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Controlling a Master Switch of adipocyte development and insulin sensitivity: Covalent Modifications of PPAR γ

Z. Elizabeth Floyd and Jacqueline M. Stephens*

¹Ubiquitin Biology laboratory and the Adipocyte Biology laboratory at the Pennington Biomedical Research Center, Louisiana State University Systems, Baton Rouge, LA 70808

Abstract

Adipocytes are highly specialized cells that play a central role in lipid homeostasis and the maintenance of energy balance. Obesity, an excessive accumulation of adipose tissue, is a major risk factor for the development of Type 2 diabetes mellitus (T2DM), cardiovascular disease, and hypertension. A variety of studies suggest that obesity and T2DM can be linked to a breakdown in the regulatory mechanisms that control the expression and transcriptional activity of PPAR γ . PPAR γ is a nuclear hormone receptor that functions as a master switch in controlling adipocyte differentiation and development. Also important in controlling glucose homeostasis and insulin sensitivity, PPAR γ is a ligand-dependent transcription factor that is the functional receptor for the anti-diabetic thiazolidinediones (TZDs). In the last fifteen years, a variety of covalent modifications of PPAR γ activity have been identified and studied. These covalent modifications include phosphorylation, ubiquitylation, O-GlcNAcylation and SUMOylation. Covalent modifications of PPAR γ represent key regulatory mechanisms that control both PPAR γ protein stability and transcriptional activity. A variety of PPAR γ transgenic models, including mice heterozygous for PPAR γ , have demonstrated the importance of PPAR γ expression in glucose homeostasis and insulin resistance. In the following review, we have highlighted the regulation of PPAR γ by covalent modifications, the interplay between these interactions and how these post-translational modifications impact metabolic disease states.

Keywords

Adipocyte; PPAR γ ; Adipogenesis; Ubiquitylation; SUMOylation

1.1. Role of PPAR γ in disease

Our understanding of adipose tissue biology and the role of adipocytes in obesity-related diseases such as type 2 diabetes (T2DM) has benefited greatly from the discovery that the lipid-activated peroxisome proliferator-activated receptor gamma (PPAR γ , NR1C3) nuclear receptor is essential for adipocyte development [1–3]. Subsequent to its discovery as a critical adipogenic transcription factor, PPAR γ was identified as the pharmacological target of the insulin-sensitizing thiazolidinediones (TZD) that have been widely used to treat insulin resistance associated with T2DM [4, 5]. Since that time, various genetic studies

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*To whom correspondence should be addressed: Louisiana State University, Pennington Biomedical Research Center, Baton Rouge, LA 70808, Phone (225)-763-2648, FAX (225)-578-2597, jsteph1@lsu.edu.

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using animal models of tissue-specific PPAR γ deletions have shown that activation of PPAR γ in adipose tissue is central to the insulin-sensitizing effects of the TZDs. Mice lacking white adipose tissue [6] are not responsive to TZDs and deletion of PPAR γ in adipose tissue causes insulin resistance in adipose and liver, but not in skeletal muscle [7]. Inhibiting PPAR γ activity in mature adipocytes leads to insulin resistance associated with decreased expression of key genes required for insulin signaling in adipocytes, reduced uptake of free fatty acids by adipocytes and increased lipolysis [8, 9]. Gain-of-function experiments show that adipocyte-specific constitutive activation of PPAR γ in mature adipocytes can regulate whole body insulin sensitivity [10] without stimulating adipogenesis. Collectively, these observations indicate that PPAR γ activation is as important to lipid and glucose metabolism in fully formed adipocytes as it is for adipocyte development.

1.2. Regulation of PPAR γ activity by protein-protein interactions and ligand binding

PPAR γ is a ligand-activated transcription factor that is expressed in adipocytes as PPAR γ 2 and PPAR γ 1; the two forms differing by a thirty amino acid N-terminal extension found in PPAR γ 2 (refer to Figure 1). While PPAR γ 1 is expressed in multiple tissues [11], PPAR γ 2 is expressed primarily in adipocytes [12]. PPAR γ 2 is the more adipogenic PPAR γ isoform *in vitro* [13] and it is also the isoform transcriptionally regulated by nutrition [11]. PPAR γ has an overall domain structure typical of nuclear hormone receptors. This includes an N-terminal activation function-1 (AF-1) domain that functions as a ligand-independent activation domain and confers specificity of target gene activation [14]. PPAR γ also contains a DNA binding domain followed by a hinge domain and a large ligand binding domain that contains a short region at the C-terminus called the activation function 2 (AF-2) domain that is responsible for ligand-dependent activation [12] (refer to Figure 1). PPAR γ transcriptional activity is regulated at multiple levels. PPAR γ forms a heterodimeric complex with RXR α that is mediated by the PPAR γ ligand binding domain. Interaction with RXR α is required for PPAR γ to bind the PPAR γ responsive element of its target gene promoters. Once bound to DNA, PPAR γ activation can be modulated by a large number of coregulators classified as corepressors and coactivators whose association with PPAR γ is determined by ligand binding to PPAR γ . Corepressor proteins that regulate PPAR γ activity include the nuclear receptor corepressor NCoR-1 and its homolog SMRT (silencing mediator for retinoid and thyroid receptors) [15, 16] and the receptor interacting protein 140 (RIP 140) [17]. In recent studies of transgenic mice lacking NCoR in adipocytes, it was shown that deletion of this corepressor leads to increased adipogenesis, reduced inflammation, and enhanced systemic insulin sensitivity [18]. These observations are reminiscent of a TZD treatment and support other studies that show transcriptionally active PPAR γ promotes insulin sensitivity. In the absence of ligand, PPAR γ can be bound to target gene promoters while associated with the nuclear corepressors that recruit chromatin-modifying enzymes to regulate access to the DNA as has been shown in the glycerol kinase promoter [19]. Of note, the most active cis-regulatory elements bound by PPAR γ differ between mouse and man [20]. Also, studies in adipocytes and macrophages have demonstrated that PPAR γ DNA binding is predominantly cell type specific [21]. Upon ligand binding, the corepressor proteins are replaced by coactivating proteins that promote PPAR γ transcriptional activity. A major category of the coactivators is the p160 family of proteins that includes the cAMP response element binding protein (CBP)/p300 and Steroid Receptor Coactivators (SRC)-1,-2,-3, which recruit histone modifiers to the chromatin structure (reviewed in [22]). A second category of coactivators includes subunits of the mediator complex such as the PPAR-binding protein (PBP)/thyroid hormone receptor-associated protein (TRAP) 220/vitamin D receptor-associated protein (DRIP) 205 [23–25].

These coactivators interact with the general transcriptional machinery to control assembly of the transcription preinitiator complex [23]. TRAP220/DRIP205, originally cloned as a coactivator of the vitamin D receptor [24], interacts directly with PPAR γ [25]. An additional coactivator, PRIP (peroxisome proliferators-activated receptor gamma interacting protein) serves to link TRAP220/DRIP205 bound PPAR γ to the CBP/p300 coactivator [26]. These coregulators orchestrate the selective binding of PPAR γ to an enormous array of gene promoters.

Our understanding of the genes regulated by PPAR γ binding has greatly expanded from the set of genes initially identified as direct targets of this nuclear receptor. Earlier studies established that PPAR γ controls the expression of genes that are required for lipid synthesis, transport, and storage [1, 27–29] in adipocytes. Recent studies that take advantage of chromatin immunoprecipitation and genome-wide sequencing technology demonstrate the PPAR γ /RXR α heterodimer binds to more than 5,000 sites, directly activating the expression of a substantial number of genes involved in lipid and glucose metabolism during adipogenesis and in mature adipocytes [30–32]. In a majority of the binding sites, C/EBP α is also present [30, 32], in agreement with the accumulated evidence that PPAR γ and C/EBP α activities coordinate to form a fully functional insulin sensitive adipocyte. The overlap between PPAR γ and C/EBP α binding sites persists in mature adipocytes [32].

1.3. Regulation of PPAR γ by covalent modifications

As indicated above, PPAR γ can be associated with a large number of proteins. These important protein-protein interactions are non covalent, but these interactions control PPAR γ activity and can be significantly regulated by covalent modifications of PPAR γ . PPAR γ is known to be modified by phosphorylation, SUMOylation, O-GlcNAcylation, and ubiquitylation (refer to Figure 1). The multiple covalent modifications of PPAR γ increase the levels of control of both PPAR γ expression and transcriptional activity that contribute to its ability to modulate adipocyte development and insulin sensitivity.

1.3.1. Phosphorylation of PPAR γ

The most well described of these modifications is phosphorylation of PPAR γ . Growth factor activation of the mitogen activated protein kinases (MAPK) results in phosphorylation of PPAR γ 2 in the N-terminal AF-1 domain at serine¹¹² (Ser⁸² in PPAR γ 1) [33–37], the single MAPK consensus recognition site in PPAR γ . The first studies on PPAR γ phosphorylation were prompted by observations of the MAPK-inducing growth factors including epidermal growth factor (EGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) inhibited adipogenesis. Mutation of serine¹¹² to alanine abolishes both the growth factor stimulated phosphorylation of PPAR γ at serine¹¹² and the inhibitory effect of PDGF and EGF on PPAR γ transcriptional activity [35]. *In vitro* studies have demonstrated that the non-phosphorylated Ser¹¹²Ala mutant form of PPAR γ is more active than wild-type PPAR γ in the presence of ligand [33, 36, 37]. The MAPK-mediated phosphorylation of PPAR γ at serine¹¹² in the AF-1 domain functions to inhibit PPAR γ activity by regulating ligand binding in the C-terminal ligand binding domain of PPAR γ [38]. The physiological relevance of PPAR γ phosphorylation at serine¹¹² was demonstrated in mice homozygous for the Ser¹¹²Ala mutation of PPAR γ 2. Although diet-induced obesity is associated with insulin resistance, elimination of the phosphorylation site protected the Ser¹¹²Ala mice from diet-induced insulin resistance even though the mice were obese [39]. Preservation of insulin sensitivity in the obese Ser¹¹²Ala mice correlated with higher expression of PPAR γ target genes and increased serum levels of adiponectin, an insulin-sensitizing factor secreted exclusively by adipocytes. An additional PPAR γ phosphorylation site at serine²⁷³ in the PPAR γ ligand-binding domain that is regulated by Cdk5 [40, 41] may have important implications for the treatment of T2DM. Cdk5-dependent phosphorylation is

known to play a role in neurobiology and neurodegenerative diseases [42, 43], but this kinase is also expressed in non-neuronal tissue, including the insulin secreting pancreatic beta cells [44], and adipocytes [40]. Cdk5 activity can be regulated by TNF- α [45], a pro-inflammatory cytokine that is up-regulated in adipose tissue in conditions of obesity and insulin resistance [46]. The Cdk5-mediated phosphorylation of PPAR γ in adipose tissue has been linked to obesity [40]. Importantly, treatment with thiazolidinediones, the insulin sensitizing drugs that act as high affinity PPAR γ ligands, increases PPAR γ transcriptional activity and decreases cdk5-dependent phosphorylation of PPAR γ 2 at serine²⁷³. However, Cdk5 phosphorylation at serine²⁷³ of PPAR γ 2 does not regulate PPAR γ transcriptional activity in general, but does alter the expression of metabolically important target genes in adipocytes such as adiponectin. Moreover, PPAR γ ligands that are less potent agonists than the thiazolidinediones, but have insulin-sensitizing effects, also inhibit Cdk5-mediated phosphorylation in PPAR γ 2. Transgenic mice lacking the corepressor NCoR in adipocytes exhibit improvements in adipogenesis, reduced inflammation, and enhanced systemic insulin sensitivity and a notable decrease in CDK5-mediated PPAR γ ser²⁷³ phosphorylation [18]. These studies suggest that NCoR can function as an adaptor protein to enhance the ability of CDK5 to associate with and phosphorylate PPAR γ . Collectively, these studies suggest that phosphorylation of PPAR γ 2 at serine²⁷³ by Cdk5 is a key determinant of whole body insulin sensitivity and that new classes of anti-diabetic drugs may emerge that are based on regulating Cdk5-mediated phosphorylation of the PPAR γ ligand binding domain. One compound that fits these criteria has recently been described [41].

In addition to modulating PPAR γ transcriptional activity, MAPK signaling also affects PPAR γ by regulating the subcellular location of PPAR γ . These data demonstrate an interaction of MEK1 with the C-terminal AF2 domain of PPAR γ , yet this interaction does not directly alter phosphorylation of PPAR γ [47, 48]. However, there is evidence that cytoplasmic-nuclear shuttling of PPAR γ may be regulated by phosphorylation of PPAR γ in the AF-1 domain at serines 46 and 51 of PPAR γ 2 (Ser 16 and Ser 21 of PPAR γ 1) [49]. In this instance, phosphorylation of PPAR γ is mediated by casein kinase II (CK-II) and leads to cytoplasmic localization of PPAR γ and decreased PPAR γ transcriptional activity. These studies were largely performed in HEK 293 cells and in adipocytes the overall majority of PPAR γ is found in the nucleus. Hence, the physiological relevance of these observations is still unclear.

1.3.2. SUMOylation of PPAR γ

PPAR γ is reversibly modified by SUMO-1 (small ubiquitin-related modifier) at lysine¹⁰⁷ (lysine⁷⁷ in PPAR γ 1) and lysine³⁹⁵ (lysine³⁶⁵ in PPAR γ 1) [50–53]. First identified in 1995 [54] as a suppressor of a centromere-associated protein, SUMO-1 is an approximately 10 kD protein that regulates protein activity, stability, or cellular location. Although found in the cytoplasm and nucleus, SUMO-1 is best described as regulating a wide range of nuclear functions, including replication, transcription, DNA repair, and cytoplasmic-nuclear protein transport [55]. SUMOylation of PPAR γ in the N-terminal AF-1 domain (lysine¹⁰⁷) strongly represses PPAR γ transcriptional activity, suggesting SUMO-1 modification of PPAR γ at lysine¹⁰⁷ accounts for the repressive effect of amino acids 100–138 [56]. The potential for “crosstalk” between SUMO-1 modification at lysine¹⁰⁷ and phosphorylation at serine¹¹² within this region is supported by at least one study showing that SUMOylation of PPAR γ is impaired in the phosphorylation deficient form of PPAR γ 2 (Ser¹¹²Ala) [52], consistent with the repressive effect of serine¹¹² phosphorylation.

SUMOylation-mediated repression of PPAR γ transcriptional activity occurs even though only a small percentage of PPAR γ protein is modified by SUMO-1 at steady-state. This disproportionate effect of SUMOylation on PPAR γ activity is consistent with the effect of SUMOylation on other transcription factors and may be due to SUMO-dependent

recruitment of “downstream” cofactors that regulate PPAR γ activity. SUMOylation of PPAR γ in the AF-1 domain occurs within a sequence specific context found in many nuclear receptors that is termed a “synergy control” motif [57, 58]. At promoters containing multiple response elements, complex protein-protein interactions with transcription factors give rise to synergistic interactions that control gene expression patterns [59, 60]. The repressive activity of the synergy control motif is determined by SUMO-1 (or SUMO-2) modification of a lysine within the synergy control region and the SUMO-1 modification provides a surface for interactions with transcriptional repressors such as p300 [61] and the histone deacetylases [62]. Evidence of the effect of SUMOylation on the PPAR γ AF-1 domain synergy control motif awaits additional experiments to examine the interactions of PPAR γ when modified at lysine¹⁰⁷ by SUMO-1. However, ligand-dependent SUMOylation of PPAR γ 1 at lysine³⁶⁵ (lysine³⁹⁵ in PPAR γ 2) has been shown to promote the interaction of PPAR γ with nuclear receptor corepressor (NCoR)-histone deacetylase-3 (HDAC3) complexes in macrophages [53]. This SUMO-1 dependent interaction prevents removal of the NCoR-HDAC3 complexes by proteasomal degradation and maintains repression of PPAR γ activity. The physiological relevance of PPAR γ SUMOylation has been observed in FGF21 null mice that have alterations in PPAR γ signaling. Mice lacking FGF21 are lipodystrophic and have less body fat and decreased expression of PPAR γ target genes [63]. These transgenic mice also have increased PPAR γ SUMOylation and FGF21 is capable of inhibiting PPAR SUMOylation at lysine¹⁰⁷. Collectively, these observations suggest that hormones including FGF21 modulate PPAR γ transcriptional activity by regulating SUMOylation at lysine¹⁰⁷ and contribute to whole body insulin sensitivity. To date, no studies have examined the SUMOylation of the PPAR γ 2 ligand-binding domain in adipocytes. Hopefully, future studies will determine if this modification occurs in adipocytes and if there is any “cross-talk” between SUMO-1 modification at lysine³⁹⁵ and Cdk5-dependent phosphorylation at serine²⁷³.

1.3.3. Ubiquitylation of PPAR γ

SUMO-1 is structurally related to ubiquitin, an 8.5 kD polypeptide that regulates myriad cellular functions ranging from receptor-mediated endocytosis to regulation of transcription when covalently attached to targeted proteins [64]. Proteins modified by multiple ubiquitin polypeptides can be targeted to a number of possible fates, including proteasomal degradation, the best described endpoint for a polyubiquitylated protein. Covalent modification of proteins by ubiquitin occurs via a highly regulated cascade of enzymes that catalyze the activation of ubiquitin followed by the transfer of ubiquitin to the targeted protein. The process of activating and transferring ubiquitin to a substrate is repeated multiple times to form the polyubiquitin chains that are recognized by the proteasome.

PPAR γ proteins have a short half-life in adipocytes [65] and the turnover rate of PPAR γ proteins is regulated by the ubiquitin-proteasome system under ligand-independent and ligand-dependent conditions [66, 67]. Ligand binding increases ubiquitin modification of PPAR γ and proteasomal degradation of PPAR γ and *in vitro* assays indicate PPAR γ is modified by ubiquitin in the ligand binding domain, although the exact site of ubiquitylation has not been identified [66, 68]. Although ubiquitylation occurs in the absence of the AF2 domain, the AF2 domain is required for maximal ubiquitin modification of the PPAR γ ligand binding domain [68]. This suggests ubiquitylation of PPAR γ may precede coactivator binding of the AF-2 domain and that ubiquitin modification of PPAR γ functions as an integral part of PPAR γ transcriptional activation. The link between ubiquitin modification of PPAR γ and activation of PPAR γ is further supported by the observations that inhibiting proteasome activity increases PPAR γ activity and ubiquitylation in general is required for PPAR γ activity in adipocytes [50, 68].

The simplest interpretation of the studies on PPAR γ ubiquitylation is that ubiquitin modification of PPAR γ regulates PPAR γ activity by controlling the abundance of PPAR γ proteins. Regulation of PPAR γ protein steady state levels by promoting ubiquitin modification of PPAR γ may represent an important aspect of the action of the insulin sensitizing thiazolidinediones. Genetic studies using mouse models of insulin resistance show decreased PPAR γ gene expression is as effective as thiazolidinedione activation of PPAR γ in improving insulin sensitivity [69–71]. The decreased PPAR γ gene expression is associated with lower steady state levels of PPAR γ protein in adipose tissue [72], lending substantial support to the possibility that modification of PPAR γ by the ubiquitin-proteasome in adipocytes is an important regulation of systemic insulin sensitivity that can be exploited in the treatment of type 2 diabetes.

1.3.4. O-GlcNAcylation of PPAR γ

A type of glycosylation that occurs on both cytosol and the nuclear proteins is the β -O-linked N-acetylglucosamine (O-GlcNAc) post-translational modification. Recent mutational analysis and mass spectrometry studies have revealed that the threonine⁵⁴ in the AF-1 domain of PPAR γ 1 (threonine⁸⁴ in PPAR γ 2) is a major O-GlcNAc site [73]. The transcriptional activity of wild type PPAR γ was inhibited in the presence of an inhibitor of this modification, whereas the threonine mutant (Thr⁵⁴Ala) was unresponsive to inhibitor treatment. Although these studies were conducted *in vitro* in cultured 3T3-L1 adipocytes, the results clearly show that O-GlcNAcylation of PPAR γ functions to reduce PPAR γ transcriptional activity.

1.3.5. Potential interplay of PPAR γ post-translational modifications

The phosphorylation and SUMOylation sites in the AF-1 domain of PPAR γ are contained within a highly conserved motif (ψ KxExxSP, where ψ is a hydrophobic residue; in PPAR γ the sequence is IK¹⁰⁷ VEPAS¹¹²P) that is considered to be a phosphorylation-dependent SUMOylation motif. Many of the proteins containing this motif are transcription factors, including the nuclear receptors [74]. In PPAR γ , phosphorylation and SUMOylation in the AF-1 domain function to repress transcriptional activity. Although phosphorylation within the “phospho-SUMO switch” in other nuclear receptors acts to block SUMOylation of the AF-1 domain [75], the evidence that SUMOylation is impaired in the phosphorylation-deficient form of PPAR γ [52] supports the possibility that phosphorylation of serine¹¹² regulates SUMOylation of lysine 107 to repress PPAR γ activity. In addition, phosphorylation of serine¹¹² may affect ubiquitylation of PPAR γ since inhibition of MAPK signaling alters the stability of PPAR γ and the level of PPAR γ ubiquitylation [66, 67]. This may also be the case for SUMOylation. While SUMOylation of lysine¹⁰⁷ can occur in the absence of ubiquitylation, inhibiting SUMO-1 modification of lysine¹⁰⁷ increases proteasomal degradation of PPAR γ [50], suggesting SUMOylation regulates ubiquitin modification of the PPAR γ ligand-binding domain. Stabilization of PPAR γ protein via SUMO-1 modification is consistent with studies that indicate SUMOylation and ubiquitylation have opposing functions, but the interplay between SUMOylation and ubiquitylation in PPAR γ may be more complex and involve cooperative as well as antagonistic functions [76].

Phosphorylation, SUMOylation, and ubiquitylation work in concert with acetylation in many transcription factors, including several of the nuclear receptors [77]. PPAR γ contains twenty potential acetylation sites [78], although no sites in PPAR γ have been definitively identified as modified by acetylation. However, the possibility of acetylation accompanying phosphorylation, SUMOylation, and ubiquitylation in regulating PPAR γ activity is intriguing given that four of the potential sites for acetylation overlap or are in close proximity to SUMOylation and phosphorylation sites in the AF-1 (at lysines 107 and 119 of

PPAR γ 2) and ligand binding domain (at lysine 268, 272 and 382). Although speculative, it seems highly likely that acetylation will participate with other covalent modifications in regulating the transcriptional activity of PPAR γ .

1.4. The role of PPAR γ post-translational modifications in metabolic disease

It appears that all of the PPAR γ covalent modifications identified to date including phosphorylation, SUMOylation, O-GlcNAcylation and ubiquitylation largely function to decrease PPAR γ transcriptional activity and/or expression levels (see Figure 2). For over a decade, we have known that PPAR γ has been implicated in the regulation of systemic insulin sensitivity. This was first demonstrated when PPAR γ was shown to be the functional receptor for the synthetic anti-diabetic thiazolidinediones (5). Moreover, direct evidence from genetic studies in humans has revealed that mutations in the ligand-binding domain of PPAR γ are associated with severe insulin resistance and T2DM [79]. To date, none of the naturally occurring genetic mutations in humans have been shown to occur at the exact residues in PPAR γ where covalent modifications are known to occur. Nonetheless, these post-translational modifications have important effects on PPAR γ activity and expression levels. However, a well documented missense mutation in the PPAR γ 2 genes results in the conversion of proline to glutamine at position 115 and some studies indicate this polymorphism is associated with obesity [80, 81]. Ectopic expression of this mutant PPAR γ results in decreased serine phosphorylation and increased PPAR γ transcriptional activity [80]. Collectively, these studies revealed that the Pro115Gln mutation in PPAR2 enhanced the differentiation of adipocytes and this phenotype was likely a result of decreased serine phosphorylation.

As reviewed above and summarized in Table 1, there are a large number of studies demonstrating how covalent modification of PPAR γ plays a role in modulating its activity and expression levels. In the last year, two new PPAR γ covalent modifications have been discovered including O-GlcNAcylation and CDK5 mediated serine phosphorylation. Also, FGF21 has recently been identified as a potent modulator of PPAR γ SUMOylation [63]. It will be interesting to learn whether other insulin sensitizers act by modulating PPAR γ covalent modifications. Transgenic models of PPAR γ modulation have shown that the activity and expression of PPAR γ plays a role in both adipocyte development and insulin sensitivity. Yet, few studies on covalent modification of PPAR γ have been performed in the whole animal setting. To date, the best evidence to support that covalent modifications of PPAR γ may contribute to metabolic disease states comes from the Lazar laboratory. In these studies, mice containing a PPAR γ 2 gene that cannot be phosphorylated on serine¹¹² developed obesity following high fat feeding but were partially protected from diet induced insulin resistance [34]. Hopefully, future studies will reveal equally important roles for other post translational modification of PPAR γ under physiological and/or pathological conditions. The ability to modulate PPAR γ activity and improve insulin sensitivity by specifically inhibiting a covalent modification of PPAR γ may represent a viable therapeutic option in the treatment of insulin resistance and T2DM.

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Highlights

- PPAR γ is important in fat cell development
- PPAR γ plays a role in glucose homeostasis and an anti-diabetic drug target.
- The activity of PPAR γ is largely effected by covalent modifications..
- The activity of PPAR γ is highly regulated by phosphorylation.
- The activity of PPAR γ is highly regulated by SUMOylation
- The activity of PPAR γ is highly regulated by ubiquitin modification.

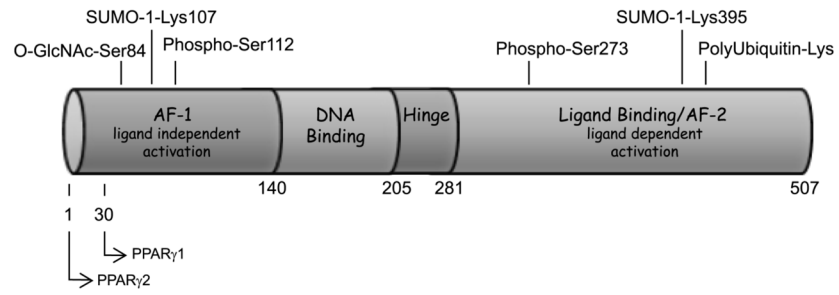


Figure 1.
Schematic of PPAR γ 2 covalent modifications

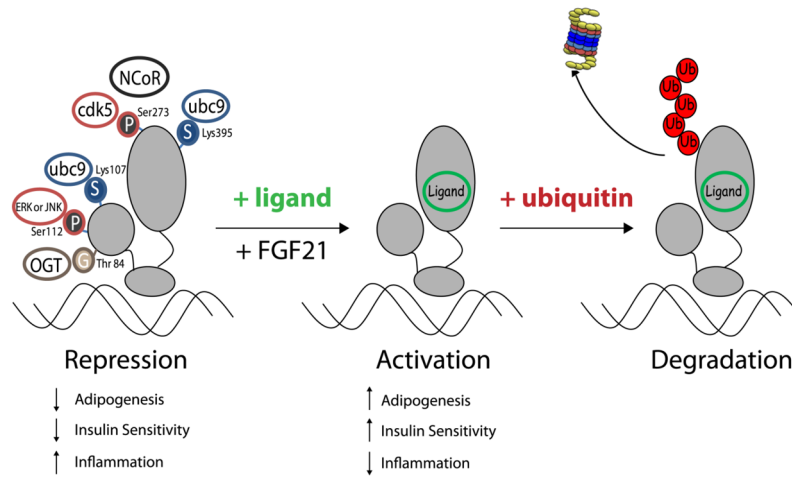


Figure 2. Covalent Modifications of PPAR γ are associated with transcriptional repression.

Table ICovalent Modifications of PPAR γ 2

| Modification of PPAR γ 2 | Site | Function |
|---------------------------------|-----------------------|--|
| Phosphorylation | Serine 112 | General decrease in transcriptional activity |
| | Serine 273 | Decrease in activation of specific target genes |
| SUMOylation | Lysine 107 | Inhibits transcriptional activity |
| | Lysine 395 | Prevents NCoR removal to maintain repression of activity |
| Ubiquitylation | Ligand Binding Domain | Targets PPAR γ for degradation to reduce protein expression |
| O-GlcNAcylation | Threonine 84 | Inhibits ligand-independent transcriptional activity |