-Luc, and Gal4 fusion expression vectors including Gal4-DBD, Gal4-PPAR α -LBD, and Gal4-PPAR γ -LBD have been described (5). The level of CAT expression was determined by measuring CAT enzyme activity. β -Galactosidase (β -gal) assays were performed by a standard colorimetric procedure with chlorophenol red- β -D-galactopyranoside (CPRG) as substrate (Boehringer Mannheim). The relative CAT and luciferase activities were normalized to the β -galactosidase activity. All the transfection experiments were performed in duplicate and repeated at least three times independently.

Ligand-Binding Assays for PPAR γ . Ligand-binding assays were performed as described previously (5, 6). Briefly, BL21(DE3) *plys* bacteria expressing a histidine-tagged ligand-binding domain of human PPAR γ protein (amino acids 176–477) was prepared by freezing/thawing in a lysis buffer containing 10 mM Tris (pH 8), 50 mM KCl, 10 mM DTT, and 1% Triton X-100. The lysate was cleared by centrifugation at 40,000 \times *g* for 30 min. Competition binding assays were performed with the thiazolidinedione PPAR γ ligand [³H]BRL 49653 (10 nM, specific activity 31 Ci/mmol; 1 Ci = 37 GBq), 300 μ g of bacterial lysate, with and without addition of extracted conditioned medium (see below), in 100 μ l of ligand-binding buffer (same buffer as above without Triton). After incubation at 4°C for 3 hr, bound and free radioactivity was separated by elution through a 1-ml Sephadex G-25 desalting column (Boehringer Mannheim). Nonspecific binding of radiolabeled ligand was assessed by using competition with cold BRL 49653 and was found to be negligible. Radioactivity was quantitated by liquid scintillation counting.

Conditioned medium from cells transfected with empty vector or vectors expressing ADD1 or C/EBP α was extracted with organic solvents by using Sep-Pak C18 column (Waters) chromatography methods as previously described (28). Briefly, 20 ml of methanol was added to 10 ml of conditioned medium and incubated at -20°C for at least 30 min. The mixture was centrifuged at 500 \times *g* for 5 min, and the supernatant was collected. The supernatant was acidified with 30 ml 0.01% glacial acetic acid and applied to a preconditioned C18 Sep-Pak cartridge. The cartridge was washed with 10 ml water and eluted in sequence with 10 ml hexane, 10 ml methyl formate, and 10 ml methanol. The methyl formate fraction, which contains fatty acids and eicosanoids, was dried down under N₂ gas and resuspended in 100 μ l methanol. A small aliquot of this extract was utilized in the ligand-binding assay described above.

RESULTS

Previous work demonstrated that coexpression of ADD1/SREBP1 with PPAR γ could increase the transcriptional activity of PPAR γ through its binding site, the PPRE. This enhanced transcriptional activity of PPAR γ by ADD1/SREBP1 appears to be indirect because ADD1/SREBP1 alone does not activate transcription of reporter-containing PPREs (23). However, it is not currently known whether this

effect is specific for PPAR γ among the PPAR isoforms. To investigate this, we cotransfected activated ADD1/SREBP1 (amino acids 1–403), along with each of the three different murine PPAR isoforms and PPAR's heterodimeric partner, RXR α . The reporter construct used is the adipose-specific enhancer region (520 bp) of the aP2 gene containing two natural PPREs, linked to CAT. As shown in Fig. 1, the transfection of each PPAR form with RXR α caused a small increase in transcriptional activity detected above the control levels. Addition of ADD1/SREBP1 caused an elevation in the transcriptional response seen only from PPAR γ , not from PPAR α or PPAR δ . This indicates that there is selectivity in the responsiveness among the PPARs to ADD1/SREBP1.

This selectivity of interaction between PPAR γ and ADD1/SREBP1 could occur at several levels, such as the transcriptional control of the enzymes responsible for the generation of an endogenous ligand(s) for PPAR γ or in the synthesis of other proteins such as coactivators, which are involved in the transcriptional response to PPAR γ . To investigate the possibility that ADD1 may be involved in the generation of ligand(s) for PPAR γ , we used a previously identified dominant-negative allele of ADD1/SREBP1 to determine whether synthetic ligands for PPAR γ could replace the functional role of ADD1/SREBP1 in adipogenesis. This dominant-negative allele of ADD1/SREBP1 contains a substitution of an alanine for an essential tyrosine residue (Y \rightarrow A, 320) in the basic DNA-binding domain (16). As reported earlier and shown in Fig. 2, the expression of this allele (amino acids 1–403, alanine substitution for tyrosine at 320; termed ADD1-DN) with a retroviral expression system strongly inhibits the differentia-

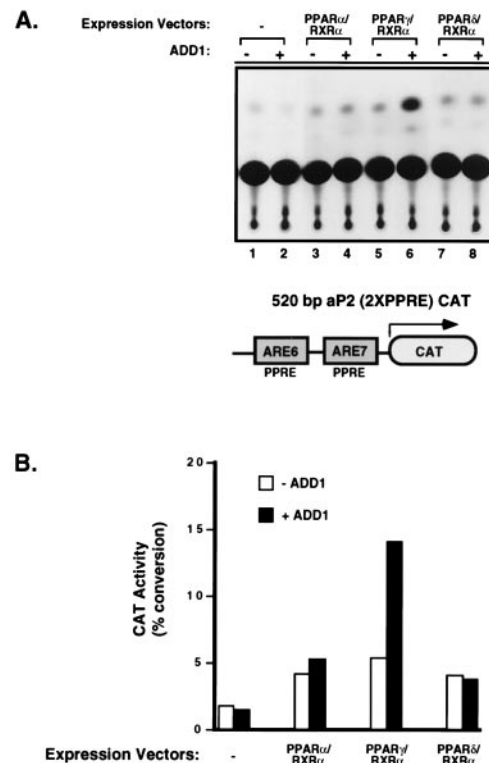


FIG. 1. Expression of ADD1/SREBP1 selectively enhances the transcriptional activity of PPAR γ . (A) NIH 3T3 cells were cotransfected with the 520-bp enhancer for aP2 gene (520-bp aP2 CAT) and indicated combinations of ADD1 with either PPAR α /RXR α , PPAR γ /RXR α , or PPAR δ /RXR α expression vectors. Transfections were performed in duplicate and repeated at least three times. A representative experiment is shown above. All CAT enzyme activities were normalized to β -gal activity. (B) Quantitation of CAT enzyme activity of A was performed by PhosphorImager analysis (Molecular Dynamics).

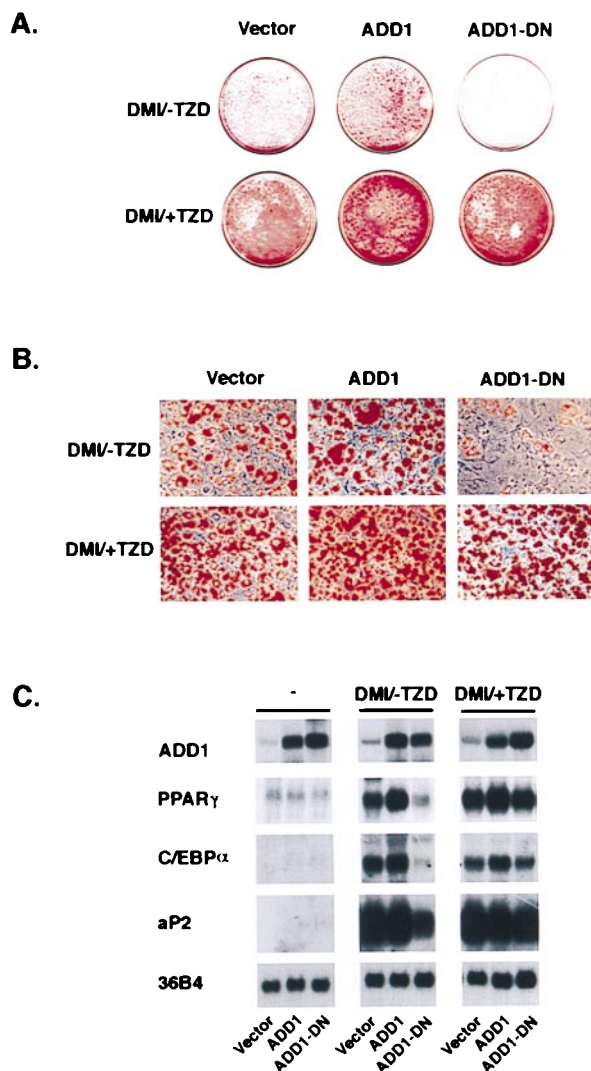


FIG. 2. A PPAR γ ligand rescues the inhibition of adipogenesis by dominant-negative ADD1/SREBP1. 3T3-L1 preadipocytes were infected with retroviruses containing either empty vector, ADD1, or dominant-negative ADD1 (ADD1-DN) as previously described (23). 3T3-L1-vector, -ADD1, and -ADD1-DN cell lines were treated with differentiation inducers in the absence (DMV/-TZD) or presence (DMV/+TZD) of the TZD ligand pioglitazone (5 μ M) for 48 hr at confluence and subsequently cultured for 5 days. Cells were fixed and stained with Oil Red O. (A) Macroscopic view of dishes. (B) Microscopic views of dishes shown in A at $\times 25$ original magnification. (C) Total RNA (10 μ g per lane) was isolated from each cell line at confluence (-) and after 5 days of postconfluent culture with treatment of either DMV/-TZD or DMV/+TZD. RNA was separated by electrophoresis, blotted to nylon membrane, and hybridized with the indicated 32 P-labeled cDNAs.

tion of 3T3-L1 cells treated with the usual differentiation inducers [labeled -TZD/DMI (dexamethasone, methylisobutyl xanthine, and insulin); see *Materials and Methods*]. Fig. 2A illustrates lipid accumulation in these cells, as shown by staining with Oil Red O. Expression of an activated form of the wild-type allele (amino acids 1–403) caused a small increase in lipid accumulation above the already robust response seen in the control (vector) cells. Addition of pioglitazone, a TZD ligand for PPAR γ , completely reverses the ability of the dominant-negative allele of ADD1/SREBP1 to block lipid accumulation and morphological differentiation of the cells (Fig. 2B). At the mRNA level, Fig. 2C shows that although the dominant-negative allele of ADD1 strongly suppresses the expression of genes linked to differentiation, such as PPAR γ ,

C/EBP α , and aP2, this effect is reversed almost completely by exposure of cells to pioglitazone during the differentiation process. These results are consistent with a role for ADD1/SREBP1 in the generation of an endogenous ligand(s) for PPAR γ during adipogenesis, though other mechanistic interpretations are also possible.

Based on these results, we therefore investigated more directly the notion that ADD1/SREBP1 may generate ligands for PPAR γ . Though no molecule has been demonstrated to serve as an endogenous ligand of PPAR γ *in vivo*, several biological ligands or activators of PPAR γ and other PPARs have been identified. These are all secreted lipids in the fatty acid family including the prostaglandins, leukotrienes, hydroxy-eicosatetraenoic acids, and polyunsaturated fatty acids (5–7). We therefore have modified the standard cotransfection protocol shown in Fig. 3A, section 1, to a protocol (Fig. 3A, section 2) where the supernatants (conditioned medium) from cells transfected with ADD1 were placed on a second dish of cells transfected with PPAR γ . As shown in Fig. 3B, supernatants from cells transfected with ADD1 stimulated a 3-fold increase in transcriptional activity obtained from PPAR γ when compared with cells not transfected, transfected with empty vectors, or transfected with C/EBP α .

To investigate the domains of PPAR γ necessary for this response, we used chimeric molecules where the ligand-binding domains (LBD) of PPAR γ or - α were fused to the DNA-binding domain (DBD) of Gal 4. As shown in Fig. 3C, transcriptional activity of the Gal 4 DBD alone or fused to the LBD of PPAR α did not respond to these supernatants, whereas Gal4 DBD fused to the LBD of PPAR γ showed a marked increase in activity. Supernatants from cells transfected with empty vectors did not show this increase. These data suggest that ADD1/SREBP1 stimulates the production of molecule(s) that can selectively activate PPAR γ through its LBD.

With the availability of synthetic, high-affinity ligands of PPAR γ , such as the TZD drugs, it is possible to look directly at ligand-binding activity. The supernatants from cells transfected with ADD1/SREBP1, C/EBP α or empty vectors were extracted with an organic solvent to extract total lipids. They then were resolubilized and put in a competition assay for binding of [3 H]BRL 49653 to PPAR γ . The supernatants from the cells transfected with ADD1 showed powerful displacement of BRL 49653, with 50% maximal displacement at 12.5% of the neat, extracted materials (Fig. 4). In contrast, cells transfected with empty vectors showed very little displacement, even when the extracted material was used in an undiluted manner. When compared at a point where these two curves overlap, such as the ED $_{25}$, there appears to be at least 8-fold more ligand activity in cells that were transfected with ADD1. C/EBP α -transfected cells showed a very small amount of displacement, much less than ADD1, but more than the vector-transfected cells. These data illustrate directly that expression of ADD1/SREBP1 triggers the generation of ligand(s) for PPAR γ .

ADD1/SREBP1 has unusual dual DNA-binding specificity, interacting both with E-box motifs and the SRE sequence. This dual DNA-binding specificity is a result of one amino acid, tyrosine 320, at a site where all other basic helix-loop-helix factors have an arginine residue (16). Mutation of tyrosine 320 to arginine converts ADD1/SREBP1 into a pure E-box binding factor and hence allows discrimination of those functions that occur through E-boxes versus those mediated by SRE-like sequences (16). Various alleles of ADD1/SREBP1 were cotransfected with PPAR γ , and transcriptional activity was determined through PPRE element. Fig. 4B indicates that the ADD1 320 Y \rightarrow R mutation is just as effective at promoting transcriptional activity of PPAR γ as the wild-type allele. These same alleles were then transfected and ligand activity was assayed from the cellular supernatants. As shown in Fig. 4C,

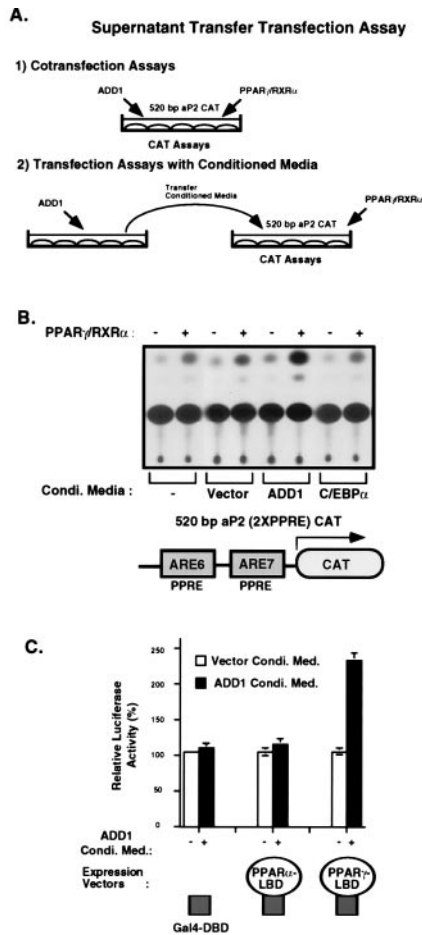


FIG. 3. Conditioned medium from cells transfected with ADD1/SREBP1 increases the transcriptional activity of PPAR γ /RXR α through its ligand-binding domain (LBD). (A) Scheme of supernatant transfer assays. Section 1 shows a cotransfection analysis; section 2 illustrates transient transfection assays with conditioned medium. To collect conditioned medium, transfected cells were incubated with 0.5% BSA in DMEM, and supernatants were isolated after 24 hr. These supernatants were used for the sequential transfection experiments with a 520-bp aP2 CAT reporter in the absence or presence of PPAR γ /RXR α . (B) As described above, conditioned medium was prepared by transient transfection of either empty expression vector or vectors expressing ADD1 or C/EBP α . Supernatants collected from different cells were incubated for 24 hr with cells transfected with the 520-bp aP2 CAT reporter and PPAR γ /RXR α expression vectors. These transfections were performed in duplicate, and the above is a representative experiment. (C) Rat1-IR cells were transfected with luciferase reporter containing four copies of the UAS_G and either Gal4 DNA-binding domain (DBD), Gal4-PPAR α LBD, or Gal4-PPAR γ LBD expression vector. After transfection, cells were incubated with conditioned medium from cells transfected with either vector or ADD1. Normalized luciferase activity was determined and plotted as luciferase activity relative to Gal4 DBD expression vector.

the ADD1-R allele stimulated the production of ligand activity in a manner that was similar to that of the wild-type allele. These data indicate that ADD1/SREBP1 can promote the function of ligand production for PPAR γ through E-box motifs of as yet unknown target genes.

DISCUSSION

Cooperation between multiple transcription factors is an important component of most, if not all, differentiation processes, including adipose differentiation. One dramatic example of this has been observed when C/EBP α and PPAR γ are coexpressed (14). This yields a powerful adipogenic response

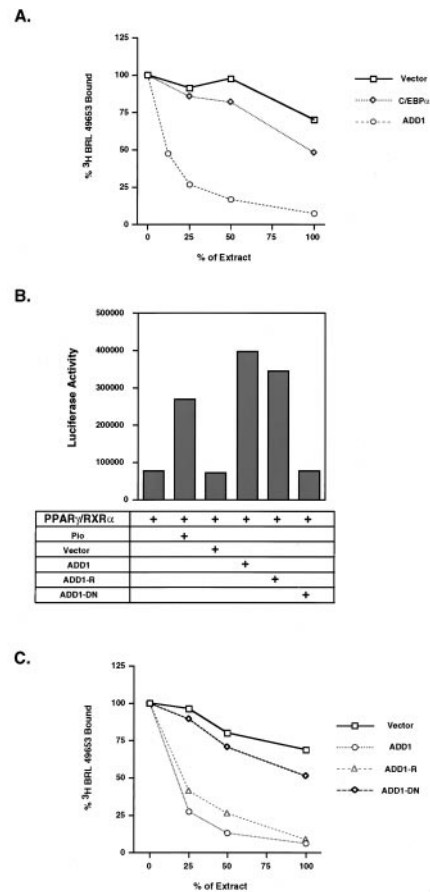


FIG. 4. ADD1/SREBP1 induces ligand activity for PPAR γ . (A) Conditioned medium from cells transfected with either empty vector, ADD1, or C/EBP α was organically extracted as described in *Materials and Methods*. Fractions of each extract were diluted in ligand-binding buffer (100% denotes 5 μ l of extract in methanol, 50% denotes 2.5 μ l, etc.) and used in competition with [3 H]BRL 49653 for binding to bacterial-produced PPAR γ (see details in *Materials and Methods*). (B) Cotransfection of several alleles of ADD1 including ADD1, ADD1-R, and ADD1-DN with PPAR γ /RXR α to determine transcriptional activity through PPRES with luciferase reporter (PPRE X3-TK-Luc). Luciferase activity was normalized with β -gal activity. (C) Ligand activity from cells transfected with either empty vector, ADD1, ADD1-R, or ADD1-DN. Conditions were the same as in A.

under conditions where neither factor alone is sufficient to promote fat cell formation. Another notable example of transcription factor cooperation is that between ADD1/SREBP1 and PPAR γ . Both of these factors are induced very early in adipogenesis, and expression of ADD1/SREBP1 enhances the transcriptional activity of PPAR γ and increases the percentage of cells undergoing differentiation (23).

The data presented here indicate clearly that expression of an activated form of ADD1/SREBP1 causes cells to produce ligand(s) for PPAR γ . These ligand(s) are likely to be fatty acid derivatives because they can be isolated with organic solvents that extract polar lipids such as eicosanoid derivatives and other fatty acids (Fig. 4). The first biological ligand described for PPAR γ was 15-deoxy- $\Delta^{12,14}$ -PGJ₂ (5, 6). This molecule, which is produced by the nonenzymatic dehydration of PGD₂, has an affinity for PPAR γ of approximately 2 μ M (6). Most recently, certain polyunsaturated fatty acids such as linoleic acid and arachidonic acid have been shown to bind to and activate PPAR γ , although the affinity here is also relatively low (K_d = 10–100 μ M) (7). These data raised the possibility that PPAR γ ligands may be provided by dietary sources through the circulation, as opposed to being produced endogenously. The experiments described here illustrate that a

PPAR γ ligand(s) is produced endogenously from cells expressing ADD1/SREBP1, a transcription factor induced early in adipogenesis. Of course, it is quite possible that ligands for this transcription factor come from both endogenous and exogenous sources, dependent on diet, physiological status, and genetic tendency toward obesity and diabetes.

One key issue to be pursued is the target gene(s) for ADD1/SREBP1 related to the biosynthesis of PPAR γ ligands. We and others previously have shown that ADD1/SREBP1 can control the expression of fatty acid synthetase and lipoprotein lipase, key genes of fatty acid metabolism (23). Because the endogenous ligand(s) may well be fatty acid derivatives, control of these genes may be rate-limiting. Consistent with this (Fig. 4), we recently have shown that ADD1/SREBP1 can control fatty acid synthetase expression through an E-box motif (29). On the other hand, the most common products of these enzymes, the dietary fatty acids, are likely to have to undergo further enzymatic transformation to function effectively as PPAR γ ligands. The genes encoding these modifying enzymes are prime candidates for regulation by ADD1/SREBP1.

Finally, the identification of the endogenous ligand described here will be important to determine. Because PPAR γ ligands potentially can be involved in both adipogenesis and insulin resistance/sensitivity, these molecules may open a unique opportunity to understand these problems. Current fractionation studies using HPLC suggest a single peak of activity, indicating one or very few components (not shown). The chromatographic behavior of this activity indicates that it is not equivalent to 15-deoxy- $\Delta^{12,14}$ -PGJ₂. Structural determination will shed light on the biochemical nature of this molecule and open the door to the study of its biosynthetic regulation.

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1. Spiegelman, B. M. & Flier, J. S. (1996) *Cell* **87**, 377–389.
2. Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I. & Spiegelman, B. M. (1994) *Genes Dev.* **8**, 1224–1234.
3. Hu, E., Tontonoz, P. & Spiegelman, B. M. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 9856–9860.
4. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M. & Kliewer, S. A. (1995) *J. Biol. Chem.* **270**, 12953–12956.
5. Forman, B. M., Tontonoz, P., Jasmine, C., Brun, R. P., Spiegelman, B. M. & Evans, R. M. (1995) *Cell* **83**, 803–812.
6. Kliewer, S. A., Lenhard, J. M., Willson, T. M., Patel, I., Morris, D. C. & Lehmann, J. M. (1995) *Cell* **83**, 813–819.
7. Krey, G., Braissant, O., L'Horsset, F., Kalkhoven, E., Perroud, M., Parker, M. G. & Wahli, W. (1997) *Mol. Endocrinol.* **11**, 779–791.
8. Wu, Z., Xie, Y., Bucher, N. & Farmer, S. R. (1995) *Genes Dev.* **9**, 2350–2363.
9. Samuelsson, L., Stromberg, K., Vikman, K., Bjursell, G. & Enerback, S. (1991) *EMBO J.* **10**, 3787–3793.
10. Umek, R. M., Friedman, A. D. & McKnight, S. L. (1991) *Science* **251**, 288–292.
11. Lin, F. T. & Lane, M. D. (1992) *Genes Dev.* **6**, 533–544.
12. Lin, F. T. & Lane, M. D. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 8757–8761.
13. Freytag, S. O., Paielli, D. L. & Gilbert, J. D. (1994) *Genes Dev.* **8**, 1654–1663.
14. Tontonoz, P., Hu, E. & Spiegelman, B. M. (1994) *Cell* **79**, 1147–1156.
15. Tontonoz, P., Kim, J. B., Graves, R. A. & Spiegelman, B. M. (1993) *Mol. Cell. Biol.* **13**, 4753–4759.
16. Kim, J. B., Spotts, G. D., Halvorsen, Y. D., Shih, H. M., Ellenberger, T., Towle, H. C. & Spiegelman, B. M. (1995) *Mol. Cell. Biol.* **15**, 2582–2588.
17. Yokoyama, C., Wang, X., Briggs, M. R., Admon, A., Wu, J., Hua, X., Goldstein, J. L. & Brown, M. S. (1993) *Cell* **75**, 187–197.
18. Briggs, M. R., Yokoyama, C., Wang, X., Brown, M. S. & Goldstein, J. L. (1993) *J. Biol. Chem.* **268**, 14490–14496.
19. Hua, X., Yokoyama, C., Wu, J., Briggs, M. R., Brown, M. S., Goldstein, J. L. & Wang, X. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 11603–11607.
20. Wang, X., Sato, R., Brown, M. S., Hua, X. & Goldstein, J. L. (1994) *Cell* **77**, 53–62.
21. Sakai, J., Duncan, E. A., Rawan, R. B., Hua, X., Brown, M. S. & Goldstein, J. L. (1996) *Cell* **85**, 1037–1046.
22. Sheng, Z., Otani, H., Brown, M. S. & Goldstein, J. L. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 935–938.
23. Kim, J. B. & Spiegelman, B. M. (1996) *Genes Dev.* **10**, 1096–1107.
24. Shimano, H., Horton, J. D., Hammer, R. E., Shimomura, I., Brown, M. S. & Goldstein, J. L. (1996) *J. Clin. Invest.* **99**, 846–854.
25. Shimano, H., Horton, J. D., Shimomura, I., Hammer, R. E., Brown, M. S. & Goldstein, J. L. (1997) *J. Clin. Invest.* **99**, 846–854.
26. Hu, E., Kim, J. B., Sarraf, P. & Spiegelman, B. M. (1996) *Science* **274**, 2100–2103.
27. Graves, R. A., Tontonoz, P. & Spiegelman, B. M. (1992) *Mol. Cell. Biol.* **12**, 1202–1208.
28. Serhan, C. N. & Sheppard, K. A. (1990) *J. Clin. Invest.* **85**, 772–780.
29. Kim, J. B., Pasha, S., Wright, M., Yao, K. M., Mueller, E., Solanes, G., Lowell, B. B. & Spiegelman, B. M. (1998) *J. Clin. Invest.* **101**, 1–9.