RESEARCH COMMUNICATION

# C/EBPα induces adipogenesis through PPARγ: a unified pathway

**Evan D. Rosen,[1,5] Chung-Hsin Hsu,[1,5] Xinzhong Wang,[2,6] Shuichi Sakai,[3,7] Mason W. Freeman,[2] Frank J. Gonzalez,[3] and Bruce M. Spiegelman[1,4,8]**

[1]Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA; [2]Lipid Unit, Massachusetts General Hospital, Boston, Massachusetts 02114, USA; [3]Laboratory of Metabolism, National Cancer Institute, Bethesda, Maryland 20892, USA; [4]Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA

**PPARγ and C/EBPα are critical transcription factors in adipogenesis, but the precise role of these proteins has been difficult to ascertain because they positively regulate each other's expression. Questions remain about whether these factors operate independently in separate, parallel pathways of differentiation, or whether a single pathway exists. PPARγ can promote adipogenesis in C/EBPα-deficient cells, but the converse has not been tested. We have created an immortalized line of fibroblasts lacking PPARγ, which we use to show that C/EBPα has no ability to promote adipogenesis in the absence of PPARγ. These results indicate that C/EBPα and PPARγ participate in a single pathway of fat cell development with PPARγ being the proximal effector of adipogenesis.**

Received September 27, 2001; revised version accepted November 9, 2001.

Adipogenesis is the process by which undifferentiated precursor cells differentiate into fat cells. This has become one of the most intensively studied developmental processes for at least two reasons: the increasing prevalence of obesity in our society has focused attention on many aspects of fat cell biology, and the availability of good cell culture models of adipocyte differentiation has permitted detailed studies not possible in other systems. Experiments using these in vitro models of adipogenesis, which include the 3T3-L1 and 3T3-F442A lines, have illustrated the transcriptional cascade that promotes fat cell differentiation (Rosen et al. 2000). Representatives of several transcription factor families have been implicated in this process, including the CCAAT/enhancer binding proteins C/EBPα, C/EBPβ, and C/EBPδ; the nuclear hormone receptor peroxisome proliferator-activated receptor γ (PPARγ); and the basic helix–loop–helix protein ADD1/SREBP1c. Studies in adipogenic cell lines have shown that hormonal induction of differentiation is rapidly followed by expression of C/EBPβ and C/EBPδ (Cao et al. 1991; Yeh et al. 1995). Within the next day or so, levels of these proteins peak and then begin to drift downward, coincident with a rise in C/EBPα and PPARγ. These latter factors induce gene expression changes characteristic of mature adipocytes and remain elevated for the life of the cell. In the present model of the transcriptional cascade leading to adipogenesis, C/EBPβ and C/EBPδ induce low levels of PPARγ and C/EBPα, which are then able to induce each other's expression in a positive feedback loop that promotes and maintains the differentiated state. This model is consistent with gain-of-function data showing that the addition of either PPARγ or C/EBPα can promote adipogenesis in fibroblast cell lines (Lin and Lane 1994; Tontonoz et al. 1994).

Loss-of-function studies have shown convincingly that PPARγ is required for adipogenesis in vivo and in vitro, and cells lacking PPARγ express greatly reduced levels of C/EBPα (Barak et al. 1999; Kubota et al. 1999; Rosen et al. 1999). Similarly, fibroblasts lacking *C/EBPα* have reduced adipogenic potential, and express reduced levels of PPARγ (Wu et al. 1999). Importantly, adding PPARγ back to *C/EBPα*$^{-/-}$ fibroblasts with a retroviral vector restores their capacity to accumulate lipid and activate markers of adipogenesis, including the endogenous *PPARγ* gene (Wu et al. 1999). These *C/EBPα*$^{-/-}$ adipocytes are normal in almost every way with the important exception that they do not show insulin sensitivity.

Importantly, because the converse manipulation has not been performed, it is not known whether the presence of exogenously applied C/EBPα is sufficient to promote adipogenesis in the absence of PPARγ. The reasons for this omission are largely technical, and relate to the fact that *PPARγ*$^{-/-}$ embryos die at embryonic day E9.5–E10, prior to the stage where the establishment of embryonic fibroblasts is generally considered feasible (Barak et al. 1999; Kubota et al. 1999). Additionally, the protocols for differentiating adipocytes directly from ES cells are cumbersome (Dani et al. 1997; Rosen et al. 1999) and are not amenable to retroviral expression of C/EBPα or other factors, as the LTRs of most available retroviruses are rapidly and irreversibly silenced after ES cell infection (Cherry et al. 2000).

It has therefore been difficult to delineate the relationship between C/EBPα and PPARγ in adipogenesis. Two competing models consistent with the available data are illustrated in Figure 1. In Figure 1A, a model is depicted in which PPARγ and C/EBPα induce each other's expression and can each act independently to promote fat cell differentiation. Figure 1B presents an alternative model in which PPARγ is the direct regulator of adipogenesis, whereas the major role of C/EBPα is centered on maintaining expression of PPARγ and promoting full insulin sensitivity.

To ascertain which model is more likely, we have created a fibroblast cell line that lacks PPARγ. We use these cells to show that the adipogenic action of C/EBPα is entirely dependent on PPARγ.

[*Key Words*: Adipogenesis; PPARγ; C/EBPα; PPARγ null fibroblasts]
**Present addresses: [5]Division of Endocrinology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215, USA; [6]Millennium Pharmaceuticals, 640 Memorial Drive, Cambridge, MA 02139, USA; [7]Fuji Gotemba Research Laboratories, Chugai Pharmaceutical Company, Shizuoka 412-8513, Japan.**
**[8]Corresponding author.**
**E-MAIL bruce_spiegelman@dfci.harvard.edu; FAX (617) 632-4655.**
Article and publication are at http://www.genesdev.org/cgi/doi/10.1101/gad.948702.

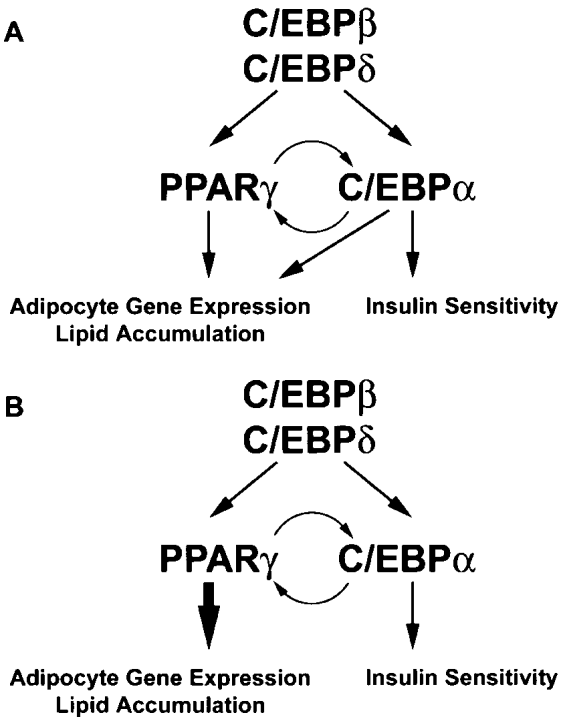


**Figure 1.** Two models of adipogenesis. Competing models are shown consistent with available data on the relative roles of PPARγ and C/EBPα in adipogenesis. See text for details.

## Results and Discussion

### *Generation of immortalized cell lines heterozygous or null for PPARγ*

Mice carrying floxed alleles of *PPARγ* were generated (data not shown). The *PPARγ* null allele was generated by crossing *PPARγ*-floxed mice with the EIIaCre transgenic mouse line as outlined previously (Hayhurst et al. 2001). Offspring that lacked exon 2 were selected by Southern blotting, and the null allele was maintained in the heterozygous state with the active *PPARγ*-floxed allele. Matings were established between mice that were heterozygous null at the *PPARγ* locus (+/–) and animals carrying a single allele of *PPARγ* with loxP sites flanking exon 2 (flox/–). At E12.5, embryos were harvested, minced, and trypsinized to generate mouse embryonic fibroblasts (MEFs). Embryonic heads were genotyped by PCR, and were found to represent the expected ratios of flox/+, flox/–, and +/– offspring (–/– embryos die at E9.5–E10.5, and are not seen by E12.5). MEFs from flox/+ and flox/– embryos were passaged repeatedly until they passed through crisis using the classic 3T3 protocol of Todaro and Green (1963). After immortalization, cells were expanded and infected with one of two different adenoviruses. One adenovirus expressed the Cre recombinase and green fluorescent protein (GFP) separated by an internal ribosome entry site (IRES) sequence, whereas the second virus expressed only GFP. After infection, cells were trypsinized and sorted by fluorescence detection; cells expressing GFP were collected, replated, and expanded. As shown in Figure 2B, Southern blotting showed that cells (flox/+ or flox/–) infected with the Cre-producing adenovirus completely lost their floxed alleles. This result was confirmed with genomic PCR (not shown), which showed the complete absence of a band associated with a residual floxed exon 2. Northern analysis of cells treated with conditions selected to induce adipogenesis (see Materials and Methods and below) shows the presence of PPARγ mRNA in –/– cells, but RT-PCR of this RNA shows that all of it is associated with loss of exon 2 (data not shown). Western blot analysis of similarly treated cells reveals no PPARγ protein (Fig. 2C); the expected translation product of PPARγ mRNA lacking exon 2 is ~10 kD and does not appear on Western blotting, perhaps because of protein instability. One distinct advantage of this approach is that the *PPARγ*$^{-/-}$ and flox/– cells were immortalized as a single line prior to exposure to Cre recombinase. This ensures that any biological changes associated with transformation are present in both flox/– and –/– cells, which in turn increases our confidence that any differences noted between these cell lines specifically reflects the presence or absence of PPARγ.

### *Ectopic PPARγ can stimulate adipogenesis in PPARγ*$^{-/-}$ *fibroblasts, but C/EBPα cannot*

Flox/– fibroblasts can be differentiated into adipocytes with low efficiency (<2%) in the presence of dexamethasone, methylisobutylxanthine, and insulin (DMI), in addition to the synthetic *PPARγ* agonist troglitazone. In contrast, however, the *PPARγ*$^{-/-}$ fibroblasts are not competent to undergo adipogenesis at all; we have never seen even a single fat cell develop in any experiment. This result is consistent with earlier observations made by our group and others showing that PPARγ is absolutely required for adipogenesis in vitro and in vivo (Barak et al. 1999; Kubota et al. 1999; Rosen et al. 1999).

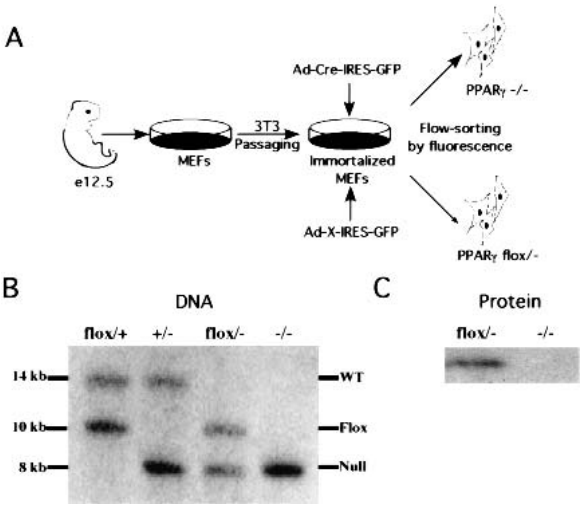


**Figure 2.** Generation of *PPARγ*$^{-/-}$ fibroblasts. (*A*) Scheme showing strategy used to make *PPARγ*$^{-/-}$ cells. PPARγ flox/– (or flox/+) embryos were harvested at day 12.5. Embryonic fibroblasts were plated and passaged repeatedly using a classic 3T3 protocol. After immortalization, cells were split into two aliqouts and infected with one of two different adenoviruses expressing Cre recombinase and GFP (Ad-Cre-IRES-GFP) or GFP alone (Ad-X-IRES-GFP). Cells were sorted by fluorescence to pick GFP-expressing cells. (*B*) Southern blot showing complete loss of floxed alleles in cells that received Ad-Cre-IRES-GFP, whereas floxed alleles were retained by cells that received Ad-X-IRES-GFP. (*C*) Immunoblot of PPARγ in PPARγ flox/– and –/– cells exposed to a prodifferentiative regimen.

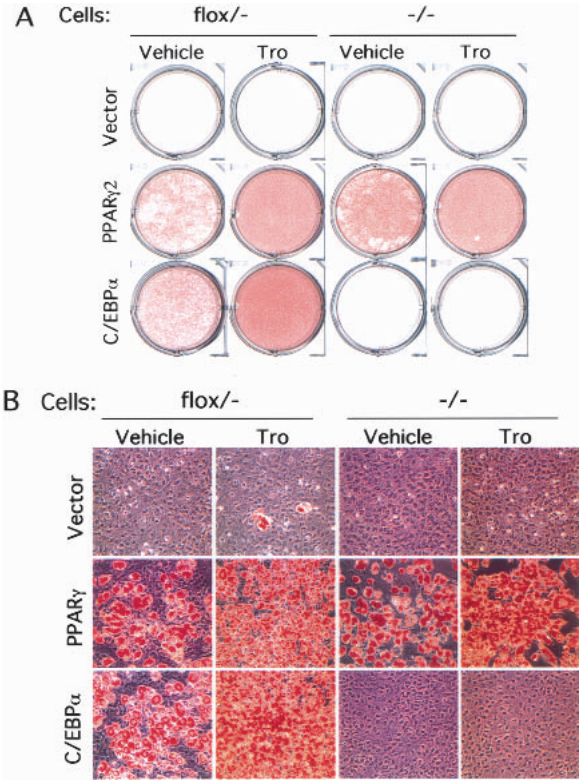


**Figure 3.** PPARγ restores adipogenesis in *PPARγ*$^{-/-}$ cells, but C/EBPα does not. (*A*) Dishes containing PPARγ flox/– or –/– cells were infected with retroviruses expressing PPARγ2, C/EBPα, or vector only. Cells were exposed to a prodifferentiative regimen with or without troglitazone, and stained with oil red O after 7 d. (*B*) Microscopic view of cells in *A*.

We next examined whether the block in differentiation seen in *PPARγ*$^{-/-}$ cells is specifically caused by the lack of PPARγ by infecting them with a PPARγ2-expressing retrovirus. Ectopic expression of PPARγ in flox/– cells resulted in a dramatic increase in adipogenic potential when DMI alone was used as the inducing cocktail. The effect was even more pronounced when troglitazone was added to the cells, suggesting that at some level of PPARγ expression, the amount of endogenous ligand for PPARγ becomes limiting. Importantly, retroviral introduction of PPARγ was able to rescue the adipogenic potential of *PPARγ*$^{-/-}$ cells, indicating that the lack of adipogenesis seen in vector-treated cells truly reflects the absence of this nuclear hormone receptor, and not some unanticipated disruption of the adipogenesis machinery at a site distinct from PPARγ.

To ascertain the adipogenic potential of C/EBPα in the absence of PPARγ, this transcription factor was added to the *PPARγ* flox/– and –/– cells using the same retroviral delivery system. C/EBPα enhanced the extent of adipogenesis in *PPARγ* flox/– cells, consistent with the gain-of-function effects seen in the past with this protein (Fig. 3A,B). C/EBPα, however, failed to induce any lipid accumulation whatsoever in the *PPARγ*$^{-/-}$ cells. This lack of adipogenic action is not caused by any impairment of C/EBPα expression in these cells, because immunoblot analysis reveals high levels of the 42-kD C/EBPα protein (Fig. 4A).

Gene expression analysis confirms the findings shown at the level of cell morphology and lipid accumulation, that is, that C/EBPα can support adipocyte-specific gene expression only when PPARγ is also present. The adipocyte-selective fatty-acid-binding protein aP2 is a direct target gene of PPARγ. Ectopic high-level expression of PPARγ is able to promote *aP2* expression at day 0, before fat cell differentiation has even begun (Fig. 4B). The lack of *aP2* expression in *PPARγ*$^{-/-}$ fibroblasts in the presence of C/EBPα reflects the lack of adipogenesis in these cells. A similar pattern is observed for adipsin, another fat-cell-specific protein induced during adipogenesis. Recent data indicate that C/EBPα is a major contributor to the expression of adipsin in fat cells (Chen et al. 2000); the fact that we do not detect adipsin message in *PPARγ*$^{-/-}$ cells even when high levels of C/EBPα are present indicates that other factors present in mature adipocytes must be required as well. PPARγ itself is unlikely to be the missing factor, in part because we do not see induction of adipsin message in PPARγ-expressing cells before the onset of adipogenesis (Fig. 4B), and because activation of PPARγ by thiazolidinedione drugs has actually been shown to decrease adipsin expression in mature fat cells. PPARγ also must be present in order for C/EBPα to maximally induce its own mRNA expression.

### *An enhanced model of the adipogenic transcriptional cascade*

Studies using NIH-3T3 fibroblasts have indicated that expression of either PPARγ or C/EBPα is sufficient to induce adipogenesis (Lin and Lane 1994; Tontonoz et al. 1994). Regardless of which factor is employed, the phenotype of the differentiated cells appears to be very simi-

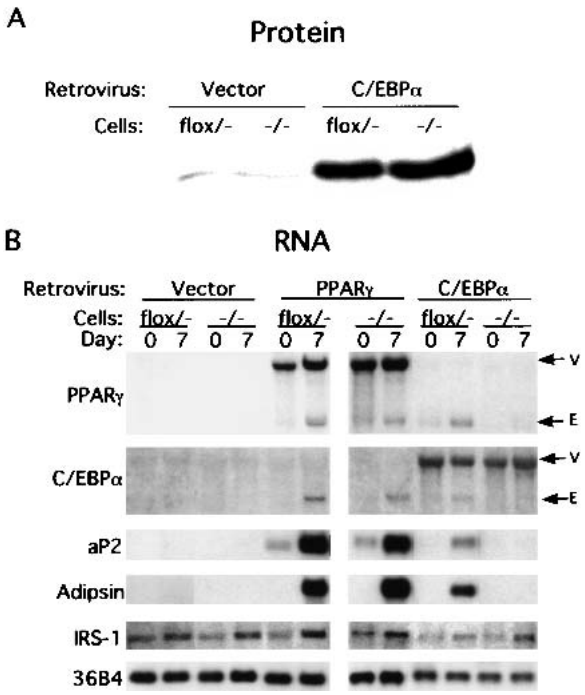


**Figure 4.** Expression of C/EBPα, PPARγ, and other adipocytic markers in PPARγ flox/– and –/– cells. (*A*) Immunoblot analysis of C/EBPα in PPARγ flox/– and –/– cell lines. (*B*) Northern analysis of multiple adipocytic markers in PPARγ flox/– and –/– cell lines. (V) Viral message, (E) endogenous message.

lar. The mechanistic interpretation of this result is difficult, however, because ectopic expression of either factor results in enhanced endogenous expression of the other. Fibroblasts that lack C/EBPα have been generated previously, and have been shown to be deficient in both *PPARγ* expression and adipogenic potential; when PPARγ is replaced, however, these cells are competent to undergo nearly all aspects of fat cell differentiation with the exception of insulin sensitivity (El-Jack et al. 1999; Wu et al. 1999). This result opened the possibility that PPARγ and C/EBPα are largely redundant in adipogenesis. In the present study, *PPARγ*$^{-/-}$ fibroblasts were generated and used to show that such is not the case.

In light of the data presented above, what role does C/EBPα play in adipogenesis? Clearly, C/EBPα is required for adipogenesis as shown by loss-of-function studies in vivo and in vitro. The fact that *C/EBPα* null fibroblasts undergo adipogenesis when PPARγ is replenished, however, strongly supports the argument that the role of C/EBPα in fat cell differentiation is limited to the induction and maintenance of PPARγ levels. Indeed, this is consistent with observations that C/EBPα can induce PPARγ2 expression by direct binding to specific sites in the *PPARγ* promoter (Elberg et al. 2000). Interestingly, recent studies on *C/EBPα* null mice indicate that the development of white adipose tissue (WAT), but not brown adipose tissue (BAT), is dependent on C/EBPα (Linhart et al. 2001); we would hypothesize based on our data that PPARγ levels in WAT are more dependent on C/EBPα than PPARγ levels in BAT.

In addition to maintaining PPARγ expression in developing adipocytes, C/EBPα is critical in the establishment of insulin sensitivity. This effect is mediated in part by direct transcriptional induction of insulin receptor and IRS-1 levels, and in part by a poorly characterized postreceptor mechanism (Wu et al. 1999). Additionally, other genes typical of the differentiated state (e.g., adipsin and leptin) are strongly promoted by the direct actions of C/EBPα (Chen et al 2000). C/EBPα, then, plays several important roles in adipocyte function, despite not being directly responsible for the process of adipogenesis per se.

Another possibility is that PPARγ may be permissive for C/EBPα action, perhaps directly through protein–protein interactions (although no evidence exists for such an interaction) or alternatively through induction of a cofactor critical for C/EBPα. We believe that the *PPARγ*$^{-/-}$ fibroblasts will be invaluable to screen for these and other factors that act downstream of PPARγ in adipogenesis; such studies are already underway.

## Materials and methods

*Generation of adenoviral constructs*

To create the Ad-Cre-IRES-GFP virus, the pLEP plasmid (Wang et al. 2000) was cleaved with *Hin*dIII and *Not*I, and a cDNA encoding Cre recombinase (kindly provided by M. Murakawa, MGH Nessel Gene Therapy Center) was inserted. A second fragment, encoding the internal ribosomal entry site sequences derived from the encephalomyocarditis virus and a codon optimized green fluorescent protein (kindly provided by E.C. Park, MGH Nessel Gene Therapy Center) was inserted at the *Not*I site (Fukumura et al. 1998). This plasmid and the plasmid pREP7 were both cleaved by PI-*Psp*I, ligated to each other using DNA ligase, and then packaged in phage packaging extracts (MaxPlax, Epicentre Technologies) as described (Wang et al. 2000). Cosmid DNA was isolated from *Escherichia coli* transduced with the packaged DNA and human 293 embryonic kidney cells were transfected with 10 μg of this DNA following cleavage by I-*Ceu*I. Virus propagation, purification, and plaque assay were performed using established adenoviral protocols (Graham and Prevec 1991).

*Generation of PPARγ flox/– and –/– cell lines*

PPARγ flox/– mice were mated to PPARγ +/– mice. At E12.5, embryos were harvested. Primary MEFs were generated by removing the heads of the embryos (saved for PCR genotyping), scratching out the viscera with a forceps, and trypsinizing the bodies after mincing. The resulting slurry was plated in 75-cm$^2$ flasks. Cells derived from flox/– and flox/+ embryos were passaged by plating $3 \times 10^5$ cells per 60-mm dish every 3 d as described (Todaro and Green 1963). After crisis and expansion, cells were split into two aliqouts and infected with adenovirus Ad-Cre-IRES-GFP or Ad-X-IRES-GFP at an m.o.i. of 100,000:1. Forty-eight hours after infection, cells were flow-sorted on a Cytomation Mo-Flo by exciting cells with a 488-nm laser and collecting at 530/480 bp. The most intensely GFP-expressing cells were replated, expanded, and frozen.

*Cell culture*

Cells were cultured in DME with 10% FBS at 10% $CO_2$. After retroviral infection and selection (see below), cells were allowed to grow to confluence in either 100-mm dishes or 6-well plates. Once confluence was reached, cells were exposed to a prodifferentiative regimen including dexamethasone (1 μM), insulin (5 μg/mL), and isobutylmethylxanthine (0.5 mM) with or without 10 μM troglitazone. After 2 d, cells were maintained in medium containing insulin until ready for harvest at day 7.

*Retroviral infections*

Retroviruses were constructed in pMSCV vectors (Clontech) using either puromycin or hygromycin selectable markers. Viral constructs were transfected into 293EBNA cells using FuGene (Roche) along with plasmids expressing gag-pol and the VSV-G protein. Supernatants were collected after 48 h, and either used immediately or frozen at –80°C for later use. Viral supernatants were added to PPARγ flox/– or –/– cells for 4 h; selection with puromycin (2 μg/mL) or hygromycin (175 μg/mL) was started 48 h later. Cells were selected, expanded, and studied immediately or frozen for later use.

*Northern and Western blots*

For Northern analysis, cells were grown to confluence and treated with a prodifferentiative regimen as noted above. Cells were lysed in Trizol and processed according to the manufacturer's instructions. For each sample, 10 μg of total RNA was loaded onto formaldehyde-agarose gels, transferred onto nylon membranes, and hybridized with the appropriate $^{32}$P-labeled probe in Ultrahyb (Ambion). Blots were stripped in boiling 0.1% SDS between different probes.

For Western analysis, cells were grown to confluence and treated with a prodifferentiative regimen as noted above. Lysis buffer (PBS with 1% Triton X-100 and complete protease inhibitor tablets; Boehringer Mannheim) was added, and the cells were triturated in Eppendorf tubes on ice. After shaking at 4°C for 10 min, lysates were spun at 14,000*g*, and supernatants were collected and snap-frozen in liquid nitrogen. Protein concentrations were determined with a BioRad assay, and 200 μg of total protein was TCA-precipitated and run on 10% SDS-PAGE. Transfer was performed using a semidry apparatus onto PVDF, and equal loading was ascertained by Ponceau staining. Membranes were blocked in 5% milk prior to incubation with primary antibody (PPARγ: E-8; C/EBPα: sc-61, Santa Cruz). After washing in TBST and exposing to secondary antibody, blots were developed with enhanced chemiluminescent reagent and exposed to film.

## Acknowledgments

We thank the Dana-Farber Flow Cytometry Core Facility, and Eric Smith for help with the figures. E.D.R. was supported by NIH DK02535. B.M.S. was supported by NIH R37DK31405.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

## References

Barak, Y., Nelson, M.C., Ong, E.S., Jones, Y.Z., Ruiz-Lozano, P., Chien, K.R., Koder, A., and Evans, R.M. 1999. PPAR gamma is required for

placental, cardiac, and adipose tissue development. *Mol. Cell* **4:** 585–595.

Cao, Z., Umek, R.M., and McKnight, S.L. 1991. Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. *Genes & Dev.* **5:** 1538–1552.

Chen, S.S., Chen, J.F., Johnson, P.F., Muppala, V., and Lee, Y.H. 2000. C/EBPβ, when expressed from the C/EBPα gene locus, can functionally replace C/EBPα in liver but not in adipose tissue. *Mol. Cell. Biol.* **20:** 7292–7299.

Cherry, S.R., Biniszkiewicz, D., van Parijs, L., Baltimore, D., and Jaenisch, R. 2000. Retroviral expression in embryonic stem cells and hematopoietic stem cells. *Mol. Cell. Biol.* **20:** 7419–7426.

Dani, C., Smith, A.G., Dessolin, S., Leroy, P., Staccini, L., Villageois, P., Darimont, C., and Ailhaud, G. 1997. Differentiation of embryonic stem cells into adipocytes in vitro. *J. Cell Sci.* **110:** 1279–1285.

Elberg, G., Gimble, J.M., and Tsai, S.Y. 2000. Modulation of the murine peroxisome proliferator-activated receptor γ 2 promoter activity by CCAAT/enhancer-binding proteins. *J. Biol. Chem.* **275:** 27815–27822.

El-Jack, A.K., Hamm, J.K., Pilch, P.F., and Farmer, S.R. 1999. Reconstitution of insulin-sensitive glucose transport in fibroblasts requires expression of both PPARγ and C/EBPα. *J. Biol. Chem.* **274:** 7946–7951.

Fukumura, D., Xavier, R., Sugiura, T., Chen, Y., Park, E.C., Lu, N., Selig, M., Nielsen, G., Taksir, T., Jain, R.K., et al. 1998. Tumor induction of VEGF promoter activity in stromal cells. *Cell* **94:** 715–725.

Graham, F.L. and Prevec, L. 1991. Manipulation of adenovirus vectors. In *Methods in molecular biology* (ed. E.J. Murray), pp. 58–71. Humana, Clifton, NJ.

Hayhurst, G.P., Lee, Y.-H., Lambert, G., Ward, J.M., and Gonzalez, F.J. 2001. Hepatocyte nuclear factor 4α (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. *Mol. Cell. Biol.* **21:** 1393–1403.

Kubota, N., Terauchi, Y., Miki, H., Tamemoto, H., Yamauchi, T., Komeda, K., Satoh, S., Nakano, R., Ishii, C., Sugiyama, et al. 1999. PPAR γ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol. Cell* **4:** 597–609.

Lin, F.T. and Lane, M.D. 1994. CCAAT/enhancer binding protein α is sufficient to initiate the 3T3-L1 adipocyte differentiation program. *Proc. Natl. Acad. Sci.* **91:** 8757–8761.

Linhart, H.G., Ishimura-Oka, K., DeMayo, F., Kibe, T., Repka, D., Poindexter, B., Bick, R.J., and Darlington, G.J. 2001. C/EBPα is required for differentiation of white, but not brown, adipose tissue. *Proc. Natl. Acad. Sci.* **98:** 12532–12537.

Rosen, E.D., Sarraf, P., Troy, A.E., Bradwin, G., Moore, K., Milstone, D.S., Spiegelman, B.M., and Mortensen, R.M. 1999. PPARγ is required for the differentiation of adipose tissue in vivo and in vitro. *Mol. Cell* **4:** 611–617.

Rosen, E.D., Walkey, C.J., Puigserver, P., and Spiegelman, B.M. 2000. Transcriptional regulation of adipogenesis. *Genes & Dev.* **14:** 1293–1307.

Todaro, G.J. and Green, H. 1963. Quantitative studies of mouse embryo cells in culture and their development into established lines. *J. Cell Biol.* **17:** 299–313.

Tontonoz, P., Hu, E., and Spiegelman, B.M. 1994. Stimulation of adipogenesis in fibroblasts by PPAR γ 2, a lipid-activated transcription factor. *Cell* **79:** 1147–1156.

Wang, X., Zeng, W., Murakawa, M., Freeman, M.W., and Seed, B. 2000. Episomal segregation of the adenovirus enhancer sequence by conditional genome rearrangement abrogates late viral gene expression. *J. Virol.* **74:** 11296–11303.

Wu, Z., Rosen, E.D., Brun, R., Hauser, S., Adelmant, G., Troy, A.E., McKeon, C., Darlington, G.J., and Spiegelman, B.M. 1999. Cross-regulation of C/EBP α and PPAR γ controls the transcriptional pathway of adipogenesis and insulin sensitivity. *Mol. Cell* **3:** 151–158.

Yeh, W.C., Cao, Z., Classon, M., and McKnight, S.L. 1995. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes & Dev.* **9:** 168–181.