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Degradation of the Peroxisome Proliferator-activated Receptor \( \gamma \) Is Linked to Ligand-dependent Activation*

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The nuclear hormone receptor peroxisome proliferator-activated receptor \( \gamma \) is a ligand-activated transcription factor that regulates several crucial biological processes such as adipogenesis, glucose homeostasis, and cell growth. It is also the functional receptor for a new class of insulin-sensitizing drugs, the thiazolidinediones, now widely used in the treatment of type 2 diabetes mellitus. Here we report that PPAR\( \gamma \) binds to specific lipids associated with the ability of ligands to activate this receptor. However, analyses of PPAR\( \gamma \) mutants show that, although degradation does not strictly depend on the transcriptional activity of the receptor, it is dependent upon the ligand-gated activation function 2 (AF2) domain. Proteasome inhibitors inhibited the down-regulation of PPAR\( \gamma \) and ligand activation enhanced the ubiquitination of this receptor. These data indicate that, although ligand binding and activation of the AF2 domain increase the transcriptional function of PPAR\( \gamma \), these same processes also induce ubiquitination and subsequent degradation of this receptor by the proteasome.

The peroxisome proliferator-activated receptor (PPAR) \( \gamma \), a member of the nuclear hormone receptor family of transcription factors, has recently been implicated in the regulation of a variety of biological processes. Two forms of PPAR\( \gamma \), PPAR\( \gamma 1 \) and PPAR\( \gamma 2 \), are generated by alternative splicing. PPAR\( \gamma 2 \) bears an additional 30 amino acids at the amino terminus and is predominantly expressed in adipose tissue. PPAR\( \gamma \) plays a central regulatory role in adipogenesis, where it acts in concert with members of the CAAT/enhancer-binding protein (C/EBP) family of transcription factors (reviewed in Ref. 1). The expression of PPAR\( \gamma \) mRNA and protein are induced early during adipogenesis. Many of the adipocyte-specific genes harbor PPAR\( \gamma \) binding sites in their promoters or upstream enhancer regions. Gain-of-function experiments have shown that PPAR\( \gamma \) is sufficient to induce adipogenesis, specifically, ectopic expression and activation of PPAR\( \gamma \) in fibroblasts efficiently induces an adipocytic phenotype (2). Recent genetic studies conclusively demonstrate that PPAR\( \gamma \) is also absolutely required for fat cell formation. Cells lacking both alleles for PPAR\( \gamma \) do not differentiate into adipocytes in vitro or in vivo; a PPAR\( \gamma \)-/- mouse lived only briefly after birth and lacked visible fat pads (3–5). The gene dosage of PPAR\( \gamma \) is apparently important, because differentiation of cells that contain only a single PPAR\( \gamma \) allele shows a phenotype intermediate to wild-type and null cells (3, 5).

PPAR\( \gamma \) has also been implicated in the regulation of systemic insulin sensitivity. This was first suggested when PPAR\( \gamma \) was found to be the functional receptor for a group of synthetic insulin-sensitizing agents, the thiazolidinediones (TZDs), which are currently used for the treatment of type 2 diabetes mellitus (6, 7). This role is now supported by the finding that certain mutations within PPAR\( \gamma \) are associated with severe insulin resistance and diabetes mellitus, even though these patients are not obese (8).

Although expressed at lower levels than in adipose cells, PPAR\( \gamma \) has also been implicated in the growth and/or differentiation of several cell types such as monocytes, breast, and colonic epithelium. The ability of PPAR\( \gamma \) to arrest growth in many of these cell types has suggested a possible connection to tumor biology (reviewed in Refs. 9, 10). Indeed, loss-of-function mutations of PPAR\( \gamma \) have been found in human colon cancers, suggesting a possible role as a tumor suppressor in this cell lineage (11). Recently, clinical data show that treatment of liposarcoma patients with TZDs induced tumor differentiation and a reduction in tumor cell growth (12).

PPAR\( \gamma \) is a ligand-activated transcription factor that binds to DR-1 sites as a heterodimeric complex with the retinoic X receptor (RXR). Synthetic ligands for PPAR\( \gamma \) are the aforementioned TZDs (e.g. troglitazone, pioglitazone, and rosiglitazone) and certain nonsteroidal anti-inflammatory drugs (6, 13). Natural ligands include 15-deoxy-\( \Delta 12,14 \)-prostaglandin \( \text{J}_2 \) (15d-PGJ2), certain polyunsaturated fatty acids such as linoleic acid, and endogenous constituents of oxidized low density lipoprotein particles such as 9- and 13-hydroxyoctadecadienoic acid and 15-hydroxyeicosatetraenoic acid (14–17). All of these natural compounds are low affinity ligands. So far, there is no consensus on the existence or the nature of a high affinity endogenous ligand.

To date, the major regulatory events identified in PPAR\( \gamma \)
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function are ligand binding, coactivator docking, and phospho-
ylation at an inhibitory serine (serine 112 of murine PPARγ) by
mitogen-activated protein (MAP) kinase (18–21). As for
most nuclear receptors, binding of agonist ligands induces a
conformational change in a conserved helix in the carboxyl
terminus of the ligand binding domain (22). This helix, also
known as the AF2 helix, is a docking site for a number of
coactivators that stimulate transcription via acetylation of core
histones or interaction with the basal transcription machinery
(reviewed in Ref. 23). PPARγ is also susceptible to negative
regulation via covalent modification. Phosphorylation of serine
112 in murine PPARγ2 by MAP kinases (Erk 1, Erk 2, and
stress-activated protein kinase/c-Jun amino-terminal kinase)
results in a strong suppression of PPARγ activity (18–21), at
least in part by influencing ligand binding (24).

We describe here a novel regulatory mechanism for PPARγ
via ligand binding: the induction of ubiquitin-dependent degrada-
tion of this receptor by the proteasome. Because ligands
induce both the transcriptional activation and the destruction
of the receptor, these results illustrate a feedback system for
balancing the transcriptional activity of PPARγ.

EXPERIMENTAL PROCEDURES

Chemicals—Pioglitazone was purchased from Upjohn, Wy14,643
from Chemsyn, and 15d-PGJ2 from Cayman Chemical. Troglitazone,
M2, and rosiglitazone were gifts from A. Sahlol (Parke-Davis Phar-
aceuticals). LG268 was a gift from R. Heyman (Ligand Pharmaceuticals).
The proteasome inhibitors MG132 and N-acetyl leucine nor-
leucine (ALLN) were obtained from Calbiochem, Calpain inhibitor II
from Roche Molecular Biochemicals. Serum for tissue culture was
purchased from Hyclone, and dexamethasone and insulin were obtained
from Sigma.

Adipocyte Differentiation and Ligand Treatment—3T3-F442A and
3T3-L1 cells were differentiated for 6–9 days as described previously
(25). Ligands and proteasome inhibitors were added at the concentra-
tion and for the times indicated in the figure legends. Degradation
occurred both in the presence (Dulbecco’s modified Eagle’s medium
(DMEM) + 10% fetal bovine serum) or the absence (DMEM + 2% bovine
serum albumin) of growth factors.

Transient Transfection Assays—Expression of all PPARγ alleles is
controlled by an SV-40 promoter. The wild-type PPARγ (2), PPARγ2-S112A (19), PPARγ2-ΔDNA (originally named
PPARγ2-M2) (2) have been described. PPARγ2-E499Q was cloned
d by polymerase chain reaction, thereby changing codon 499 from GAG to
GAG to CAG.

To analyze PPARγ degradation in NIH-3T3 cells, 60-mm dishes
(Figs. 2B, 3B–D, and 4A) or 100-mm dishes (Fig. 5) at 60–70% conflu-
ence were transiently transfected by mixing plasmid DNA (see figures)
with Superfect transfection (Qiagen) reagent for 3 h according to the
manufacturer’s instructions. Ligands were added 3 h after transfection
(Fig. 3, B–D), and whole cell lysates were prepared 15 h after treatment.
For Figs. 2B, 4A, and 5, ligands and inhibitors were added 24 h after
transfection for the time indicated in the figure legends.

To test for transcriptional activity of PPARγ, NIH-3T3 cells, grown in
24-well cell culture plates in DMEM + 10% bovine calf serum, were
transfected with pSV-Sport plasmids (500 ng each) encoding PPARγ,
and DR-1 Luciferase, and 100 ng of β-galactosidase plasmid utilizing
Superfect transfection reagent 3 h after transfection, cells were exposed
to ligands for 15 h, lysed, and assayed for luciferase and β-galactosidase
activity using a 96-well luminometer and spectrophotometer.

Whole Cell Lysates, Immunoprecipitations, and Western Blot Analy-
sis—Cells were washed once in phosphate-buffered saline (PBS) and
then lysed in PBS supplemented with 1% Triton X-100, 0.5% deoxy-
cholate, 10 mM sodium pyrophosphate, 2 mM sodium vanadate, 100 mM
sodium fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 100 μg/ml
phenylmethylsulfonyl fluoride (and 25 μg/ml ALLN for Figs. 4 and 5).
Concentration of the soluble proteins was determined by Bradford
assays (Bio-Rad), and for each sample the same amount of protein
(50–100 μg) was analyzed by SDS gel electrophoresis after trichloro-
acetic acid precipitation (19). Western blots were performed as de-
scribed previously (19). The antibody against PPARγ is described in
a previous study (19), antibodies against RXRα, C/EBPα, and C/EBP
response element binding protein (CREB) were obtained from Santa
Cruz. Supernatant of 9E10 hybridoma cultures was a gift from S.
Gaubatz.

Immunoprecipitations were performed with an anti-PPARγ antibody
diluted 1:100 in lysis buffer. After 4 h of incubation, 50 μl of a 50:50
slurry of protein A-Sepharose (Amersham Pharmacia Biotech) was
added, and the mixture was incubated for another 2 h. Immunoprecipi-
tations were then washed three times with PBS + 1% Nonidet P-40.
Immunocomplexes were analyzed by SDS-polyacrylamide gel electrophoresis and Western blot as described above.

RESULTS

PPARγ Protein Amount Is Decreased by Its Specific Li-
gands—To investigate the effect of PPARγ ligands on the level of
this receptor, differentiated adipocytes were treated with either vehicle or pioglitazone, a ligand of the TZD class. Whole
cell lysates were analyzed by Western blot analysis with an
anti-PPARγ antibody. PPARγ2 appears as a doublet in solvent-
treated cells; as shown earlier, the upper band is an inactive
form of PPARγ, phosphorylated by MAP kinases on serine 112
(19). The lower band represents the nonphosphorylated, active
form of this protein (Fig. 1A, lanes 1, 11, and 12). Interestingly,
treatment with pioglitazone resulted in a dramatic loss of
PPARγ protein, with the active, nonphosphorylated form of
this receptor lost preferentially (Fig. 1A, lanes 2 and 3). The
levels of two other proteins, RXRα, the heterodimerization
partner of PPARγ, and C/EBPβ did not change when the cells
were exposed to pioglitazone. This indicates that the decrease
in protein is selective or specific for PPARγ.

To analyze more critically whether the decrease of PPARγ
protein is related to specific ligand binding, differentiated adi-
pocytes were treated with several different TZDs (pioglitazone,
troglitazone, and rosiglitazone) and the natural ligand 15-
dPGJ2. As controls we also used M2, a urinary metabolite of
troglitazone that no longer binds to PPARγ, the PPARα-selec-
tive ligand Wy14,643, and the RXR-selective ligand LG268.
Treatment with the different TZDs and with 15dPGJ2 again
resulted in a loss of the nonphosphorylated, active form of
PPARγ (Fig. 1A, compare lanes 2–7, 13, and 15 with lanes 1, 11,
and 12). In contrast, treatment with the inactive troglitazone
metabolite M2 or Wy14,643 and LG268 did not down-regulate
PPARγ (Fig. 1A, lanes 8–10 and 14). Interestingly, LG268 did
decrease the amount of its own receptor, RXRα (Fig. 1A, com-
pare lane 10 with lanes 1–9 and 11). Thus, the decrease of the
PPARγ protein appears to be specific for PPARγ ligands.

The experiments described were performed with concentra-
tions of ligand (1 and 5 μM) that are above the Kd of most
of these ligands for PPARγ (40 nM for rosiglitazone to 3 μM for
15-dPGJ2). Therefore, a dose response was performed on dif-
ferentiated adipocytes to determine the relationship between
this reduced amount of protein and the affinity for a particular
ligand. As shown in Fig. 1B, an effect of pioglitazone on PPARγ
levels could be detected at 50 nM, whereas strong effects could
be seen at 500 nM (Fig. 1B, compare lanes 1 and 4), which is
close to its EC50 of 690 nM (26). Recently, Shao et al. (24)
proposed that the phosphorylated form of PPARγ binds to
ligand with lower affinity than the nonphosphorylated form.
In our experiments, ligand leads to the preferential loss of
the nonphosphorylated PPARγ. Thus, the loss of the receptor
encoded by PPARγ correlates well with ligand binding.

Decrease of PPARγ Is Regulated at the Protein Level—Rosen-
baum and Greenberg (27) and Camp et al. (28) recently re-
ported that PPARγ mRNA levels are decreased upon exposure
of cells to specific ligands; hence the loss of protein shown here
could be a consequence of mRNA metabolism. To address this,
we first performed time course studies of the amount of PPARγ

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protein and PPARγ mRNA in response to a PPARγ ligand. Total protein and RNA of differentiated adipocytes were prepared after 10 or 24 h of treatment with either pioglitazone or vehicle. Fig. 2A shows that, in agreement with the published reports, a 24-h treatment of adipocytes with a TZD downregulated PPARγ mRNA and both bands of PPARγ protein. However, 10 h of pioglitazone treatment left PPARγ mRNA largely intact. In contrast, most of the unphosphorylated form of the PPARγ protein has disappeared by this time, whereas the phosphorylated form was barely affected. These data strongly suggest that PPARγ ligands have an effect on PPARγ protein that is separate from effects at the mRNA levels.

To separate the effects of PPARγ ligands at transcriptional and post-translational levels more definitively, we expressed PPARγ ectopically with the SV-40 promoter, which is not subject to regulation by PPARγ ligands. Fibroblasts transiently transfected with the empty vector or with a PPARγ expression plasmid were treated for 24 h with pioglitazone and analyzed by Western and Northern blot. As can be seen in Fig. 2B (compare lanes 1 and 3), substantial PPARγ mRNA and protein could only be detected in cells receiving the expression vector for PPARγ. Northern blot analysis showed that the PPARγ mRNA is expressed at similar levels in the absence or presence of ligand (Fig. 2B, lanes 3 and 4). However, following the treatment with ligand, the amount of nonphosphorylated PPARγ protein was reduced, whereas the amount of phosphorylated PPARγ was largely unaffected (Fig. 2B, lanes 3 and 4), similar to what is described alone for endogenous PPARγ in adipocytes (see Fig. 1). Importantly, the protein levels of two other transcription factors, RXRα and C/EBPα, were not affected by the treatment (Fig. 2B). These data clearly demonstrate an effect of PPARγ ligands on PPARγ protein that is separable from effects at the mRNA level; in addition these results show that the decrease of PPARγ protein is not unique to adipocytes.

The AF2 Domain of PPARγ Is Important for Ligand-induced Down-regulation—To study the structural requirements for PPARγ down-regulation, we transfected wild-type PPARγ and various mutant alleles into NIH-3T3 cells (Fig. 3A). Although the experiments above indicate that the nonphosphorylated form of PPARγ is preferentially degraded, any conversion between phosphorylated and nonphosphorylated forms upon ligand binding would complicate these data. To clarify and simplify this issue, we used an allele of PPARγ with the serine 112 phosphorylation site converted to alanine (PPARγ2-S112A) (19). Transient transfections and subsequent treatment with pioglitazone revealed that both the wild-type PPARγ2 as well as PPARγ2-S112A were transcriptionally active under the conditions used (Fig. 3B). Both were also subject to down-regulation (Fig. 3C). Phosphorylation of PPARγ2 on serine 112 is therefore not required for its down-regulation.

Because ligand binding results in transcriptional activation, we next analyzed whether transcriptional activation of PPARγ is required for the ligand-dependent down-regulation. Two different point mutants were used to address this question: PPARγ2-DΔDNA, a double point mutant in the DNA binding region that can bind ligand but is unable to bind to DNA (2), and PPARγ2-E499Q, a mutant in the AF2 domain of the receptor. The latter also binds to PPARγ ligands with comparable affinity as the wild-type receptor (data not shown). Both of these mutants showed no ligand-dependent transcriptional activation (Fig. 3B). Upon ligand exposure, PPARγ2-DΔDNA was subject to normal ligand-dependent degradation, whereas the levels of PPARγ2-E499Q did not change (Fig. 3C). These data indicate that transcriptional activation of the receptor per se is not a prerequisite for ligand-induced down-regulation. However, an intact AF2 domain is required, suggesting that a ligand-dependent conformational change of the AF2 region and/or docking of cofactors to the AF2 region is important for the down-regulation.

Proteins known to associate with the AF2 region of nuclear hormone receptors are coactivators and some corepressors. We therefore investigated whether coexpression of a ligand-dependent coactivator (SRC-1) or corepressor (RIP140) would influence the degradation of PPARγ. Although most corepressors bind to nuclear hormone receptors in a nonligand-dependent way, RIP140 is unusual in that it is a corepressor whose association through the AF2-domain is stimulated by ligand binding. As shown in Fig. 3D, down-regulation in the presence of SRC-1 occurs rather normally. In contrast, RIP140 completely blocked degradation of PPARγ. These results illustrate that protein down-regulation is not linked simply to ligand binding but is closely associated with a transcriptionally active conformation of the AF2 domain of the receptor. Two different ways of interfering with the activity of the AF2 region but not ligand binding, mutation in the AF2-domain or binding of RIP140, prevent protein down-regulation.

PPARγ Is Degraded by the Proteasome—We next addressed the mechanisms by which PPARγ is degraded. Because the
ubiquitin-proteasome pathway regulates the stability of many proteins, including transcription factors (29), we analyzed whether proteasome inhibitors, ALLN and lactacystin, can block the down-regulation. NIH-3T3 cells expressing PPARγ2-S112A were treated for 15 h in the absence or presence of ligand with or without proteasome inhibitors. Because ALLN is known to also inhibit calpains, we also studied the effects of calpain inhibitor II, which does not affect proteasome-dependent degradation (30). As shown in Fig. 4A, ligand-induced down-regulation was largely inhibited by all three proteasome inhibitors but was not affected by calpain inhibitor II. In addition to the inhibition of the ligand-induced degradation, basal

FIG. 2. Ligands stimulate a reduction of PPARγ protein that is distinct from mRNA regulation. A, differentiated 3T3-F442A cells were treated for the times indicated with 5 μM pioglitazone or vehicle. Whole cell lysates were analyzed by Western blot with an antibody against PPARγ. Total RNA was prepared and analyzed in Northern blots with a probe against PPARγ. Ethidium bromide staining of the 28 S rRNA is shown as a loading control. B, NIH-3T3 cells were transiently transfected with either the empty vector or an expression vector for PPARγ2. On the following day cells were treated overnight with or without 5 μM pioglitazone. Whole cell lysates were analyzed in Western blots with antibodies against PPARγ, CREB, and RXRα. Total RNA was analyzed in Northern blots with a probe against PPARγ. Ethidium bromide staining of the 28 S rRNA is shown as a loading control.
levels of PPARγ are increased by the presence of the proteasome inhibitors for the long-term treatment. This suggests that the normal turnover of the receptor is also mediated by the proteasome.

To test the importance of the proteasome in ligand-induced degradation of PPARγ in adipocytes, differentiated 3T3-L1 cells were incubated with pioglitazone or vehicle following treatment with or without the proteasome inhibitor MG132. To minimize cell stress caused by the proteasome inhibitors in fat cells, we used a shorter treatment with proteasome inhibitors and PPARγ ligands at higher concentrations. As shown in Fig. 4B, PPARγ is effectively degraded under these conditions (Fig. 4B, lanes 1 and 2). Treatment with the proteasome inhibitor completely inhibited the down-regulation (Fig. 4B, lanes 3–6).

These experiments suggest that PPARγ is degraded by the proteasome upon ligand-mediated activation in both fibroblasts and fat cells.

**Ligand Enhances Ubiquitination of PPARγ**—Most proteasome substrates are ubiquitinated prior to their degradation and can be detected by the formation of high molecular weight complexes (for review see Ref. 31). To investigate whether PPARγ is ubiquitinated in response to ligand, we expressed either PPARγ, myc-tagged ubiquitin, or both in NIH-3T3 cells. The cells were treated with or without ligand in the absence or presence of ALLN, a proteasome inhibitor that has proven useful in preserving short-lived ubiquitin conjugates. Immunoprecipitations were performed with an antibody against PPARγ, followed by a Western blot analysis with the myc-tag specific antibody 9E10. In the absence of myc-tagged ubiquitin, no higher weight molecular complexes were observed (Fig. 5, lanes 1–4). Similarly, in the presence of both PPARγ and myc-tagged ubiquitin, but without proteasome inhibitor, almost no high molecular weight complex can be detected (Fig. 5, lanes 5 and 6). When the cells were treated with ALLN, a small amount of a high molecular weight ubiquitin complex could be observed without ligand treatment (Fig. 5, lanes 7). Following ligand addition, the amount of this complex was greatly increased (Fig. 5, lane 8). As expected, the overall levels of PPARγ detected by Western blots were unchanged in the presence of ALLN (data not shown). The formation of the higher molecular weight complex in response to ligand depends upon PPARγ, because it cannot be observed when no PPARγ was expressed (Fig. 5, lanes 9–12). We conclude that PPARγ is ubiquitinated prior to degradation by the proteasome, and that ubiquitination is increased by a PPARγ ligand.

**DISCUSSION**

Homeostasis in many physiological systems is maintained by the action of hormones, including those that bind to either cell surface or nuclear receptors. The precise balance of hormone actions requires both positive and negative controls. Negative modulation of signaling can occur through several mechanisms. In one case, receptor signaling is reduced through a covalent modification such as phosphorylation, as for example the phosphorylation of PPARγ on serine 112 (18–21). Downstream signaling can also be prevented by receptor destruction, which may occur after ligand binding via direct proteolytic degradation of a receptor; for cell surface receptors this often requires the internalization of the receptors bound to their ligands (32).

Nuclear hormone receptors are transcription factors whose activity can be induced by ligand binding. Many of these receptors have ligands with long half-lives. Several mechanisms have been proposed to attenuate or terminate the actions of hormones through this class of receptors. Chen et al. (33) recently proposed a possible mechanism for attenuation of the estrogen receptor (ER) function, involving signaling through the acetylation of the coactivator ACTR, which causes the disruption of this ER transcriptional complex. Another mechanism that has been reported recently is the reduction of the amount of nuclear hormone receptors through their ligand-dependent degradation by the proteasome. This has been shown for the ER, the retinoic acid receptor (RAR), the RXR, and the progesterone receptor (PR) (34–38). Generally, the structural requirements for the turnover of nuclear hormone receptors have not been well established. For RAR, however, it was shown that its degradation is dependent on the DNA binding function and certain amino acids in the AF2 region (35), suggesting that transcriptional activation of this receptor is required for its degradation.

We show here that PPARγ ligands induce the degradation of this receptor by the proteasome. This process is induced only by ligands that bind specifically to this receptor and in a concentration range that correlates well with ligand binding. Our experiments using proteasome inhibitors demonstrate that PPARγ is degraded by the proteasome upon ligand activation. Proteins targeted for proteasomal degradation are often modified by polymers of ubiquitin, which confer specificity for the degradation process (31). Indeed, PPARγ is ubiquitinated upon ligand binding prior to its degradation by the proteasome. In the current model of the ubiquitination process, attachment of
ubiquitin to lysine residues in the substrate is mediated by the serial action of three enzymes, the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2), and the ubiquitin-protein ligase (E3). There are many E2 and E3 enzymes, and a complex of E2 and E3 mediates substrate-specificity (31). PPARγ degradation might very well require a specific E2-E3 complex, because different E2 enzymes have already been shown to mediate the basal turnover of ER, PR, and thyroid hormone receptors (38).

A crucial question here is which features of PPARγ are recognized by the proteolytic machinery upon ligand binding. One possibility is that the signal for receptor degradation is the recruitment of PPARγ into transcriptionally activated complexes on target promoters, a process known to be stimulated by ligand binding. However, we can essentially rule out this possibility, because a PPARγ mutant defective in DNA binding is degraded as well as the wild-type receptor. This is in direct contrast to the proposed mechanisms for the degradation of RAR, which requires intact DNA binding capacity (35). We conclude, based on the data shown here, that transcriptional activity of PPARγ is not required for its degradation.

However, our results make it likely that a key requirement for PPARγ degradation is the ligand-induced conformational change associated with transcriptional activation. Crystal structures of ligand-bound nuclear receptors, including that of PPARγ, suggest that an important result of ligand binding is the translocation of the AF2 helix (22). It is this feature that is recognized by many coactivator and corepressor proteins, particularly those with LXXLL motifs. We show here that a point mutant of PPARγ in the AF2 helix that is transcriptionally inactive is not degraded upon ligand exposure. Furthermore, a corepressor protein, RIP140, which docks via the AF2 motif, blocks PPARγ degradation. However, a coactivator protein such as SRC-1 does not interfere with receptor destruction. Hence, it is tempting to speculate that a protein associated with the degradation machinery might also bind to the AF2 domain and compete with a corepressor like RIP140 for receptor docking. A coactivator might bind differently to the AF2 region and still allow the degradation machinery to make contact with the AF2 domain. Consistent with this is recent data illustrating that the contact of coactivators and corepressors in the AF2 domain are not identical (39). In an alternative model, the degradation apparatus might recognize a complex of coactivator and ligand-activated PPARγ. There is precedence for the involvement of a coactivator in the degradation of a transcription factor; the turnover of p53 requires the formation of a ternary complex between p53, the coactivator p300/CBP, and the ubiquitin ligase mdm2 (40). Clearly, more study is required to understand the role of the AF2 in the docking of a very large array of proteins that can potentially bind there in vivo.

The complex biology of PPARγ is only beginning to be understood. However, it is notable that ligands that activate this receptor are already clinically useful. Many patients with type 2 diabetes mellitus can achieve improved glucose homeostasis through the use of synthetic PPARγ ligands, and increasing clinical data suggest dramatic improvement in patients with polycystic ovarian syndrome, another disease involving insulin resistance (7). Most recently, differentiation of human tumors in patients with liposarcoma was achieved (12), raising the possibility that PPARγ ligands may also have utility in the cancer clinic. PPARγ ligands also modulate several important functions in monocytes, including cytokine generation, differentiation to macrophages, and lipid accumulation (9, 10). However, even in many clinical situations where benefits have been established, such as in type 2 diabetes, the effects of PPARγ ligands are still far from ideal. In particular, many patients do not achieve a large enough response to eliminate the use of insulin or other insulinotropics (7). Hence, an understanding of how to achieve a more robust response through the PPARγ response system holds particular interest and promise. A further understanding of the receptor destruction process induced by PPARγ ligands could eventually offer the possibility of therapeutic modulation of receptor number and improved responses through this system.

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