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Inhibition of Adipogenesis by a COOH-Terminally Truncated Mutant of PPARγ2 in 3T3-L1 Cells

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Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor that is thought to be an important regulator of adipocyte differentiation. This ligand-dependent transcription factor is also activated by thiazolidinediones, a new class of synthetic antidiabetic drugs, resulting in a marked adipogenic response in cultured cells and enhanced insulin sensitivity *in vivo*. The importance of the COOH-terminal region of PPARγ2 in thiazolidinedione-induced adipogenesis has now been investigated by expression of a mutant protein (PPARγ2-ΔC) that lacks the COOH-terminal 16 amino acids of full-length PPARγ2. The mutant protein failed to bind a thiazolidinedione ligand, but its ability to bind the peroxisome proliferator response element was similar to that of the wild-type protein. Expression of PPARγ2-ΔC inhibited the thiazolidinedione-induced increase in trans-activation activity of endogenous PPARγ in CV-1 cells. Furthermore, the mutant protein prevented thiazolidinedione-induced adipogenesis in 3T3-L1 cells, whereas expression of recombinant wild-type PPARγ2 promoted adipogenesis. These data show not only that the COOH-terminal region of PPARγ2 is indispensable for thiazolidinedione-induced adipogenesis mediated by this protein in 3T3-L1 cells, but also that the PPARγ2-ΔC mutant acts in a dominant negative manner by interfering with the access of endogenous PPARγ to the peroxisome proliferator response element of target genes.

Nuclear hormone receptors constitute a large family of transcription factors that are regulated by various endogenous or synthetic ligands and mediate diverse biological responses. Peroxisome proliferator-activated receptors (PPARs)[2] are members of this family of proteins (1, 2) and are encoded by three distinct genes: The PPARα gene is expressed predominantly in liver, heart, kidney, and adipose tissue, and the encoded protein is thought to be important for fatty acid homeostasis (7, 8). The PPARβ (also known as NUC1 or PPARδ) gene is expressed in many tissues, including adipose tissue, intestine, skeletal muscle, heart, brain, and kidney, but the function of the encoded protein is not fully understood (9, 10). The PPARγ gene gives rise to two protein isoforms, γ1 (6, 11) and γ2 (2), as a result of alternative RNA splicing (12, 13). The mouse PPARγ1 and PPARγ2 proteins differ by only 30 amino acid residues at their NH_2-termini. Whereas PPARγ1 is expressed in a broad range of tissues, including liver, heart, kidney, skeletal muscle, adipose tissue, and white blood cells, PPARγ2 is expressed predominantly in adipose tissue (2, 6, 10, 14).

Among PPAR isoforms, PPARγ is the only one that is highly enriched in adipose tissue. Furthermore, PPARγ plays a central role in activating the program of adipogenesis (15). Adipocyte differentiation is characterized by coordinated increases in adipocyte-specific gene expression. In most instances, these increases in gene expression are due to activation of gene transcription. Expression of PPARγ is increased relatively early during differentiation of preadipocytes into adipocytes. Furthermore, ectopic expression and ligand activation of PPARγ in nonadipogenic fibroblasts promotes their transformation into lipid-filled adipocytes and induces a full program of adipogenic gene expression (14). The promoters or enhancers of several adipogenic genes contain PPARγ binding sites (16–18). The known agonists and ligands for PPARγ include thiazolidinediones, a synthetic class of insulin sensitizers, as well as prostaglandin J_2 and certain polyunsaturated fatty acids (19–22). One of the thiazolidinediones, troglitazone, is used in the treatment of type II diabetes mellitus. Thus, PPARγ may contribute to the amelioration

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[2] Abbreviations used: PPAR, peroxisome proliferator-activated receptor; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HA, hemagglutinin; GST, glutathione *S*-transferase; PCR, polymerase chain reaction; RXR retinoid X receptor; RAR, retinoic acid receptor; PPRE, peroxisome proliferator response element; TR, thyroid hormone receptor.

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of insulin resistance in individuals with type II diabetes or obesity who are treated with troglitazone.

To investigate the functional role of the COOH-terminal region of PPARγ2, we have now produced a PPARγ2 mutant that lacks the COOH-terminal 16 amino acids of the full-length protein. This mutant protein was shown to act in a dominant negative manner, and its overexpression in 3T3-L1 cells inhibited thiazolidinedione-induced adipogenesis.

EXPERIMENTAL PROCEDURES

Materials. Cells were obtained from American Type Culture Collection. Insulin was obtained from Sigma (St. Louis, MO); Dulbecco's modified Eagle's medium (DMEM) and Lipofectin were from Life Technologies (Rockville, MD), and monoclonal antibodies (12CA5) to the hemagglutinin (HA) epitope were from Boehringer Mannheim (Indianapolis, IN). Polyclonal antibodies to PPARγ2 were generated by immunizing rabbits with a glutathione *S*-transferase (GST) fusion protein of mouse PPARγ2. Glutathione-Sepharose 4B was from Pharmacia. The pSV-β-galactosidase vector was from Promega. The pAxCAwt vector was kindly provided by I. Saito (University of Tokyo, Japan), the PPRE3-TK-LUC vector by K. Umesono (University of Kyoto, Japan), troglitazone by Sankyo (Tokyo, Japan), and BRL49653 by SmithKline Beecham (Harlow, UK). Mouse PPARγ2 cDNA was obtained by reverse transcription and the polymerase chain reaction (PCR) with mRNA from 3T3-L1 adipocytes.

Preparation of recombinant adenoviruses. PCR method was utilized to produce the PPARγ2 constructs tagged with HA epitope at their COOH termini with the oligonucleotide primers [5′-attctagagttatgggtgaaactctggga-3′ (sense) and 5′-taggtacctcatgcgtagtcgggaacatcgtacggatacaagtccttgtagatctcctg-3′ (anti-sense) for wild-type PPARγ2; 5′-attctagagttatgggtgaaactctggga-3′ (sense) and 5′-ctggtaccctacgcgtaatcggggacatcgtacgggtatgtctctgtcttcttgatcacatg-3′ (antisense) for COOH-terminally truncated PPARγ2]. The sequence validity of both constructs was confirmed by sequencing the PCR products. Complementary DNA encoding HA-tagged wild-type PPARγ2 (PPARγ2-WT) or its COOH-terminally truncated mutant (PPARγ2-ΔC) was subcloned into the SwaI site of pAxCAwt (23), which contains the CAG promoter (24). The resulting vectors were introduced into cells by transfection as described previously (25). A single clone of recombinant adenovirus was isolated for each protein through serial dilution in a plaque assay. The isolated recombinant adenoviruses (Adex-PPARγ2-WT and Adex-PPARγ2-ΔC) were expanded and twice purified by ultracentrifugation on a CsCl gradient. Viral titer was determined by plaque assay (26).

Immunoblot analysis. CHO or 3T3-L1 cells were infected with adenoviruses (multiplicity of infection, 20 plaque-forming units per cell) for 4 h, incubated in the absence of virus for 48 h, washed twice with phosphate-buffered saline, harvested, and lysed for 15 min at 4°C in a solution containing 50 mM Tris-HCl (pH 7.5), 150 mM KCl, 1% (v/v) Triton X-100, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. The lysate was centrifuged at 20,000 × *g* for 15 min to remove cell debris, and the resulting supernatant was fractionated by SDS-polyacrylamide gel electrophoresis on a 10% gel. The separated proteins were transferred electrophoretically to a nitrocellulose membrane and, after blocking of nonspecific sites on the membrane, were exposed to primary antibodies. Immune complexes were detected with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (ECL; Amersham, Little Chalfont, UK).

Ligand binding assay. Approximately 10 μg of purified GST or GST-PPARγ2 (PPARγ2-WT or PPARγ2-ΔC) fusion protein were incubated at 4°C for 6 h in a reaction mixture (50 μl) containing 50 mM Tris-HCl (pH 7.5), 50 mM KCl, 10 mM dithiothreitol, and 200 nM [^{3}H]BRL49653 (40 Ci/mmol) in the absence or presence of a 500-fold excess of unlabeled BRL49653. Protein-bound radioactivity was isolated by centrifugation of the reaction mixture through Quick Spin Sephadex G-25 columns (Boehringer Mannheim) and quantitated by liquid scintillation counting.

Gel retardation assay. The PPRE oligonucleotides 5′-GTTACTAGGACAAAGGTCACAGAT-3′ and 3′-AATGATCCTGTTTCCAGTGTCTAG-5′ were annealed and labeled with [α-^{32}P]dCTP by one cycle of the polymerase chain reaction (4 min of denaturation at 94°C, 10 min of annealing at 55°C, 10 min of elongation at 72°C). CHO cells were infected with Adex-PPARγ2-WT or Adex-PPARγ2-ΔC and lysed 48 h later. Cell extracts (30 μg of protein) containing equal amounts of the two recombinant PPARγ proteins were incubated for 20 min at room temperature with the ^{32}P oligonucleotide probe in a total volume of 15 μl containing 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 2 mM EDTA, 5 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride, 10% (v/v) glycerol, and 1 μg of poly(dI:dC). For competition experiments, various amounts (10- to 200-fold molar excess) of unlabeled PPRE oligonucleotide probe were included in the reaction mixture. DNA-protein complexes were resolved from free probe by electrophoresis on a 6% polyacrylamide gel, after which the gel was dried and exposed to X-ray film.

Luciferase assay. CV-1 cells, cultured in DMEM supplemented with 10% FBS in 24-well plates, were transfected with the use of Lipofectin with the reporter plasmid PPRE3-TK-LUC (250 ng); with the expression vector pcDL-SRα (27) containing PPARγ2-WT or PPARγ2-ΔC cDNA (or pcDL-SRα alone) (200 ng); and with the internal control plasmid pSV-β-galactosidase (300 ng). After 24 h, the medium was replaced by FBS-free DMEM containing 1 μM troglitazone and the cells were incubated for an additional 24 h. Cells were then lysed and assayed for luciferase and β-galactosidase activities. Luciferase activity was normalized by β-galactosidase activity and expressed as fold activation.

RESULTS

Preparation of Wild-Type and COOH-Terminally Truncated PPARγ2 Constructs

PPARγ forms heterodimers with the retinoid X receptor (RXR), binds to enhancer, promoter, or silencer sequences of DNA, and thereby controls the expression of various genes. Like other nuclear hormone receptors, PPARγ contains distinct functional domains, which include an NH_2-terminal A/B domain, a DNA-binding domain, a hinge domain, and a ligand-binding domain. The COOH-terminal end of the ligand-binding domain contains a highly conserved motif known as AF2 (activating function 2). Retinoic acid (28, 29), estrogen (30), or thyroid hormone (31–33) receptors that contain deletions or point mutations in their AF2 regions have been shown to act in a dominant negative manner. We therefore decided to delete the AF2 region of PPARγ2 in an attempt to generate a dominant negative mutant. The mutant PPARγ2 (PPARγ2-ΔC) lacked the COOH-terminal 16 amino acids of the full-length protein and therefore terminated at residue 489; a retinoic acid receptor α (RARα) mutant that terminates at the corresponding residue 403 was shown to inhibit the activity of the endogenous receptor (28). Both the wild-type PPARγ2 receptor (PPARγ2-WT) and PPARγ2-ΔC were tagged with the HA epitope (YPYDVPDYA) at their COOH-termini (Fig. 1).

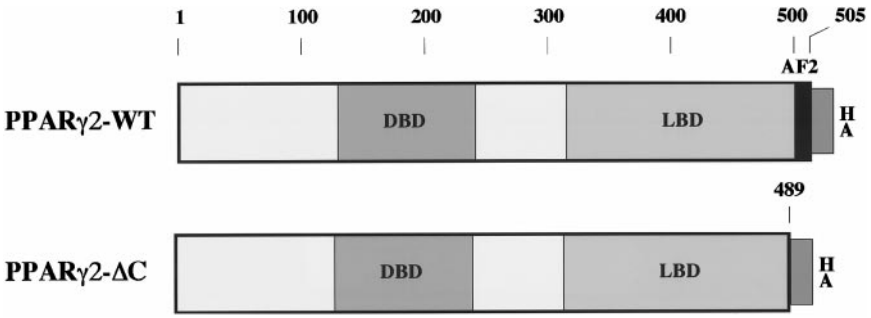

FIG. 1. Schematic representation of PPARγ2-WT and PPARγ2-ΔC constructs. PPARγ2-ΔC lacks the COOH-terminal 16 amino acids of the wild-type protein. Both PPARγ2-WT and PPARγ2-ΔC were engineered at the cDNA level to contain the HA epitope tag at their COOH termini. DBD and LBD indicate the DNA-binding and ligand-binding domains, respectively. AF2 indicates activating function 2. Numbers refer to amino acid residues.

Ligand Binding to PPARγ2-WT and PPARγ2-ΔC

The thiazolidinedione BRL49653 binds to PPARγ with an affinity in the nanomolar range (22). To investigate the ligand binding ability of the mutant PPARγ2, we first expressed both PPARγ2-WT and PPARγ2-ΔC in *Escherichia coli* as GST fusion proteins. We then incubated 10 μg of GST-PPARγ2-WT or GST-PPARγ2-ΔC fusion proteins immobilized on glutathione-Sepharose beads with [^{3}H]BRL49653 in the absence or presence of a 500-fold excess of unlabeled ligand. The extent of [^{3}H]BRL49653 binding to GST-PPARγ2-WT was ~500 times the extent of that to GST alone; 99% of this binding was abolished in the presence of unlabeled BRL49653, suggesting that the binding was specific (Fig. 2). In contrast, the extent of [^{3}H]BRL49653 binding to GST-PPARγ2-ΔC did not differ from the extent of that to GST alone and was not affected by the presence of unlabeled ligand. These results demonstrate that the COOH-terminal 16 amino acids of PPARγ2 are important for ligand binding.

DNA Binding Activities of PPARγ2-WT and PPARγ2-ΔC

We next examined the ability of the PPARγ2 mutant to bind DNA with the use of a gel retardation assay. Extracts of CHO cells infected with adenoviruses encoding PPARγ2-WT or PPARγ2-ΔC were first confirmed to express similar amounts of the two proteins by immunoblot analysis with antibodies to the HA epitope tag (Fig. 3A). The extracts were then incubated with a ^{32}P-labeled oligonucleotide probe containing a peroxisome proliferator response element (PPRE), after which protein-DNA complexes were detected by electrophoresis and autoradiography. Both PPARγ2-WT and PPARγ2-ΔC bound to the ^{32}P-labeled PPRE probe, and this binding was inhibited by the presence of unlabeled competitor oligonucleotide in a concentration-dependent manner (Figs. 3B and 3C). The two PPARγ2 proteins thus exhibited similar abilities to bind to the PPRE.

Trans-activation Activities of PPARγ2-WT and PPARγ2-ΔC

To assess the trans-activation activities of PPARγ2-WT and PPARγ2-ΔC, we performed luciferase reporter assays in CV-1 cells. Each PPARγ2 protein was transiently expressed in CV-1 cells with the use of the expression vector pcDL-SRα (27) and assayed for its ability to activate transcription of the luciferase gene positioned downstream of three copies of a PPRE. Troglitazone (1 μM) induced an ~15-fold increase in luciferase activity in cells expressing PPARγ2-WT (Fig. 4). This drug also increased luciferase activity 2.3-fold in control cells transfected with the empty pcDL-SRα vector, an effect likely mediated by endogenous PPARγ. In contrast, troglitazone had no effect on luciferase activity in cells expressing PPARγ2-ΔC. These results suggest that PPARγ2-ΔC acts in a dominant negative manner to inhibit the trans-activation activity of endogenous PPARγ.

Inhibition of Adipogenesis by PPARγ2-ΔC in 3T3-L1 Cells

To examine whether PPARγ2-ΔC also exerts a dominant negative effect on thiazolidinedione-induced adipogenesis, we expressed the mutant protein or PPARγ2-WT in 3T3-L1 preadipocytes with the use of the corresponding adenovirus vectors. We verified that equal amounts of PPARγ2-WT and PPARγ2-ΔC proteins were expressed in the infected cells by immunoblot analysis with antibodies to PPARγ2 (Fig. 5A). The cells were then incubated for 4 days with 100 nM insulin and 5 μM BRL49653 to induce adipogenesis, and the percentage of differentiated cells was determined after incubation in the absence of inducers for an additional 8 days. As a result of this treatment, ~50% of noninfected, control 3T3-L1 preadipocytes differentiated into adipocytes (Figs. 5B and 5C). Whereas ~100% of preadipocytes expressing PPARγ2-WT dif-

ferentiated into adipocytes, expression of PPARγ2-ΔC almost completely inhibited differentiation. These results suggest that PPARγ2-ΔC acts in a dominant negative manner to inhibit the function of endogenous PPARγ in thiazolidinedione-induced adipogenesis in 3T3-L1 cells.

DISCUSSION

We have shown that the COOH-terminal region of PPARγ2 is important for the function of this protein. The COOH-terminally truncated mutant PPARγ2-ΔC prevented not only trans-activation by endogenous PPARγ but also adipogenesis induced in 3T3-L1 cells by BRL49653, a potent thiazolidinedione ligand for PPARγ (22). Moreover, this latter observation, together with the demonstration that PPARγ2-WT promoted BRL49653-induced adipogenesis in 3T3-L1 cells, indicates that thiazolidinedione-induced adipogenesis in these cells is mediated by activation of endogenous PPARγ.

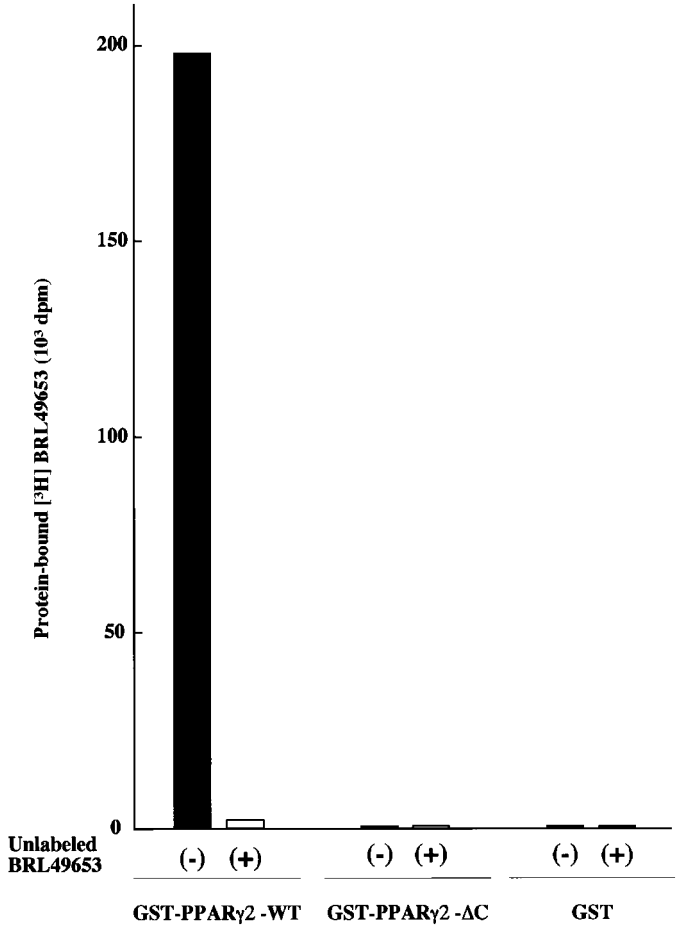

FIG. 2. Ligand binding activities of PPARγ2-WT and PPARγ2-ΔC. Approximately 10 μg of purified GST, GST-PPARγ2-WT, or GST-PPARγ2-ΔC were incubated for 6 h at 4°C with [^{3}H]BRL49653 in the absence or presence of a 500-fold excess of unlabeled BRL49653. Protein-bound radioactivity was separated and quantitated by liquid scintillation counting. Data are means of triplicates from an experiment that was repeated three times with similar results.

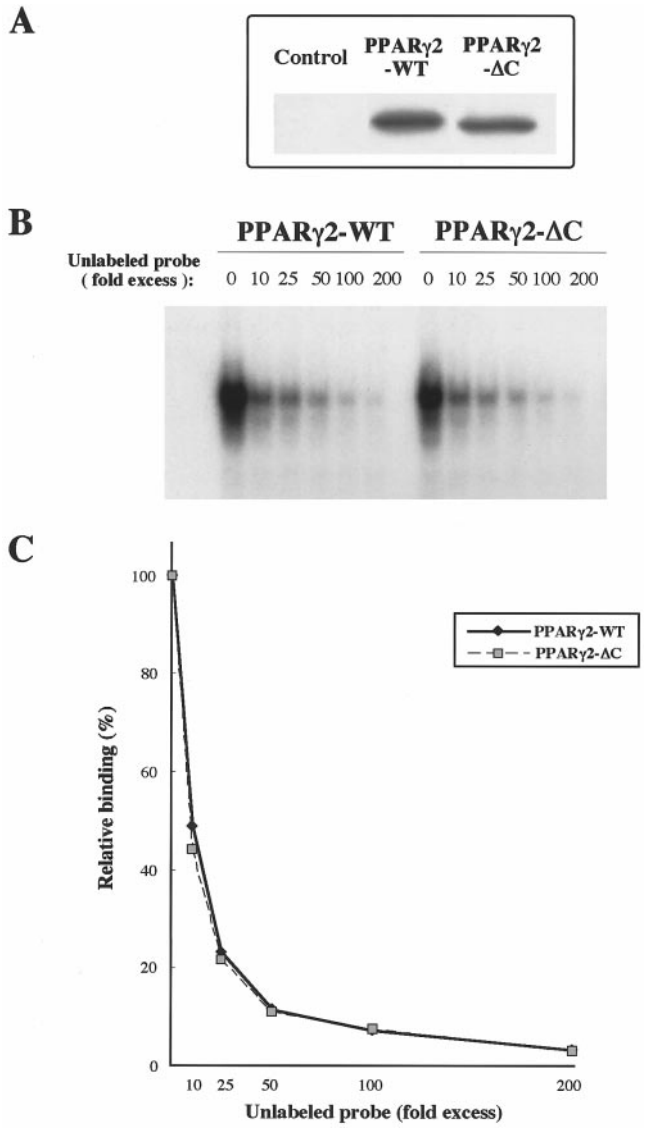

FIG. 3. DNA binding activities of PPARγ2-WT and PPARγ2-ΔC. (A) Immunoblot analysis of PPARγ2-WT and PPARγ2-ΔC expression in CHO cells. Equal amounts of extract protein of control CHO cells or of CHO cells that had been infected with Adex-PPARγ2-WT or Adex-PPARγ2-ΔC were subjected to immunoblot analysis with antibodies to the HA epitope tag. (B) Gel retardation assay of the DNA binding activities of PPARγ2-WT and PPARγ2-ΔC. CHO cell extracts containing equal amounts of the two PPARγ2 proteins were incubated for 20 min at room temperature with a ^{32}P-labeled PPRE oligonucleotide probe in the absence or presence of the indicated molar excesses of unlabeled oligonucleotide. DNA–protein complexes were resolved from free probe by polyacrylamide gel electrophoresis and detected by autoradiography. (C) Quantitation of the DNA binding activities of PPARγ2-WT and PPARγ2-ΔC. The data shown in (B) were quantitated by NIH image. Binding measured in the absence of unlabeled oligonucleotide was defined as 100%. Similar results were obtained in two additional experiments.

An example of a nuclear receptor that acts in a dominant negative manner is provided by the viral protein v-ErbA, which blocks the function of its cellular counterpart, the thyroid hormone receptor (TR) (31, 33, 34). The v-ErbA and TR proteins differ in their COOH-terminal regions, most prominently in that v-ErbA is truncated in this region relative to the TR. A COOH-terminally truncated mutant of RARα, which terminates at amino acid 403, has also been generated and shown to inhibit the activity of wild-type RARα in a dominant negative manner (28). Both TR and RAR proteins form stable heterodimers with RXR and bind to direct repeats of AGGTCA that are separated by specific numbers of nucleotides (35). PPARγ also forms heterodimers with RXR (36). Thus, PPARγ2-ΔC resembles these dominant negative mutants of both TR and RARα in terms of both structure and function. Both v-ErbA and the RARα mutant truncated at residue 403 exhibit reduced ligand binding activity compared with the corresponding wild-type proteins. PPARγ2-ΔC exhibited no ligand binding activity. The effect of COOH-terminal truncation on ligand binding was more marked for PPARγ2 than for RARα; the RARα mutant exhibited a ligand binding activity about one-twelfth

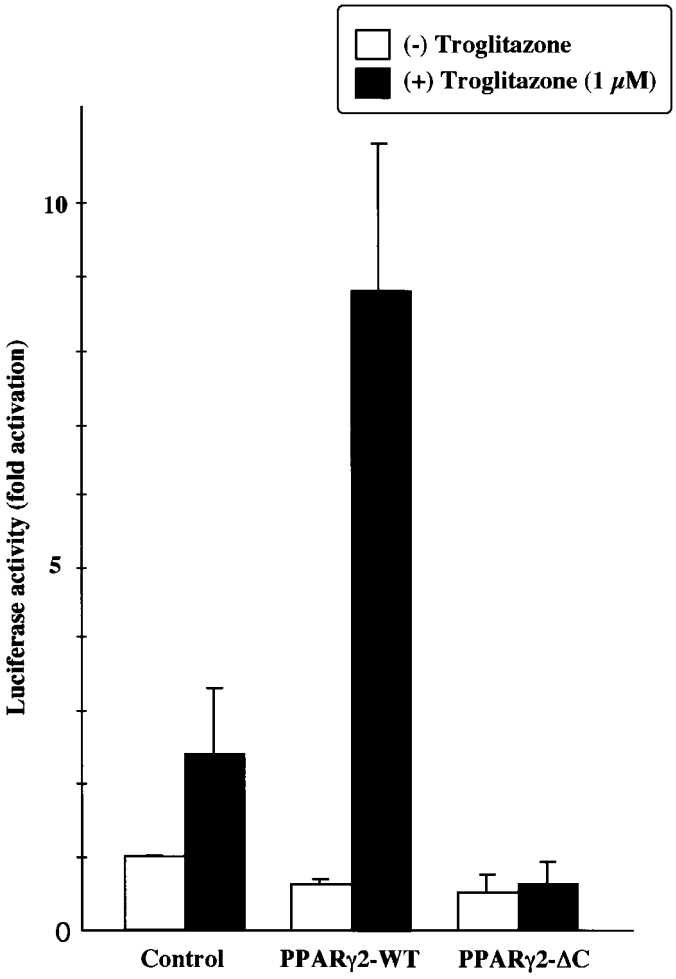

FIG. 4. Trans-activation activities of PPARγ2-WT and PPARγ2-ΔC. CV-1 cells were transfected with the reporter plasmid PPRE3-TK-LUC, pcDL-SRα containing PPARγ2-WT or PPARγ2-ΔC cDNA (or pcDL-SRα alone), and pSV-β-galactosidase. The transfected cells were incubated for 24 h in the absence or presence of 1 μM troglitazone, after which cell lysates were assayed for luciferase and β-galactosidase activities. Luciferase activity was normalized on the basis of β-galactosidase activity, and was expressed as fold activation relative to the value for control cells (transfected with empty pcDL-SRα) incubated in the absence of troglitazone. Data are means ± SD of values from three independent experiments.

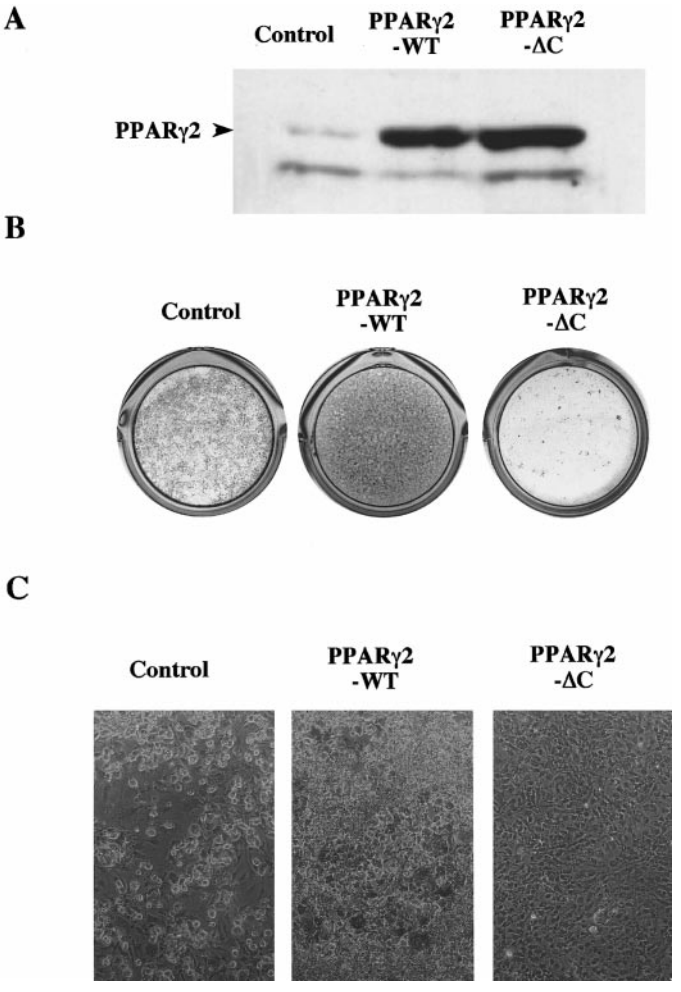

FIG. 5. Inhibition of adipogenesis in 3T3-L1 cells by PPARγ2-ΔC. (A) Immunoblot analysis of PPARγ2-WT and PPARγ2-ΔC expression in 3T3-L1 preadipocytes. Equal amounts of extract protein of control 3T3-L1 preadipocytes or cells that had been infected with adenovirus vectors encoding PPAR-γ2-WT or PPARγ2-ΔC were subjected to immunoblot analysis with antibodies to PPARγ2. The position of the recombinant PPARγ2 proteins is indicated. (B and C) Effects of PPARγ2-WT and PPARγ2-ΔC on adipogenesis. Two days after infection with Adex-PPARγ2-WT or Adex-PPARγ2-ΔC, 3T3-L1 preadipocytes were incubated for 4 days with insulin (100 nM) and BRL49653 (5 μM). The cells were then incubated for an additional 8 days in the absence of inducers, after which adipogenesis was evaluated by oil red O staining and macroscopic (B) or microscopic (C) observation. Adipogenesis in control (noninfected) cells was similarly analyzed. Magnification in (C), 100×.

that of the wild-type protein (28). Another dominant negative mutant of RARα that contains a one-amino acid substitution (G303E) in the ligand binding domain also exhibits a marked decrease in ligand binding activity compared with wild-type RARα (29).

The likely mechanistic explanation for the dominant negative action of PPARγ2-ΔC is that the mutant protein forms stable heterodimers with RXR at PPREs of target genes, as suggested by our gel retardation assay data, but is unable to interact with specific ligands, as indicated by our ligand binding data. Thus, the interaction of PPARγ2-ΔC-RXR complexes with PPREs not only fails to activate transcription of the target genes, but also inhibits the binding of endogenous PPARγ to these response elements. Furthermore, the homologous cofactors p300 and CREB-binding protein (CBP), which are essential for the regulation of nuclear receptor transactivation activity, have been shown to associate with the COOH-terminal region of PPARγ2 in a ligand-dependent manner (37). Thus, it is also likely that PPARγ2-ΔC is unable to bind these cofactors.

Deletion of the NH_2-terminal domain of PPARγ was shown to activate the adipogenic activity of the protein when expressed in NIH 3T3 cells (14). Furthermore, phosphorylation of the serine residue at position 112 of mouse PPARγ2 by mitogen-activated protein (MAP) kinase inhibited PPARγ2-mediated adipogenesis (38). A missense mutation (P115Q) in human PPARγ2 (equivalent to P113Q in the mouse protein) is associated with marked obesity. Moreover, this mutation was shown to prevent phosphorylation of the serine residue at position 114 (S112 in the mouse protein) and to promote adipogenesis (39, 40). We have previously failed to detect mutations that affect the amino acid sequence of PPARγ2, including that of the COOH-terminal domain, in individuals with lipoatrophic diabetes, a condition characterized by the complete absence of adipose tissue, alterations in lipid metabolism, and extreme insulin resistance (41). To date, no disorders have been attributed to mutations of the COOH-terminal domain of PPARγ2.

PPARγ is expressed not only during adipogenesis but also in mature adipocytes, suggesting that it also plays an important role in maintenance of adipocyte characteristics. Therefore, PPARγ may have two different functions which depend on the differentiation of adipogenesis. It will be interesting to know whether PPARγ2-ΔC also acts as a dominant-negative mutant in mature adipocytes and inhibits several effects of thiazolidinediones, a new class of antidiabetic insulin-sensitizing drugs and ligands for PPARγ (42) in mature adipocytes. These data will be very important to understand how thiazolidinediones improve insulin sensitivity in a whole body.

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REFERENCES

1. Isseman, I., and Green, S. (1990) *Nature* **347,** 645–650.
2. Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M. (1994) *Genes Dev.* **8,** 1224–1234.
3. Saltiel, A. R., and Olefsky, J. M. (1996) *Diabetes* **45,** 1661–1669.
4. Gearing, K. L., Crickmore, A., and Gustafsson, J. A. (1994) *Biochem. Biophys. Res. Commun.* **199,** 255–263.
5. Schmidt, A., Endo, N., Rutledge, S. J., Vogel, R., Shinar, D., and Rodan, G. A. (1992) *Mol. Endocrinol.* **6,** 1634–1641.
6. Zhu, Y., Alvares, K., Huang, Q., Rao, M. S., and Reddy, J. K. (1993) *J. Biol. Chem.* **268,** 26817–26820.
7. Djouadi, F., Weinheimer, C. J., Saffitz, J. E., Pitchford, C., Bastin, J., Gonzalez, F. J., and Kelly, D. P. (1998) *J. Clin. Invest.* **102,** 1083–1091.
8. Motojima, K., Passilly, P., Peters, J. M., Gonzalez, F. J., and Latruffe, N. (1998) *J. Biol. Chem.* **273,** 16710–16714.
9. Amri, E. Z., Bonico, F., Ailhaud, G., Abumrad, N. A., and Grimaldi, P. A. (1995) *J. Biol. Chem.* **270,** 2367–2371.
10. Braissant, O., Foufelle, F., Scotto, C., Dauça, M., and Wahli, W. (1996) *Endocrinology* **137,** 354–366.
11. Chen, F., Law, S. W., and O'Malley, W. (1993) *Biochem. Biophys. Res. Commun.* **196,** 671–677.
12. Zhu, Y., Qi, C., Korenberg, J. R., Chen, X. N., Noya, D., Rao, M. S., and Reddy, J. K. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7921–7925.
13. Elbrecht, A., Chen, Y., Cullinan, C. A., Hayes, N., Leibowitz, M. D., Moller, D. E., and Berger, J. (1996) *Biochem. Biophys. Res. Commun.* **224,** 431–437.
14. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) *Cell* **79,** 1147–1156.
15. Schoonjans, K., Staels, B., and Auwerx, J. (1996) *Biochim. Biophys. Acta* **1302,** 93–109.
16. Graves, R. A., Tontonoz, P., and Spiegelman, B. M. (1992) *Mol. Cell. Biol.* **12,** 1202–1208.
17. Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A. M., Heyman, R. A., Briggs, M., Deeb, S., Staels, B., and Auwerx J. (1996) *EMBO J.* **15,** 5336–5348.
18. Tai, T.-A. C., Jennermann, C., Brown, K. K., Oliver, B. B., MacGinnitie, M. A., Wilkinson, W. O., Brown, H. R., Lehmann, J. M., Kliewer, S. A., Morris, D. C., and Graves, R. A. (1996) *J. Biol. Chem.* **271,** 29909–29914.
19. Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) *Cell* **83,** 803–812.
20. Kliewer, S. A., Sundseth, S. S., Jones, S. A., Brown, P. J., Wisely, G. B., Koble, C. S., Devchand, P., Wahli, W., Willson, T. M., Lenhard, J. M., and Lehmann, J. M. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 4318–4323.
21. Kliewer, S. A., Lenhard, J. M., Willson, T. M., Patel, I., Morris, D. C., and Lehmann, J. M. (1995) *Cell* **83,** 813–819.
22. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkinson, W. O., Willson, T. M., and Kliewer, S. A. (1995) *J. Biol. Chem.* **270,** 12953–12956.
23. Kanegae, Y., Lee, G., Tanaka, M., Nakai, M., Sakai, T., Sugano, S., and Saito, I. (1995) *Nucleic Acids Res.* **23,** 3816–3821.

24. Niwa, H., Yamamura, K., and Miyazaki, J. (1991) *Gene* **108,** 193–199.
25. Miyake, S., Makimura, M., Kanegae, Y., Harada, S., Sato, Y., Takamori, K., Tokuda, C., and Saito, I. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 1320–1324.
26. Kanegae, Y., Makimura, M., and Saito, I. (1994) *J. Med. Sci. Biol.* **47,** 157–166.
27. Takebe, Y., Seiki, M., Fukisawa, J., Hoy, P., Yokota, K., Arai, K., Yoshida, M., and Arai, N. (1988) *Mol. Cell. Biol.* **8,** 466–472.
28. Damm, K., Heyman, R. A., Umesono, K., and Evans, R. M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2989–2993.
29. Saitou, M., Narumiya, S., and Kakizuka, A. (1994) *J. Biol. Chem.* **269,** 19101–19107.
30. Ince, B. A., Zhuang, Y., Wrenn, C. K., Shapiro, D. J., and Katzenellenbogen, B. S. (1993) *J. Biol. Chem.* **268,** 14026–14032.
31. Damm, K., Thompson, C. C., Evans, R. M. (1989) *Nature* **339,** 593–597.
32. Mu–oz, A., Zenke, M., Gehring, U., Sap, J., Beug, H., and Vennström, B. (1988) *EMBO J.* **7,** 155–159.
33. Zenke, M., Mu–oz, A., Sap, J., Vennström, B., and Beug, H. (1990) *Cell* **61,** 1035–1049.
34. Zenke, M., Kahn, P., Disela, C., Vennström, B., Leutz, A., Keegan, K., Hayman, M. J., Choi, H. R., Yew, N., Engel, J. D., and Beug, H. (1988) *Cell* **52,** 107–119.
35. Umesono, K., Murakami, K. K., Thompson, C. C., and Evans, R. M. (1991) *Cell* **65,** 1255–1266.
36. Mangelsdorf, D. J., and Evans, R. M. (1995) *Cell* **83,** 841–850.
37. Gelman, L., Zhou, G., Fajas, L., Raspé, E., Fruchart, J.-C., and Auwerx, J. (1999) *J. Biol. Chem.* **274,** 7681–7688.
38. Hu, E., Kim, J. B., Sarraf, P., and Spiegelman, B. M. (1996) *Science* **274,** 2100–2103.
39. Ristow, M., Müller-Wieland, D., Pfeiffer, A., Krone, W., and Kahn, C. R. (1998) *N. Engl. J. Med.* **339,** 953–959.
40. Adams, M., Reginato, M. J., Shao, D., Lazar, M. A., and Chatterjee, V. K. (1997) *J. Biol. Chem.* **272,** 5128–5132.
41. Okazawa, H., Mori, H., Tamori, Y., Araki, S., Niki, T., Masugi, J., Kawanishi, T., Kubota, T., Shinoda, H., and Kasuga, M. (1997) *Diabetes* **46,** 1904–1906.
42. Saltiel, A. R., and Olefsky, J. M. (1996) *Diabetes* **45,** 1661–1669.