THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 272, No. 32, Issue of August 8, pp. 20230–20235, 1997

Printed in U.S.A.

Ligand-independent Activation Domain in the N Terminus of Peroxisome Proliferator-activated Receptor γ (PPARγ)

DIFFERENTIAL ACTIVITY OF PPARγ1 AND -2 ISOFORMS AND INFLUENCE OF INSULIN*

(Received for publication, April 8, 1997, and in revised form, May 19, 1997)

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Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear hormone receptor superfamily, and is an important regulator of adipogenesis and adipocyte gene expression. PPARγ exists as two isoforms, PPARγ1 and PPARγ2, that differ only in their N termini. Both isoforms are activated by ligands that include the antidiabetic thiazoladinedione drugs and 15-deoxy-$\Delta^{12,14}$-prostaglandin J2, and potential differences in their function have yet to be described. We report that, in addition to a ligand-activated transcriptional activity, when studied under conditions of ligand depletion, intact PPARγ has a ligand-independent activation domain. To identify the basis for this ligand-independent activation, we used GAL4-PPARγ chimeric expression constructs and UAS-TK-LUC in CV1 cells and isolated rat adipocytes. In both cell systems, isolated PPARγ1 and PPARγ2 N termini have activation domains, and the activation function of PPARγ2 is 5–6-fold greater than that of PPARγ1. Insulin enhances the transcriptional effect mediated by both PPARγ1 and PPARγ2 N-terminal domains. These data demonstrate that 1) PPARγ has an N-terminal (ligand-independent) activation domain; 2) PPARγ1 and PPARγ2 N termini have distinct activation capacities; and 3) insulin can potentiate the activity of the N-terminal domain of PPARγ.

The peroxisome proliferator-activated receptor γ (PPARγ)[1] is a member of the nuclear receptor superfamily that plays a pivotal role in the molecular determination of adipogenesis and the regulation of adipocyte gene expression (1–5). Under appropriate conditions, expression of PPARγ through retroviral infection of fibroblastic cell lines is sufficient to cause differentiation along an adipocyte lineage, as assessed by expression of adipocyte-specific genes, accumulation of lipid, and acquisition of adipocyte morphology (6). Recently, it has been shown that 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (PG J2) is a high affinity ligand for PPARγ (7, 8) and that PPARγ is also the receptor for the thiazoladinedione class of insulin-sensitizing drugs (7, 8). PPARγ resembles other members of the nuclear receptor superfamily in that ligand-dependent receptor activation alters the rates of transcription of genes, specifically those that have peroxisome proliferator response elements (PPREs) within their promoters (*e.g.* aP2, phosphoenolpyruvate carboxykinase, and uncoupling protein) (9–11).

PPARγ exists as two isoforms, PPARγ1 and PPARγ2, that differ only in their N termini, with PPARγ2 having an additional 30 amino acids that are encoded by a single exon (9, 12). Expression of mRNA encoding the two isoforms is driven by alternative promoters within a single PPARγ gene (12), and their expression is differentially regulated in a tissue-specific manner. PPARγ2 is most abundantly expressed in adipocytes and is relatively specific for this tissue (9, 13). In contrast, while PPARγ1 is also expressed at a high level in adipocytes, it is also found at significant but lower levels in a number of other tissues, including muscle (13–15). Considering the relative abundance of PPARγ1 in many nonadipose tissues, it is likely that this isoform is capable of subserving roles apart from regulation of adipogenesis. In addition, although no functional differences between the γ1 and γ2 isoforms have been described to date, it is possible that these isoforms subserve different functions under some conditions. However, deletion of the N-terminal 129 amino acids of PPARγ did not diminish the adipogenic potency of PPARγ that was introduced into 3T3 fibroblasts by retroviral infection (6), and it has therefore been viewed as unlikely that the N terminus of PPARγ subserves a functionally important role.

Here, we provide evidence that PPARγ, in addition to being activated in a ligand-dependent manner, can also be activated in a ligand-independent manner, and we define a ligand-independent activation domain within the N terminus of PPARγ. We also demonstrate the first potential functional difference between the two PPARγ isoforms, wherein the N terminus of PPARγ2 more potently activates a heterologous promoter than does PPARγ1. We have mapped the overall activation domain to a region common to the two isoforms and demonstrate that the 30 amino acids unique to PPARγ2 can activate a heterologous promoter only in concert with the main N-terminal activation domain. Finally, we provide evidence that the ligand-independent activation function of the N terminus of PPARγ is augmented when cells are treated with insulin. We propose a model for the activation of PPARγ and discuss its possible implications.

MATERIALS AND METHODS

Plasmid Construction—The PPRE reporter construct consisted of two copies of the DR1 element upstream of the TK109 promoter in the vector pA_3Luc (16). All GAL4 constructs were constructed by inserting

* This work was supported by National Institutes of Health Grant DK 28082 (to J. S. F.) and Grants DK 02354 and 46200 (to A. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by the Paul Dudley White Fellowship Award from the American Heart Association, Massachusetts Affiliate.

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[1] The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; PCR, polymerase chain reaction; TK, thymidine kinase; DBD, DNA binding domain.

a PCR-generated fragment into the GAL4 vector (17). The γ1 N terminus, γ2 N terminus, each of the GAL4 constructs made to map the activation domain, and the PPARδ N terminus were constructed in a similar manner. 5′ primers were designed to contain an *Eco*RI (GAATTC) site after a random pentamer (CGCGG) to ameliorate restriction digestion. 3′ primers were designed to contain a stop codon before a *Pst*I (CTATAG) site and the same pentamer. The C-terminal-GAL4 construct was designed in a similar manner except both primers contained a *Bam*HI (GGATCC) site instead of *Eco*RI and *Pst*I. All restriction sites were in frame with the template, which kept an open reading frame from GAL4 through the entire PCR fragment. PCR was performed under the following conditions: after heating to 94 °C, buffer and enzyme (Takara) were added, and 30 PCR cycles were performed, each as follows: 94 °C for 30 s, 54 °C for 1 min, and 72 °C for 1.5 min. Each PCR was purified from the buffer and enzyme using Qiaquick (Qiagen) and digested with appropriate restriction enzymes, run on an agarose gel, purified, and ligated into the GAL4 vector, which has been previously linearized using the same enzymes, thus keeping the GAL4 DNA binding domain (DBD) in frame with the appropriate PCR fragment. UAS-TK-Luc contains five copies of the 17-base pair upstream activating sequence upstream of TK luciferase (14). The integrity of each construct was confirmed by restriction endonuclease digestion and dideoxy sequencing. All plasmids for transfection were prepared using column (Qiagen) purification. The murine PPARγ2 plasmid and the murine PPARδ plasmid, which were used as templates for PCR reactions, were a gift from B. M. Spiegelman.

Preparation and Transient Transfection of Rat Adipocytes—Adipocytes were isolated from male Sprague-Dawley rats (8–10 weeks) using a procedure previously described with modifications (18). Briefly, epididymal adipose tissue was minced and digested in Krebs Ringer buffer containing 1.5 mg/ml collagenase (Worthington) and 1% bovine serum albumin (Intergen) at 37 °C for 30 min. The adipocytes were strained through nylon mesh and washed three times with Krebs Ringer buffer containing 1% bovine serum albumin and three more times with Dulbecco's modified Eagle's medium containing 2 mM glutamine, 400 mM N^6-[*R*-(−)-1-methyl-2-phenyl]adenosine, 25 mM HEPES, 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.). After the final wash, cells were resuspended as a 50% solution in Dulbecco's modified Eagle's medium. Adipocytes were transfected by electroporation as described previously (19), with some modifications (electroporation at 400 V, 500 microfarads). We used 6 μg of UAS-TK-Luc and 1.5 μg of empty GAL4, PPARγ1 N terminus GAL4, or γ2 N terminus GAL4. The incubation was carried out at 37 °C in 5% CO_2. 2 h after electroporation, Dulbecco's modified Eagle's medium with 3.5% bovine serum albumin (final concentration) was added, and the indicated cells were treated with 10 nM insulin. The cells were harvested for luciferase activity 20 h after transfection.

Cell Culture and Transient Transfection—CV-1 cells were maintained in Dulbecco's modified Eagle's medium supplemented with L-glutamine, 10% fetal calf serum, 100 μg/ml penicillin, and 0.25 μg/ml streptomycin. Transient transfections were performed using the calcium phosphate technique in six-well plates, with each well receiving 1.60 μg of reporter and 80 ng of the γ1 N terminus, the γ2 N terminus, each of the indicated constructs containing a part of the N terminus, or empty GAL4 vector. 20 h after transfection, the cells were washed with phosphate-buffered saline and refed with Dulbecco's modified Eagle's medium with 10% fatty acid/growth factor-depleted fetal bovine serum and the indicated concentration of BRL49653. To deplete PPAR ligands and serum growth factors, fetal bovine serum was treated for 24 h at 4 °C with 5 mg/ml of activated charcoal (Sigma) and 30 mg/ml of anion exchange resin (type AGX-8, analytical grade, Bio-Rad). After centrifugation, anion exchange resin was added again for an additional 5 h. The resulting fetal bovine serum was centrifuged again and filtered before use. 44–48 h after transfection, the cells were harvested in extraction buffer and assayed for luciferase activity (20). All experiments were performed in triplicate and repeated between 2–6 times. The results shown are the mean ± the S.E.

Western Blot Analysis—CV-1 cells were passed to six-well dishes the day before transfection and transfected in duplicates as described above. Cells were harvested 72 h post-transfection by aspirating the medium, rinsing in ice-cold phosphate-buffered saline, and scraping into 250 μl of ice-cold lysis buffer (1% Nonidet P-40, 0.5% Triton X-100, 10% glycerol, 150 mM NaCl, 2 mM Na_3VO_4, 20 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 5 μg/ml aprotinin, 50 mM Tris-HCl, pH 7.4). The lysate was clarified by centrifugation at 23,000 × *g* for 15 min, and finally 125 μl of 3 × Laemmli buffer (21) was added to the supernatant.

Proteins were boiled for 5 min and subjected to SDS-polyacrylamide gel electrophoresis (21), followed by transfer of the resolved polypeptides to nitrocellulose membranes using the system of Towbin *et al.* (22). The membranes were blocked with 10% nonfat dried milk in Towbin buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% Tween 20) for 2 h at room temperature and then incubated with a polyclonal anti-yeast GAL4 DNA binding domain antibody (Upstate Biotechnology, Inc., Lake Placid, NY) (1:1000) in 5% milk overnight at 4 °C. After removal of unbound antibodies by three washes of 20 min in Towbin buffer at room temperature, membranes were incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin (1:1000) in 2.5% milk for 1.5 h at room temperature and washed five times in Towbin buffer. The targeted proteins were detected using enhanced chemiluminescence (ECL) as described by the manufacturer (Amersham International, Buckinghamshire, UK).

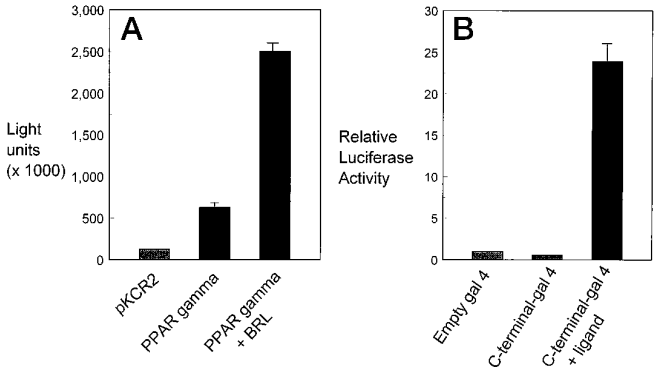

FIG. 1. **The activation domain of PPARγ may not be confined to its C terminus.** *A*, CV-1 cells were transfected with a cDNA encoding full-length PPARγ2 or the vector plasmid pKCR2 and cotransfected with PPRE-LUC. Cells were washed 24 h post-transfection and treated with double-stripped serum with or without ligand (BRL49653). 24 h later, cells were lysed and assayed for luciferase activity. *B*, CV-1 cells were transfected with a chimera containing the GAL4 DNA binding domain alone (empty GAL4), or the GAL4 DBD upstream of the PPARγ2 ligand binding domain (amino acids 193–505) with or without BRL, together with UAS-TK-LUC. Cells were treated in an identical manner to those in *A*. The data shown are the mean of a representative experiment performed in triplicate ± S.E. Very similar results were obtained in two additional experiments.

RESULTS

We first assessed the capacity of PPARγ to mediate transcriptional activation under conditions of varying availability of endogenous ligand. To do this, we transfected CV-1 cells with an expression plasmid for PPARγ2 and assayed its ability to activate a reporter plasmid consisting of a PPRE upstream of a thymidine kinase (TK) promoter and the luciferase reporter gene. In an attempt to limit the availability of PPARγ ligand, we charcoal-stripped the serum twice before use (Fig. 1*A*). Despite this treatment of the serum, PPARγ transactivated the reporter plasmid in the absence of added ligand. Activity was further enhanced by the addition of the thiazoladinedione BRL49653, which is known to bind to and activate PPARγ (7, 8). Since this basal activation could have been due to residual ligand in the serum or to ligand produced by the cells during the course of the experiment, we constructed a plasmid containing the yeast GAL4 DBD upstream of the isolated PPARγ ligand binding domain (amino acids 193–505). This construct was co-transfected with a reporter gene containing the yeast GAL4 response element upstream of the TK promoter and luciferase as a reporter (UAS-TK-LUC), under serum and cell conditions identical to those of the previous experiment (Fig. 1*B*). Although BRL was able to activate this construct very efficiently, indicating the intactness of the ligand binding domain, there was no activation in the absence of BRL, suggesting that the cellular level of endogenous ligand was extremely minimal under these conditions. The results additionally suggest that the high level of reporter activation mediated by the full-length PPARγ in the presence of the same stripped serum

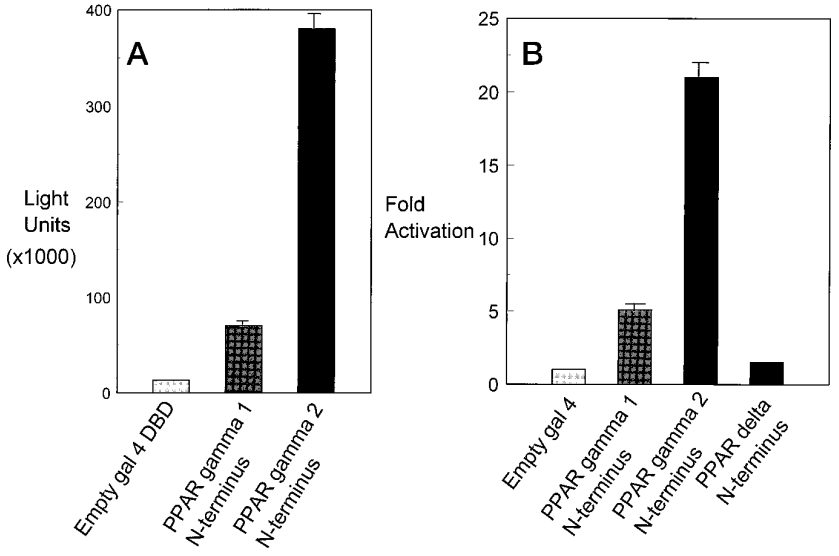

FIG. 2. **Evidence for an activation domain in PPARγ1 and -2 N termini.** *A*, CV-1 cells transfected with plasmids containing the GAL4 DNA binding domain, either alone or upstream of PPARγ1 and -2 N termini (amino acids 1–99 and 1–129, respectively), and co-transfected with UAS-TK-LUC. *B*, the same experiment as *A*, with the addition of PPARδ N terminus downstream of the GAL4 DNA binding domain. Both *A* and *B* represent mean light units or -fold activation of three experiments, each of which was performed in triplicate.

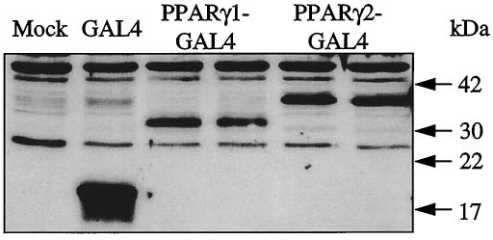

FIG. 3. **Expression of chimeric GAL4 DBD proteins as detected by Western blots.** Cells were transiently transfected with nothing (*Mock*), GAL4 DNA binding domain (*GAL4*), or PPARγ1 or -2 N termini downstream of the GAL4 DBD (the latter two in duplicate). Clarified lysates were subjected to 15% SDS-polyacrylamide gel electrophoresis and and then probed with an antibody against yeast GAL4 DBD.

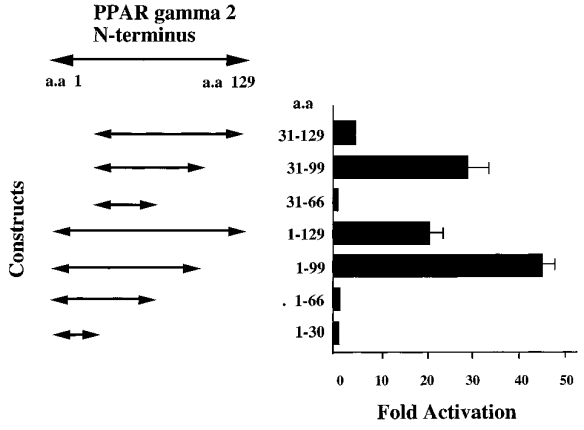

FIG. 4. **Analysis of PPARγ2 N-terminal activation domain.** CV-1 cells were transfected with a plasmid encoding the GAL4 DNA binding domain either alone or upstream of amino acids 1–30, 1–66, 1–129, 31–66, 31–99, or 31–129 of PPARγ2, respectively, and cotransfected with UAS-TK-LUC. Cells were treated as in Fig. 1. Data are expressed as the mean of fold activation over the luciferase activity obtained in cells transfected with the GAL4 DNA binding domain. The results are the mean of two experiments, each performed in triplicate.

and without any added ligand was indeed ligand-independent and did not map to the ligand binding domain. To further explore the possible existence of a ligand-independent activation domain in PPARγ, we constructed two separate plasmids containing sequence encoding the N termini of PPARγ1 or -2 (amino acids 1–98 and amino acids 1–128, respectively) downstream of GAL4's DNA binding domain. Upon cotransfecting each of these constructs with UAS-TK-LUC, reporter activation was seen with both N termini, with the activation by the PPARγ2 N terminus being about 5-fold greater than that of PPARγ1 (Fig. 2). In Western blotting experiments, using antibodies against the GAL4 DBD of the proteins, the expression levels of the two fusion proteins were the same (Fig. 3). PPARγ1 DBD and PPARγ2 DBD migrated with molecular masses of ~32 and ~36 kDa, respectively.

It is possible that the N-terminal 30 amino acids that are unique to PPARγ2 define a self-contained transactivation domain that might fully account for the difference between the activation potency of the two isoforms. A construct containing amino acids 1–30 of PPARγ2 downstream of the GAL4 DBD was therefore tested by co-transfection with UAS-TK-LUC. This construct failed to show any transactivation ability on its own (Fig. 4). We therefore further dissected the N terminus of PPARγ into several constructs, each containing a unique segment of the PPARγ N terminus in the same GAL4 fusion construct (Fig. 4). The activation domain maps to the region common to the two isoforms. Although the 30 N-terminal amino acids had no activation ability on their own, constructs lacking those 30 amino acids were consistently less active, suggesting a role for this region in the overall active conformation of the domain. Interestingly, amino acids 99–129 seemed to repress the ability of these constructs to activate transcription. Together, these results suggest a complex basis for the structural determinants of the PPARγ ligand-independent activation domain.

To test whether N-terminal activation ability was present in a structurally homologous molecule, we constructed a plasmid containing the A/B domain of PPARδ (amino acids 1–72) downstream of GAL4's DBD, and this construct was unable to activate transcription of the reporter gene (Fig. 2*B*). Since the extent of transcriptional activation mediated by the PPARγ N termini might be dependent on the presence of tissue-specific co-activators, we transfected the PPARγ2 N-terminal constructs together with UAS-TK-LUC into freshly isolated rat adipocytes (Fig. 5*A*). The PPARγ2 N terminus showed a greater fold activation in adipocytes, the cell most relevant for PPARγ2 function, than in CV-1 cells. To determine whether the ligand-independent transcriptional activation mediated by PPARγ2 is regulated by insulin, we added 10 nM insulin to the transfected cells (Fig. 5*B*). Insulin enhanced the ligand-independent activation mediated by both PPARγ1 and -2 constructs, while having little or no effect on the vector containing the GAL4 DBD alone.

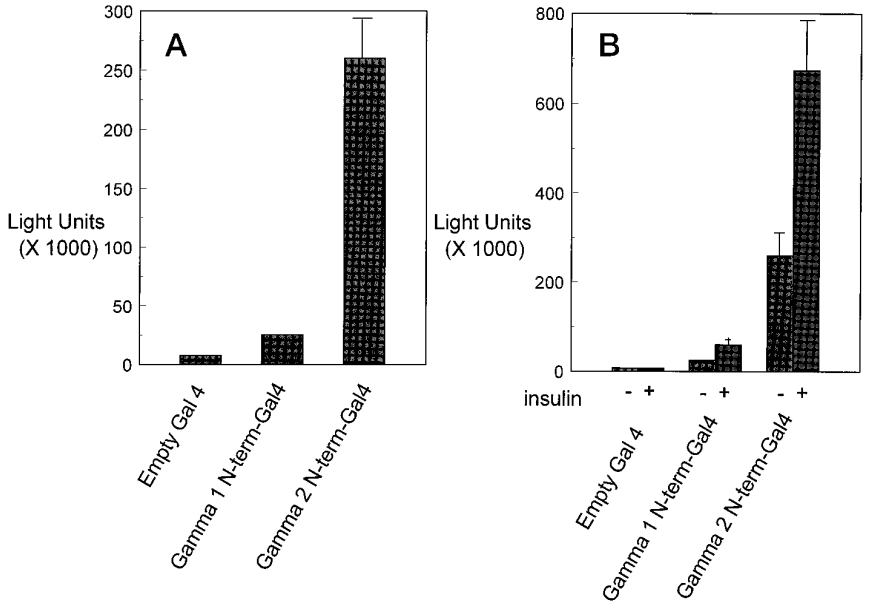

FIG. 5. **PPARγ N terminus activates transcription in primary rat adipocytes and this is enhanced by insulin.** *A*, isolated rat adipocytes were electroporated with a plasmid containing either the GAL4 DNA binding domain alone or GAL4 DBD upstream of the N-terminal amino acids of PPARγ1 or -2 (amino acids 1–99 and 1–129, respectively), and these were cotransfected with UAS-TK-LUC. *B*, 2 h after electroporation, some cells were treated with 10 nM insulin as indicated. Cells were harvested 20 h after transfection and assayed for luciferase activity. The data represent three separate experiments, each done in triplicate.

DISCUSSION

In this report we show that PPARγ resembles other members of the nuclear receptor superfamily in having the capacity to activate transcription in a ligand-independent manner. We have shown this in a number of ways. First, by co-transfecting intact PPARγ with a reporter plasmid under conditions designed to limit the availability of endogenous PPARγ ligand, we found that PPARγ activated transcription constitutively. To determine whether this activation was caused by residual endogenous ligand in the cells or media, and to identify a possible activation domain in the N terminus, we utilized the sensitive GAL4/UAS system. First, we created a construct that included PPARγ's C terminus with both the LBD and its activation domain, but without the DNA binding and N-terminal domains. By cotransfecting this plasmid with the reporter UAS-TK-LUC, we created a sensitive assay for the presence of PPARγ ligand. Using this paradigm, we showed that, under identical cell and serum conditions to those of the experiment with intact PPARγ, this construct showed no transactivation activity. However, the addition to these cell transfectants of the PPARγ ligand BRL49653 produced more than 50-fold activation. Taken together, these results suggest that the activation seen with intact PPARγ in the presence of charcoal-stripped serum and without added ligand is due neither to residual ligand nor to a constitutively active ligand binding domain.

To pursue this possibility further, we created chimeras containing the GAL4 DBD with PPARγ1 and -2 A/B domains, in the absence of their DNA binding, ligand-binding, and C-terminal activation domains. Using these constructs, it is apparent that the N termini of both PPARγ1 and -2 do indeed contain activation domains, as assessed by the ability of these constructs to activate transcription of a GAL4-responsive reporter gene. Thus, we have shown that PPARγ can enhance transcription in both a ligand-dependent and ligand-independent manner.

The potential importance of this ligand-independent activation is worthy of discussion. Many members of the nuclear receptor superfamily, including receptors for estrogen, progesterone, thyroid hormone, glucocorticoids, vitamin D_3, and retinoic acid contain two distinct domains responsible for activating transcription of specific genes (23). A critical activation domain is present in the receptor C terminus (the E domain), and this is activated upon binding of the cognate hormone/ligand to the adjacent ligand binding domain (reviewed in Ref. 24). In addition to this ligand-activated function, these receptors can also activate transcription independent of ligand binding, via an activation domain that resides within their N termini (the A/B domain). This domain can be constitutively active and/or be regulated independently of ligand via phosphorylation (25, 26). Ligand-independent activation (AF-1) domains have been shown to have several attributes. In some cases they act synergistically with the ligand-dependent activation domain, as with the human estrogen receptor in HeLa cells (27), where neither activation domain in isolation was capable of activating transcription significantly but both domains together produced marked activation. The ligand-independent activation domain may also be necessary for the regulation of complex cellular events, as in the case of glucocorticoid-induced apoptosis in lymphocytes (28). Transfection of glucocorticoid receptors in which the N terminus was deleted ablated the apoptosis caused by the intact receptor, while the same receptor remained responsive to a number of other dexamethasone effects to induce gene expression, suggesting that regulation of different genes may be mediated by distinct activation domains of the same receptor (29). In the case of the vitamin D_3 receptor, it has been shown that mutating serine 51 and thus preventing phosphorylation reduces activity (30), suggesting that phosphorylation/dephosphorylation may be involved in regulation of this activation domain.

These data also provide evidence that the two PPARγ isoforms may have differential abilities to activate target genes. To date, there have been no reports of functional differences between the PPARγ 1 and 2 isoforms, which have been described as having very similar capacities to be activated by known activators in co-transfection assays (14) and to be fully capable of bringing about the adipogenic program of differentiation in 3T3 cells under appropriate conditions. Here, we show that the N terminus of PPARγ2 is much more potent at activating a reporter independent of ligand than is the N terminus of PPARγ1. Interestingly, this difference between the two PPARγ isoforms is even more pronounced after transient transfection into isolated rat adipocytes, cells that normally express markedly higher levels of PPARγ2 than other tissues (1, 13). Whether this observation is due to higher expression in adipocytes of relevant but currently unknown co-activators is not known at this time. It is interesting to note that in adipocytes from both rodents (13) and humans (15), PPARγ2 is the isoform whose expression is more influenced by obesity and

nutritional perturbations, while the expression of PPARγ1 is relatively stable under these conditions *in vivo*. The preferential regulation of PPARγ2 expression in physiologic states such as starvation is likely to serve a physiologic purpose that would not be evident if the two isoforms had identical profiles of biologic activity.

Tontonoz *et al.* (6) have shown that deletion of amino acids 1–127 in the N terminus of PPARγ2 does not reduce, but actually increases, the capacity of retrovirally expressed PPARγ2 to induce adipocyte differentiation of NIH-3T3 cells, as assessed by lipid accumulation and expression of adipose-specific genes. This could be viewed as conflicting with our results by suggesting that the N terminus does not play a physiological role *in vivo*. This apparent conflict can be resolved in several ways, however. First, Tontonoz *et al.* (6) performed their studies under conditions of excess ligand, including ETYA, which was at the time the best PPARγ activator, and they used nonstripped serum, another potential source of PPARγ ligand. Thus, while their results demonstrate that under conditions of abundant ligand, the N terminus of PPARγ is not necessary for fat cell differentiation, they do not conflict with the possibility that the N-terminal activation domain of PPARγ plays an important role under distinctly different conditions, *i.e.* when ligand is not abundant. Studies of differentiation induced by isolated PPARγ N termini using the retroviral approach will be necessary to further resolve the ability of the PPARγ N terminus to bring about the full program of adipogenesis or perhaps reveal an adipogenesis-unrelated role.

Finally, we observed in both CV-1 cells and isolated rat adipocytes that insulin is capable of regulating the ligand-independent transcriptional activity of PPARγ through its N-terminal activation domain. To understand the possible mechanism for this finding, it is necessary to review recent observations on the regulation of PPARγ activity by covalent modification. Shalev *et al.* (31) demonstrated that PPARγ is a phosphoprotein and that phosphorylation is capable of enhancing its transcriptional activation potency, although mapping of the responsible sites was not carried out. Zhang *et al.* (32) showed that insulin treatment of cells enhanced the ability of full-length PPARγ2 to stimulate aP2 gene expression and speculated that this might be mediated by the mitogen-activated protein kinase-dependent phosphorylation of PPARγ that they also demonstrated. In contrast, Hu *et al.* (33) clearly demonstrated that mitogen-activated protein kinase-dependent phosphorylation of PPARγ2 took place on serine 112 in the N-terminal activation domain in response to mitogens, but this phosphorylation inhibited, rather than stimulated, the ability of full-length PPARγ2 to promote specific gene expression and the process of adipogenesis. Very recently, Adams *et al.* (34) reported that mutation of the consensus mitogen-activated protein kinase site (serine 82) of PPARγ1 to alanine resulted in increased ligand-dependent transcriptional activity. In addition, these authors also described a weak constitutive transcriptional activity of the isolated PPARγ1 N terminus, and this was also increased when serine 82 was mutated. Whether or not the activity of insulin to enhance transcription by intact PPARγ2 (32) or PPARγ2 N terminus as reported here is mediated by phosphorylation of PPARγ2, it is clear that signaling by PPARγ and insulin produce a number of common effects, including the ability to promote adipocyte differentiation (35, 36). Another indication of convergent pathways for these molecules is the fact that the insulin-sensitizing thiazoladinediones are now known to act by binding to and activating PPARγ (7, 8). The data presented here suggest a novel molecular basis for such a link, *i.e.* through convergence of their signaling pathways by an ability of insulin to enhance the function of the AF-1 activation domain of PPARγ, whether by direct phosphorylation or more likely through some other mechanism, such as an ability of insulin to modify a co-activator protein.

Interestingly, our mapping experiments of the N-terminal activation domain provide evidence for a complex mechanism by which PPARγ's N terminus can influence transactivation. Thus, the PPARγ2 GAL4-(1–128) construct was less active than the PPAR γ2 GAL4-(1–99), and the PPARγ2 GAL4-(1–66) construct was less active than the PPARγ2 GAL4-(31–99). Although such results could be the consequence of a number of factors, they could be consistent with the existence of an N-terminal repression moiety under some conditions.

Prior to these studies and the report of Adams *et al.* (34), a ligand-independent N-terminal activation domain had not been described in the PPAR gene family. Our results raise many questions about the functional roles of the ligand-independent and -dependent PPARγ activation domains under varying metabolic conditions that are likely to confront adipocytes and other cells that express these receptors. For example, it is possible that, under conditions of abundant PPARγ ligand, PPARγ would favor differentiation by causing growth arrest and transcription of adipocyte genes via the ligand-dependent AF-2 domain, whereas when ligand is limiting, as might be predicted to occur during starvation, PPARγ would act on promoters of genes needed for basal adipocyte homeostasis via the ligand-independent AF-1 domain. Such speculations on the possible functional domains of PPARγ will now need to be tested experimentally.

In summary, we have demonstrated that PPARγ, like many other members of the steroid receptor superfamily, contains a ligand-independent activation domain in its N terminus. We have further defined a functional difference between the two PPARγ isoforms; PPARγ2, which is expressed mainly in adipose tissue, activates ~5–10-fold more potently via its AF-1 activation domain than does PPARγ1. Lastly, we have shown that the N-terminal ligand-independent activation domain can be regulated by insulin, providing a new basis for the convergent signaling pathways of these molecules. Together, these results suggest a more complex regulation of PPARγ action than previously known and raise the possibility that differential regulation of PPARγ isoforms *in vivo* might account for different molecular activities and phenotypes.

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