

# Modulation of the Transcriptional Activity of Peroxisome Proliferator-Activated Receptor Gamma by Protein-Protein Interactions and Post-Translational Modifications

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Peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) belongs to a nuclear receptor superfamily; members of which play key roles in the control of body metabolism principally by acting on adipose tissue. Ligands of PPAR $\gamma$ , such as thiazolidinediones, are widely used in the treatment of metabolic syndromes and type 2 diabetes mellitus (T2DM). Although these drugs have potential benefits in the treatment of T2DM, they also cause unwanted side effects. Thus, understanding the molecular mechanisms governing the transcriptional activity of PPAR $\gamma$  is of prime importance in the development of new selective drugs or drugs with fewer side effects. Recent advancements in molecular biology have made it possible to obtain a deeper understanding of the role of PPAR $\gamma$  in body homeostasis. The transcriptional activity of PPAR $\gamma$  is subject to regulation either by interacting proteins or by modification of the protein itself. New interacting partners of PPAR $\gamma$  with new functions are being unveiled. In addition, post-translational modification by various cellular signals contributes to fine-tuning of the transcriptional activities of PPAR $\gamma$ . In this review, we will summarize recent advancements in our understanding of the post-translational modifications of, and proteins interacting with, PPAR $\gamma$ , both of which affect its transcriptional activities in relation to adipogenesis.

**Key Words:** PPAR $\gamma$ , coregulator, post-translational modifications, transcriptional activity, adipogenesis, metabolic syndrome

## INTRODUCTION

### Structure and function of peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) are known to be lipid sensors, and their ligands are used in the treatment of type 2 diabetes mellitus (T2DM) and other metabolic syndromes. PPARs are a family of nuclear receptors that act as transcription factors, controlling the genes involved in energy homeostasis.<sup>1</sup> PPARs share a high degree of structural homology with other types of nuclear hormone receptors.<sup>2</sup> PPARs comprise a DNA-binding domain (DBD), an agonist-independent activation domain (AF-1), and an agonist-dependent activation domain

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(AF-2), which contains the ligand-binding domain (LBD). PPARs heterodimerize with the retinoid X receptor (RXR)- $\alpha$  and activate the transcription of target genes by binding to the PPAR response element (PPRE).

The PPAR family has three isoforms; PPAR $\alpha$ ,  $\gamma$ , and  $\beta/\delta$ . PPAR $\alpha$  is expressed mainly in the liver, heart, kidney, brown adipose tissue (BAT), and skeletal muscle,<sup>3</sup> and participates in fatty acid oxidation ( $\beta$ - and  $\omega$ -oxidation).<sup>4</sup> The PPAR $\beta/\delta$  isoform is expressed ubiquitously and is involved in fatty acid oxidation in muscle.<sup>5</sup> PPAR $\gamma$  is expressed predominantly in adipose tissue and plays key roles in lipogenesis and adipocyte differentiation. It also stimulates glucose oxidation and decreases plasma free fatty acid level.<sup>5</sup> PPAR $\gamma$  consists of two isoforms; PPAR $\gamma$ 1 is expressed in adipocytes, skeletal muscle, liver, and heart, whereas PPAR $\gamma$ 2 is mostly found in adipose tissue.<sup>6</sup> PPAR $\gamma$ 2 plays a more important role than does PPAR $\gamma$ 1 in adipogenesis.<sup>7</sup>

### Physiological significance of PPAR $\gamma$

PPAR $\gamma$  was first identified as a trans-acting factor binding to a gene encoding a fat-specific enhancer of aP2 (adipocyte-specific fatty acid binding protein).<sup>8</sup> Homozygous PPAR $\gamma$  knockout mice exhibit an embryonic lethal phenotype due to placental dysfunction. Heterozygous PPAR $\gamma$  deficient mice are resistant to high-fat diet-induced insulin resistance due to adipocyte hypertrophy and increased leptin expression.<sup>9</sup> The ectopic expression of PPAR $\gamma$  was found to enhance the differentiation of preadipocytes into adipocytes, with PPAR $\gamma$  acting as an essential factor for differentiation.<sup>10</sup> In addition, PPAR $\gamma$  is known to block the clonal expansion that occurs via mitosis, an essential stage of adipocyte differentiation.<sup>11,12</sup>

Thiazolidinediones (TZDs) are a class of compounds that function as ligands of PPAR $\gamma$ . These compounds improve insulin sensitivity *in vivo* and have been introduced as therapeutic agents for the treatment of T2DM.<sup>13,14</sup> TZDs increase the expression of PPAR $\gamma$  and its transcriptional activity in adipose tissue, resulting in the upregulation of the expression of genes involved in the metabolism of lipids, carbohydrates, steroids, and amino acids.<sup>15-17</sup> TZDs increase insulin sensitivity by upregulating the expression of multiple genes, such as adiponectin, Cbl-associated protein, insulin receptor substrate 2, and glucose transporter 4.<sup>18-23</sup> TZDs also promote fatty acid storage and lipid metabolism, such as fatty acid translocase (CD36), perilipin, fatty acid binding protein 4 (Fabp4/aP2), lipoprotein lipase, acyl-CoA synthase, phosphoenol pyruvate carboxykinase (PEPCK),

and glycerol kinase (GyK).<sup>24-31</sup>

However, the activation of PPAR $\gamma$  results in the repression of the genes encoding leptin, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-6.<sup>32-35</sup> PPAR $\gamma$  decreases serum free fatty acid level and increases the number of small adipocytes, with a concomitant decrease in the number of large adipocytes in white adipose tissue (WAT). In addition to the role of PPAR $\gamma$  in adipose tissue, PPAR $\gamma$  directly activates the genes of the glucose-sensing apparatus in the liver and pancreatic  $\beta$ -cells. TZDs increase the expression of the genes encoding glucokinase (LGK and  $\beta$ GK) and glucose transporter 2 (GLUT2) in the liver<sup>36,37</sup> and pancreatic  $\beta$ -cells, respectively (see Table 1 for summary).<sup>38,39</sup> The transcriptional activity of PPAR $\gamma$  is subject to control at various levels; i.e., via modification of the receptor itself or interactions with other proteins.

In this review, we will limit our discussion to the regulation of PPAR $\gamma$  activity by various interacting proteins including coregulators, and by post-translational modifications (PTMs) that result in transcriptional regulation of PPAR $\gamma$  target genes.

## INTERACTING PROTEINS MODULATING TRANSCRIPTIONAL ACTIVITIES OF PPAR $\gamma$

The transcriptional activity of PPAR $\gamma$  is principally modulated by agonists, which recruit either coactivators or corepressors. In general, ligand-bound PPAR $\gamma$  recruits coactivators, whereas ligand-free PPAR $\gamma$  is bound to corepressors. These coregulators function as histone-modifying enzymes or bridging groups between the basal transcriptional machinery and PPAR $\gamma$ .<sup>40</sup> Moreover, additional proteins are recruited to these coregulators that may affect tissue-specific activities of PPAR $\gamma$ .

### Coactivators of PPAR $\gamma$

#### *Coactivators with histone acetyltransferase activity*

Ligand-bound PPAR $\gamma$  undergoes conformational changes, providing contact sites for LXXLL motifs that are present in coactivators such as p160/steroid receptor coactivator-1 (SRC-1) and p300/CREB-binding protein (CBP).<sup>41</sup> These coactivators have intrinsic histone acetyltransferase activities, which enhance the transcriptional activities of PPAR $\gamma$ . Members of the p160/SRC-1 family including SRC-1 (also known

**Table 1.** Selected PPAR $\gamma$  Target Genes Involved in Metabolism

Genes	PPAR $\gamma$ effect	Organ/cell type	Metabolic effects	Reference
Adiponectin	Upregulation	Adipocyte	Decrease in atherogenesis	18
CAP	Upregulation	Adipocyte	Improved insulin sensitivity	20
IRS2	Upregulation	Adipocyte	Anti-diabetic effect	21
GLUT4	Upregulation	Adipocyte	Glucose uptake	22, 23
CD36	Upregulation	Adipocyte	Fatty acid uptake	29
aP2	Upregulation	Adipocyte	Lipid oxidation	25
LPL	Upregulation	Adipocyte, muscle	Decrease in triglyceride	23, 30
ACS	Upregulation	Adipocyte	Decrease in triglyceride	31
PCK2	Upregulation	Adipocyte, muscle	Decrease in triglyceride Increase in lipid oxidation	23-25
GyK	Upregulation	Adipocyte	Decrease in free fatty acid	26
Perilipin	Upregulation	Adipocyte	Decrease in free fatty acid	27, 28
Leptin	Downregulation	Adipocyte	Improved insulin sensitivity	32, 33
TNF- $\alpha$	Downregulation	Adipocyte, liver	Improved insulin sensitivity	32, 34, 35
IL-6	Downregulation	Adipocyte, liver	Improved insulin sensitivity	32, 34, 35
GK	Upregulation	Liver, pancreatic $\beta$ -cell	Improved glucose homeostasis	37, 38
GLUT2	Upregulation	Liver, pancreatic $\beta$ -cell	Increase in glucose sensing	36, 39

PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma; CAP, Cbl-associated protein; IRS2, insulin receptor substrate 2; GLUT4, glucose transporter 4; LPL, lipoprotein lipase; GyK, glycerol kinase; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-6, interleukin-6; GLUT2, glucose transporter 2; CD36, fatty acid translocase; ACS, acetyl-CoA synthetase; PCK2, phosphoenolpyruvate carboxykinase 2; GK, glucokinase.

as NcoA-1), SRC-2 (also known as TIF2, GRIP-1, or NcoA-2), and SRC-3 (also known as p/CIP, ACTR, RAC-3, AIB-1, or TRAM-1), belong to this category.<sup>42</sup> SRC-1 knockout (KO) mice showed increased WAT mass and a decrease in the expression of genes involved in thermogenesis in brown adipose tissue (BAT). These KO mice also showed decreased expression of the genes encoding uncoupling protein (UCP-1), PPAR $\gamma$  coactivator-1 (PGC-1 $\alpha$ ), and acyl-CoA oxidase, as well as those encoding enzymes involved in fatty acid oxidation.<sup>42</sup> LXXLL motifs in SRC-1 interact directly with the AF-2 domain of PPAR $\gamma$ , recruiting CBP, which is required for PPAR $\gamma$  function.<sup>43</sup> SRC2<sup>-/-</sup> mice exhibit increased insulin sensitivity and are resistant to the development of obesity. These mice show increased lipolysis and decreased fatty acid uptake and storage which are related to the reduction of PPAR $\gamma$  activity.<sup>42</sup> When SRC-3 is deficient, corepressors such as nuclear receptor co-repressor (NCoR) and nuclear receptor interacting protein 1 (NRIP1 or RIP140) are recruited to the PPRE of the UCP1 gene, resulting in a decrease in its transcription.<sup>44</sup> SRC-3 and SRC-1 double KO mice are resistant to high-fat diet-induced obesity, due to the decreased expression of PPAR $\gamma$  target genes.<sup>44</sup> PGC-1 $\alpha$  activates PPAR $\gamma$  by increasing the binding of SRC-1 both *in vivo* and *in vitro*,<sup>45</sup> whereas SRC-2 attenuates the formation of the PGC-1 $\alpha$ -PPAR $\gamma$  complex by competing with SRC-1.<sup>42</sup> This study suggests that the ratio of SRC-2/SRC-1 could be

a critical metabolic determinant in the development of obesity and insulin resistance.<sup>42</sup>

CBP/p300 indirectly increases the transcriptional activity of PPAR $\gamma$  through its interaction with PGC-1 $\alpha$ . The docking of PGC-1 $\alpha$  to PPAR $\gamma$  induces a conformational change in PGC-1 $\alpha$  that promotes the binding of SRC-1 and CBP/p300.<sup>45</sup> SRC-1 is also required for a functional interaction between CBP/p300 and PPAR $\gamma$ .<sup>43</sup> CBP/p300 not only binds to the AF-2 domain of PPAR $\gamma$  in a ligand-dependent manner but also binds directly to the AF-1 domain in a ligand-independent manner,<sup>46</sup> increasing the transcriptional activities of PPAR $\gamma$ <sup>46</sup> and thereby inducing adipogenesis in NIH3T3 fibroblasts.<sup>47</sup> The recruitment of PPAR $\gamma$  along with CBP/p300 to the aP2 gene promoter results in adipocyte differentiation.<sup>48</sup>

#### *TRAP mediator complex*

The thyroid hormone receptor-associated protein (TRAP) complex was first discovered in yeast and shown to be essential for RNA polymerase II-dependent transcription. TRAPs were first purified by affinity chromatography from cells overexpressing the thyroid hormone receptor. They are components of the TRAP/vitamin D receptor-interacting protein (DRIP)/activator-recruited cofactor/Mediator (Med) complex, functioning as mediators between RNA polymerase II and CBP/p300 or p160/SRC.<sup>49</sup> TRAPs also

interact with nuclear receptors, such as the vitamin D receptor (VDR), retinoic acid receptor  $\alpha$  (RAR $\alpha$ ), RXR $\alpha$ , PPAR $\alpha$ , and PPAR $\gamma$ , in a ligand-dependent manner.<sup>50</sup> Both TRAP220 and TRAP100 interact with PPAR $\gamma$  through their respective LXXLL motifs.<sup>50</sup>

TRAP220 is also referred to as the PPAR-binding protein/DRIP205/Med1 subunit of the TRAP complex, functioning as a bridging protein between various mediator complexes and nuclear receptors.<sup>51</sup> TRAP220<sup>-/-</sup> mice are embryonically lethal at day 11.5, suggesting that TRAP is essential for development. The ligand-dependent transcriptional activity of PPAR $\gamma$  is decreased in TRAP220<sup>-/-</sup> mouse embryonic fibroblasts (MEFs).<sup>51</sup> TRAP220<sup>-/-</sup> MEF cells were not able to induce adipogenic genes via PPAR $\gamma$ . The PPAR $\gamma$ 2-TRAP220 interaction is essential for adipogenesis<sup>52</sup> and increases PPAR $\gamma$ -mediated transactivation of the promoter reporter construct.<sup>53</sup> Although PPAR $\gamma$  acts by forming heterodimers with RXR $\alpha$ , treatment with the cognate PPAR $\gamma$ - and RXR $\alpha$ -selective ligands results in the recruitment of different coactivators. RXR $\alpha$ -specific ligands recruit SRC-1/p160 to PPAR $\gamma$ -RXR, whereas PPAR $\gamma$  ligands recruit TRAP220, but not SRC-1/p160.<sup>54</sup>

Regulation of PPAR $\gamma$  is achieved by the combinatorial actions of the coactivator and its ligands. Ligand-mediated selective recruitment of the coactivator may be responsible for fine-tuning of target gene expression.

#### *The switching/sucrose nonfermenting (SWI/SNF) chromatin remodeling complex*

The mating type SWI/SNF complex is an ATP-dependent chromatin remodeling enzyme that activates transcription by promoting the access of transcription factors to their cognate binding sites.<sup>55</sup> The core components of the complex include either the Brg1 or Brm ATPases and several Brg1/Brm-associated factors (BAFs). Brg1 and/or Brm can interact with a number of different transcriptional regulatory proteins.<sup>56</sup> For example, CCAAT-enhancer binding protein alpha (C/EBP $\alpha$ ), a critical factor for adipogenesis, is known to interact with hBrm.<sup>57</sup>

The Brg1/Brm-associated factors (BAFs) family is an accessory subunit of the SWI/SNF complex, acting as a connector between transcription factors and SWI/SNF complexes.<sup>49</sup> BAF180 binds PPAR $\gamma$ -RXR $\alpha$ . The factor contains six bromodomains that bind selectively to acetylated histone tails, an important protein modification for targeting the coregulator complex to chromatin.<sup>58</sup> In addition, the presence of Brg1 and Brm in the PPAR $\gamma$  promoter suggests that these

coregulator complexes may contribute to adipogenesis. Transcriptional regulation by PPAR $\gamma$  during adipogenesis critically depends on the SWI/SNF complex, which plays a key role in the formation of preinitiation complexes.<sup>56</sup>

BAF60c2 (a BAF of 60 kDa, subunit 2) is also known to interact with the LBD of PPAR $\gamma$ . The N-terminal of BAF60c binds to the C-terminal of PPAR $\gamma$ , and the C-terminal of BAF60c interacts with the N-terminal of PPAR $\gamma$  in a ligand-independent manner. BAF acts as an anchor between SWI/SNF complexes and PPAR $\gamma$ . BAF60c increases the transcriptional activity of PPAR $\gamma$  in the presence of ligand but does not affect adipocyte differentiation.<sup>59</sup>

#### *Other interacting proteins*

ADP-ribosylation factor (ARF6), a key regulator of the aP2 gene, is a novel transcription factor that is purified from BAT.<sup>60</sup> ARF6 binding sites are present in the aP2 and PEPCK gene promoters.<sup>25,61</sup> The PPAR $\gamma$ /RXR $\alpha$  heterodimer interacts with ARF6 during adipogenesis.<sup>60</sup>

Menin, encoded by the multiple endocrine neoplasia type 1 (*MEN1*) tumor suppressor gene, is involved in activation of gene transcription as a component of the mixed-lineage leukemia (MLL) 1/MLL2 (also known as KMT2A/B) protein complexes, and exhibits methyltransferase (HMT) activity.<sup>62</sup> Ectopic expression of menin increases the transcription of PPAR $\gamma$  target genes, and knock down of menin inhibits the differentiation of 3T3L1 preadipocytes into mature adipocytes. Menin interacts directly with the AF-2 domain of PPAR $\gamma$  and enhances PPAR $\gamma$ -mediated transcriptional activities in a ligand-dependent fashion. Menin increases histone H3K4 methylation in the PPAR $\gamma$  target gene, *Fabp4*, through a direct interaction with the AF-2 domain of PPAR $\gamma$ .<sup>62</sup>

Multiprotein bridging factor-1 (MBF-1) is a cofactor that was first identified in *Bombyx mori* (Bm). It has been shown to interact with LXR $\alpha$  or PPAR $\gamma$ , and stimulate their ligand-dependent transcriptional activities.<sup>63</sup> MBF-1 does not have either histone acetyltransferase or methyltransferase activity but interacts with transcription factor IID (TFIID). MBF-1 acts as a bridging protein between PPAR $\gamma$  and TFIID, increasing the transcriptional activity of PPAR $\gamma$ . Since MBF-1 is also known to interact with LXR $\alpha$  and liver receptor homolog 1 (LRH-1), a detailed investigation of the role of MBF-1 is important to understand its function in the context of lipid metabolism. The central domain of MBF-1 is necessary and critical for interaction with LRH-1, LXR $\alpha$ , and PPAR $\gamma$ .<sup>64</sup>

PPAR $\gamma$  and thromboxane synthase (TXS) are expressed in macrophages; therefore, they may be involved in atherogenesis. PPAR $\gamma$  binds to nuclear factor E2-related factor 2 (NRF2), which results in decreasing TXS gene expression by preventing the binding of NRF2 to the TXS gene. The suppression of TXS gene expression by PPAR $\gamma$  was increased by treatment with 15-deoxy- $\Delta^{12,14}$ -prostaglandin J<sub>2</sub> and troglitazone.<sup>65</sup> TXS is increased in an inflammatory model of hydronephrosis, which is characterized by infiltration of macrophages into the kidney, and produces thromboxane.<sup>66,67</sup> Thromboxane inhibitors are shown to suppress the progression of experimental diabetic nephropathy in rats<sup>68</sup> and ameliorate microalbuminuria in patients with T2DM.<sup>69</sup> Hence, PPAR $\gamma$  ligands could be used as drugs for treating renal complications of T2DM.

PPAR-interacting protein (PRIP, also known as RAP250/ASC-2/TRBP/NRC) is expressed in the reproductive organs (testis, prostate, and ovary) and identified as a novel, direct interacting coactivator of PPAR $\gamma$ , RXR $\alpha$ , PPAR $\alpha$ , RAR $\alpha$ , estrogen receptor (ER), and thyroid hormone receptor  $\beta$ .<sup>70,71</sup> Knock out of PRIP resulted in embryonic lethality and vascular dysfunction of the placenta.<sup>72</sup> PRIP<sup>-/-</sup> MEFs exhibit repression of the transcriptional activity of RXR $\alpha$  rather than PPAR $\gamma$  activity. Although PRIP was isolated in the yeast two-hybrid screen using PPAR $\gamma$  as a bait, PRIP has a preference for RXR $\alpha$  over its heterodimeric partner, PPAR $\gamma$ .<sup>73</sup>

PPAR $\gamma$ -DBD interacting protein 1 (PDIP1) was isolated using the yeast two-hybrid system with the DBD and hinge regions of human PPAR $\gamma$  as bait. Two isoforms ( $\alpha$  and  $\beta$ ) of the *PDIP1* gene are generated by alternative splicing. PDIP1  $\alpha$  and  $\beta$  increase the PPAR $\gamma$ -mediated transactivation of the PPRE, and treatment with PDIP1 siRNA significantly reduced the transcriptional activity of PPAR $\gamma$ . Because PDIP1 shows an expression pattern similar to that of CBP and TRAP220 during adipocyte differentiation, it might be involved in PPAR $\gamma$ -mediated adipogenesis.<sup>74</sup>

PGC-1 $\alpha$  also binds to DBD and hinge regions of PPAR $\gamma$  in a ligand-independent fashion, similar to PDIP.<sup>75</sup> PGC-1 $\alpha$  was isolated from a BAT cDNA library and has been shown to increase the transcriptional activity of PPAR $\gamma$  on the UCP-1 gene. UCP-1 increases mitochondrial DNA content and  $\beta$ -oxidation.<sup>75</sup> PGC-1 $\alpha$ -deficient mice exhibit a reduced number of mitochondria and lower respiratory capacity, and fail to maintain core body temperature following exposure to cold.<sup>76</sup> Overexpression of PGC-1 $\alpha$  in WAT resulted in phenotypic changes into BAT.<sup>77,78</sup> This phenotypic change provides a defense mechanism against obesity.<sup>79</sup> In adipocytes,

PPAR $\gamma$  acts as a master regulator of adipogenesis upregulating the *aP2* and *GyK* genes. However, aP2 expression is increased in mature adipocytes whereas that of the *GyK* gene is not. PPAR $\gamma$ -mediated induction of *GyK* requires the recruitment of the PPAR $\gamma$  ligand and PGC-1 $\alpha$  to PPAR $\gamma$  to replace corepressors with coactivators. In contrast, aP2 gene expression by PPAR $\gamma$  does not require its ligands. Differential regulation of target genes by ligands may determine the selective recruitment of coregulators.<sup>80</sup> The interaction between PGC-1 $\alpha$  and PPAR $\gamma$  induces a conformational change in PGC-1 $\alpha$ , facilitating the recruitment of SRC-1 and CBP/p300.<sup>45</sup> Although PGC-1 $\alpha$  is known to interact with various nuclear receptors, PGC-1 $\alpha$  is an essential cofactor for the transactivation of PPAR $\gamma$ , acting as a hub linking nutritional and hormonal signals to energy metabolism.<sup>81</sup>

### Corepressors of PPAR $\gamma$

*Nuclear receptor co-repressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptor (SMRT)*  
The PPAR $\gamma$  antagonist T0070907 covalently binds to PPAR $\gamma$  at Cys<sup>313</sup> in helix 3, and was shown to decrease PPAR $\gamma$  activity in a cell-based reporter assay. T0070907 blocks the recruitment of the coactivator and promotes the recruitment of NCoR to PPAR $\gamma$ .<sup>82</sup> In the absence of ligand, NCoR and silencing mediator of retinoid and thyroid hormone receptor (SMRT) are recruited to PPAR $\gamma$ , resulting in a decrease in its transcriptional activity. In cells treated with pioglitazone, SMRT and NCoR dissociate from PPAR $\gamma$ . In addition, treatment with siRNA against SMRT and NCoR increased adipogenesis and the accumulation of lipid droplets in 3T3L1 adipocytes.<sup>83</sup>

NAD-dependent deacetylase sirtuin-1 (SIRT1) is known to be responsible for calorie restriction and mobilizing WAT. SIRT1 activation by resveratrol decreases fat accumulation in differentiated adipocytes. SIRT1 represses PPAR $\gamma$  transcriptional activity by recruiting NCoR and SMRT.<sup>84</sup> Since a reduction in fat accumulation is sufficient to extend life span in mice,<sup>85</sup> the role of SIRT1 in fat mobilization constitutes a possible molecular pathway connecting calorie restriction to life extension.<sup>84</sup>

Adipocyte-specific NCoR knockout (AKO) mice exhibit an increase in the expression of PPAR $\gamma$ -responsive genes and a decrease in cyclin-dependent kinase (Cdk5)-mediated PPAR $\gamma$  Ser<sup>273</sup> phosphorylation, resulting in constitutive activation of these genes. Although AKO mice show an increase in adiposity, they also exhibit improved systemic in-

sulin sensitivity and glucose tolerance, and decreased adipose tissue inflammation. These studies suggest that the dominant function of adipocyte NCoR is to transrepress PPAR $\gamma$  and promote Cdk5-mediated PPAR $\gamma$  phosphorylation, similar to the effects of TZDs.<sup>86</sup>

#### *Other interacting proteins*

RIP140 is a liver protein that interacts with the AF-2 domain of PPAR $\gamma$  and also with PPAR $\gamma$ . BRL49653, a PPAR $\gamma$  ligand, strengthens the interaction between PPAR $\gamma$  and RIP140.<sup>87,88</sup> Because RIP140 is generally known to inhibit nuclear receptor activity through competition with SRC-1, transrepression of PPAR $\gamma$  by RIP140 occurs indirectly.<sup>88</sup> Although RIP140 inhibits the transcriptional activity of PPAR $\gamma$ , it does not affect adipogenesis. However, RIP140 KO mice showed increased *UCP1* gene expression and resistance to high-fat diet-induced obesity and hepatic steatosis.<sup>89</sup>

The forkhead transcription factor Foxo1 was identified as a PPAR $\gamma$ -interacting protein that disrupts the binding of PPAR $\gamma$  to the target gene. In addition, PPAR $\gamma$  plays a negative role in the transactivation of Foxo1, suggesting that there is a reciprocal interaction between these factors. Ectopic expression of the constitutively active form of Foxo1 in preadipocytes prevents adipogenesis and heterozygous Foxo1 KO mice are less susceptible to diet-induced insulin resistance.<sup>90</sup>

The retinoblastoma protein (Rb) plays a negative role during mitotic clonal expansion in the cell cycle by increasing the transactivation of C/EBP.<sup>91,92</sup> PPAR $\gamma$  has been shown to interact directly with Rb in 3T3L1 adipocytes, recruiting histone deacetylase HDAC3 which attenuates adipogenic gene expression. Dissociation of the PPAR $\gamma$ -Rb-HDAC3 complex by phosphorylation of Rb or inhibition of HDAC3 activity resulted in the activation of PPAR $\gamma$ .<sup>93</sup>

Lipin1 is known to be expressed in adipose tissue.<sup>94</sup> The null mice of lipin1 show lipodystrophy with severely reduced adipose tissue mass.<sup>95</sup> Lipin1 is increased in the later stages of adipocyte differentiation and increases transcriptional activity of PPAR $\gamma$ 2 through direct protein-protein interaction.<sup>96</sup>

Small heterodimer partner (SHP) is an atypical orphan nuclear receptor that inhibits gluconeogenesis by interacting with Foxo1, hepatocyte nuclear factor 4, or C/EBP $\alpha$ .<sup>97,98</sup> SHP is also known to increase PPAR $\gamma$  activity by interacting with PPAR $\gamma$  in a ligand-independent manner. SHP competes with NCoR for binding to the DBD/hinge region of PPAR $\gamma$ . It has been suggested that SHP may act as an en-

dogenous activator of PPAR $\gamma$ .<sup>99</sup> However, a contradictory report states that SHP represses the transcriptional activity of PPAR $\gamma$  and does not interact with PPAR $\gamma$ .<sup>100</sup> SHP decreases LGK gene expression by inhibiting the transcriptional activity of LXR $\alpha$  and PPAR $\gamma$  via interaction with their common partner, RXR $\alpha$ . Thus, SHP may play a role in fine-tuning glucose homeostasis.<sup>100</sup> The diverse functions of PPAR $\gamma$  cofactors are summarized in Table 2.

## REGULATION OF PPAR $\gamma$ ACTIVITY BY POST-TRANSLATIONAL MODIFICATION

### **Phosphorylation**

Phosphorylation of nuclear receptors is one of the principal modifications determining their transcriptional activities. Adipocyte differentiation is inhibited by growth factors<sup>101-103</sup> and cytokines,<sup>104-106</sup> which are known to phosphorylate PPAR $\gamma$  through their respective signaling pathway (Fig. 1). The site of phosphorylation is Ser<sup>112</sup> in the N-terminal transactivation domain (AF-1), which is well conserved among species ranging from fish to man.<sup>107,108</sup> Ser<sup>112</sup> phosphorylation by mitogen-activated protein kinase (MAPK) results in a decrease in transcriptional activity and adipogenesis.<sup>109-112</sup> MAPK is activated by extracellular signal-regulated kinase 1/2 (ERK1/2) that is stimulated by growth factors such as epidermal growth factor, platelet-derived growth factor, transforming growth factor- $\beta$ , insulin, or the prostaglandin PGF2 $\alpha$ .<sup>109,110,113-116</sup> Phosphorylation of Ser<sup>112</sup> by other signals including stress (UV, anisomycin) is mediated by c-Jun N-terminal kinase 1/2 and p38.<sup>107,113</sup>

Insulin plays a key role in adipogenesis.<sup>109</sup> Although preadipocytes express a limited number of insulin receptors, the cells require insulin or insulin-like growth factor-1 for optimal differentiation.<sup>117,118</sup> After maturation, large numbers of insulin receptors are expressed, transmitting insulin signals for the induction of lipogenic genes.<sup>119,120</sup> Although insulin is a pivotal player in adipogenesis, Ras/MAPK activation by insulin represses PPAR $\gamma$  activity<sup>109</sup> as shown in the growth factor-induced phosphorylation of PPAR $\gamma$  at Ser<sup>112</sup>.

Specifically, downstream tyrosine kinase-1 (Dok1), a multi-site adapter molecule in insulin receptor signaling,<sup>121-123</sup> acts as a negative regulator of MAPK.<sup>124-126</sup> In mice fed a high-fat diet, Dok1 expression is markedly increased in WAT. A lower mass of WAT is seen in Dok1-deficient mice than in wild-type mice, and the level of PPAR $\gamma$  phosphory-

**Table 2.** List of Regulatory Factors for PPAR $\gamma$  Activity

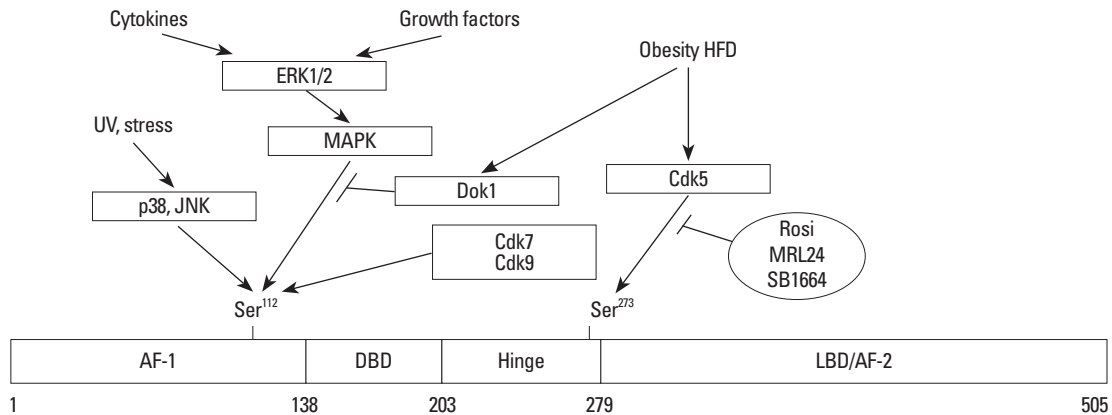
Role	Coregulators	Metabolic effects	Reference
Coactivators	SRC-1	Essential for functional interaction between CBP/p300 and PPAR $\gamma$	43
	PGC-1 $\alpha$	Increases the binding of SRC-1 and CBP/p300 to PPAR $\gamma$ Increases the transcriptional activity of PPAR $\gamma$ on the UCP-1 gene Plays a role in maintaining the number of mitochondria, respiratory capacity, body temperature in BAT	45, 75, 76
	CBP/p300	Binds to the AF-2 domain of PPAR $\gamma$ (ligand-dependent) Binds to the AF-1 domain to PPAR $\gamma$ (ligand-independent) Induces adipogenesis in NIH3T3 cells Increases aP2 gene expression	46-48
	TRAP220	Increases PPAR $\gamma$ transcriptional activity Essential for PPAR $\gamma$ -mediated adipogenesis	51, 52
	BAF60c2	Interacts with LBD of PPAR $\gamma$ Does not affect adipocyte differentiation	59
	ARF6	Regulates the expression of aP2 and PEPCK gene Interacts with PPAR $\gamma$ /RXR $\alpha$ during adipogenesis	25, 60, 61
	Menin	Increases histone H3K4 methylation at the PPAR $\gamma$ target gene, Fabp4 Directly interacts with the PPAR $\gamma$ AF-2 domain	62
	MBF-1	Acts as a bridging protein between PPAR $\gamma$ and TFIID	64
	NRF2	Decreases TXS gene expression by forming complex with PPAR $\gamma$	65
	PRIP	Critical for embryonic development and survival Represses the transcriptional activity of RXR $\alpha$	73
	PDIP	Increases the PPAR $\gamma$ -mediated transactivation	74
	SHP	Compete with NCoR in binding to the DBD/hinge region of PPAR $\gamma$ Endogenous activator of PPAR $\gamma$	99
	Lipin1	Increases the transcriptional activity of PPAR $\gamma$	96
	Corepressor	NCoR	Decreases the transcriptional activity of PPAR $\gamma$
SMRT		Decreases adipogenesis and lipid accumulation Interacts with PPAR $\gamma$ and increases the ability of PPAR $\gamma$ to associate with Cdk5 Increase adiposity and exhibit improved insulin sensitivity and glucose tolerance in adipocyte specific NCoR knock out (AKO) mice	83, 86
Sirt1		Decreases lipid accumulation in differentiated adipocytes Represses transcriptional activity of PPAR $\gamma$ by recruiting NCoR and SMRT	84
RIP140		Decreases the transcriptional activity of PPAR $\gamma$ by competing with SRC-1 Does not affect adipocyte differentiation	88, 89
Foxo1		Disrupts binding of PPAR $\gamma$ to the target genes	90
Rb		Recruits histone deacetylase HDAC3 and decreases the expression of adipogenic genes	93
	SHP	Represses the PPAR $\gamma$ /RXR $\alpha$ transactivation by interacting with RXR $\alpha$	100

PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma; SRC-1, steroid receptor coactivator-1; PGC-1 $\alpha$ , PPAR $\gamma$  coactivator-1; CBP, CREB-binding protein; TRAP, thyroid hormone receptor-associated protein; BAF, Brg1/Brm-associated factor; MBF-1, multiprotein bridging factor-1; NRF2, nuclear factor E2-related factor 2; PRIP, PPAR-interacting protein; PDIP, PPAR $\gamma$ -DBD interacting protein; SHP, small heterodimer partner; NCoR, nuclear corepressor; SMRT, silencing mediator of retinoid and thyroid hormone receptor; Rb, retinoblastoma protein; SHP, small heterodimer partner; DBD, DNA-binding domain; UCP, uncoupling protein; BAT, brown adipose tissue; AF-1, agonist-independent activation domain; AF-2, agonist-dependent activation domain; LBD, ligand-binding domain; PEPCK, phosphoenol pyruvate carboxykinase; RXR, retinoid X receptor; TFIID, transcription factor IID; TXS, thromboxane synthase; Cdk5, cyclin-dependent kinase; ARF6, ADP-ribosylation factor; RIP140, receptor-interacting protein 140.

lation was increased by ERK.<sup>127</sup> These data suggest that an increase in Dok1 gene expression caused by a high-fat diet inhibits the insulin-mediated activation of Ras/MAPK signaling, resulting in increased PPAR $\gamma$  activity.<sup>127</sup>

In contrast to the MAPK-mediated phosphorylation of Ser<sup>112</sup>, the cyclin-dependent kinases Cdk7 and Cdk9 phosphorylate the same Ser<sup>112</sup> in PPAR $\gamma$  and increase PPAR $\gamma$

activity.<sup>128,129</sup> Trichothiodystrophy (TTD) is a rare autosomal recessive disease caused by mutations in the xeroderma pigmentosum (XP) group-D (*XPD*) gene. The clinical manifestations include immature sexual development, mental retardation, skeletal abnormalities, and dwarfism. A number of patients with TTD exhibit a lack of subcutaneous fat tissue mass. XPD helicase is a subunit of the transcription



**Fig. 1.** Modulation of PPAR $\gamma$  activity by phosphorylation. Positions of phosphorylation sites in PPAR $\gamma$  and the implicated signaling pathways are indicated. Ser<sup>112</sup> phosphorylation by growth factors, cytokines, and stress signals are related to decreased PPAR $\gamma$  activity, whereas phosphorylation by Cdk7 and Cdk9 is related to increased PPAR $\gamma$  activity. Obesity or high-fat diet-mediated phosphorylation of PPAR $\gamma$  at Ser<sup>273</sup> is related to decreased insulin sensitivity. AF-1 and 2, activation function 1 and 2, respectively; Cdk5, 7 and 9, cyclin-dependent kinase 5, 7 and 9, respectively; DBD, DNA binding domain; Dok1, downstream of tyrosine kinase-1; ERK1/2, extracellular signal-regulated kinase 1/2; HFD, high fat diet; JNK, c-Jun N-terminal kinase; LBD, ligand binding domain; MAPK, mitogen-activated protein kinase; p38, p38 MAP kinase; Rosi, rosiglitazone; PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma.

factor IIIH (TFIIH) complex bridging the core-TFIIH [containing particular form of xeroderma pigmentosum B (XPB) helicase] subcomplex and the Cdk-activating kinase containing Cdk7.<sup>130</sup> When the C-terminus of XPD is mutated, XPD helicase cannot perform nucleotide excision repair.<sup>131</sup> In the process of transcription, Cdk7 in the TFIIH complex phosphorylates the C-terminal domain of the largest subunit of RNA polymerase II<sup>132</sup> and nuclear receptors such as ER, VDR, and RAR $\alpha$ .<sup>133-136</sup> PPAR $\gamma$  phosphorylation by Cdk7 is decreased in XPD patients.<sup>128</sup> The activity of a PPAR $\gamma$  promoter reporter was rescued by PPAR $\gamma$ -112<sup>S→E</sup>, a constitutively active form of PPAR $\gamma$ , in fibroblasts isolated from patients with TTD.<sup>128</sup>

In addition, Cdk9, a component of positive transcription elongation factor b, has been shown to participate in adipogenesis by directly interacting with PPAR $\gamma$  and phosphorylating Ser<sup>112</sup>.<sup>129</sup> Overexpression of Cdk9 in 3T3L1 cells increased adipogenesis, whereas inhibition of Cdk9 by specific Cdk inhibitors or a dominant-negative Cdk9 mutant inhibited adipogenesis.<sup>129</sup> These data suggest that the transcriptional activity of PPAR $\gamma$  is either activated or inhibited depending on the types of kinases involved.

In the adipose tissues of mice fed a high-fat diet, phosphorylation of Ser<sup>273</sup> by Cdk5 results in a reduction of adiponectin gene expression, without affecting adipogenesis.<sup>137</sup> Cdk5-mediated phosphorylation of PPAR $\gamma$  is blocked by full agonists such as rosiglitazone or partial agonists such as MRL24 or SR1664.<sup>137,138</sup>

Partial agonists, like MRL24 and SR1664, have been shown to have excellent anti-diabetic activity without in-

creasing adipogenesis.<sup>137,138</sup> These compounds are known to block the phosphorylation of Ser<sup>273</sup> by Cdk5<sup>137,138</sup> and can therefore potentially be used as therapeutic drugs for T2DM without causing weight gain and fluid retention, which are major side effects of full agonist-antidiabetic drugs.

It is worth note that strong PPAR $\gamma$  activators are not necessary to increase insulin sensitivity. Understanding the regulation of Ser<sup>273</sup> phosphorylation in PPAR $\gamma$  could provide a hint for the development of drugs to treat T2DM that have fewer side effects.<sup>138</sup>

### Sumoylation

SUMOylation is one of the post-translational modifications responsible for regulating the stability, nuclear-cytosolic distribution, and activity of transcription factors. Small ubiquitin-like modifier (SUMO) family proteins (SUMO-1, -2, and -3 in mammals) affect the interaction between target proteins and their substrates or the DNA that they bind. SUMO binds to proteins by forming isopeptide bonds between the C-terminal glycine residue of SUMO and the  $\epsilon$ -amino group of a lysine in the target protein.<sup>139,140</sup> Currently, a number of transcription factors including nuclear receptors, such as PPARs,<sup>141,142</sup> LXR,<sup>143</sup> glucocorticoid receptor,<sup>144</sup> androgen receptor,<sup>145</sup> and RXR $\alpha$ <sup>146</sup> are known to be SUMOylated.

Selective modulation of the transcriptional activity of PPAR $\gamma$  by SUMOylation is now beginning to be understood.<sup>142,147</sup> The transcriptional activities of PPAR $\gamma$  isoforms in the presence or absence of ligands are regulated by SUMOylation.<sup>142</sup> PPAR $\gamma$ 2 is SUMOylated by protein inhibitor of activated STAT 1 (PIAS1) or PIASx, belonging to the



PIAS family, regardless of its ligand. PPAR $\gamma$ 2 is SUMOylated at Lys<sup>107</sup> in the AF-1 domain, and at Lys<sup>395</sup> in the AF-2 domain (equivalent to Lys<sup>77</sup> and Lys<sup>365</sup> of PPAR $\gamma$ 1, respectively). SUMOylation of PPAR $\gamma$ 2 at Lys<sup>107</sup> negatively regulates the transcriptional activity of PPAR $\gamma$ 2, because the 107<sup>K→R</sup> mutation showed increased transcriptional activity.<sup>142</sup> This observation is further supported by a promoter reporter assay performed using the variant PPAR $\gamma$ 2 107<sup>K→R</sup> in NIH3T3 fibroblasts.<sup>148,149</sup> Furthermore, fibroblast growth factor21 (FGF21)-KO mice exhibit impaired insulin sensitivity in adipocytes and reduced fat mass and adipocyte size. This phenomenon occurs because PPAR $\gamma$ 2-induced adipogenesis is inhibited by SUMOylation in WAT. These results indicate that FGF21 is a key regulator of PPAR $\gamma$ 2 in the context of SUMOylation.<sup>150</sup> In addition, the transcriptional activity of PPAR $\gamma$ 2 is increased by overexpressing SUMO1/sentrin/SMT3-specific peptidase 2 (SEN2), a SUMO-specific protease, in C2C12 myotubes.<sup>147</sup> Interestingly, the inhibition of PPAR $\gamma$ 2 transcriptional activity by SUMOylation is augmented when PPAR $\gamma$ 2 is phosphorylated at Ser<sup>112</sup>.<sup>148,149</sup> This indicates an interrelationship between the SUMOylation and phosphorylation of PPAR $\gamma$ 2.

The SUMOylation of PPAR $\gamma$ 1 at Lys<sup>365</sup> (equivalent to Lys<sup>395</sup> of PPAR $\gamma$ 2) is important in the regulation of inflammatory gene expression. This SUMOylation mediates the repression of inflammatory genes like inducible nitric oxide synthase (iNOS) and TNF- $\alpha$ , which are regulated by nuclear factor kappa B in macrophages.<sup>151,152</sup> In the basal state, *iNOS* gene is repressed by TBL1/TBLR1/HDAC3/NCoR complex. Treatment of lipopolysaccharide (LPS) resulted in the removal of HDAC3/NCoR from the complex in a TBL1/TBLR1 and Ubc5-dependent fashion, allowing activation of *iNOS* gene.<sup>148</sup> When RAW264.7 macrophages or primary cultured macrophages were treated with LPS and rosiglitazone, PPAR $\gamma$ 1 was found to be SUMOylated on Lys<sup>365</sup> by Ubc9, which forms a complex with NCoR/HDAC3 on the promoters of the *iNOS* gene. Thus, the formation of the NCoR/HDAC3/SUMOylated PPAR $\gamma$ 1 complex inhibits the ubiquitination of NCoR/HDAC3, resulting in the repression of the *iNOS* and TNF- $\alpha$  genes.<sup>151,152</sup>

Ligand-dependent SUMOylation of PPAR $\gamma$ 1 therefore directly represses the promoters of inflammatory genes by stabilizing the NCoR and HDAC3 complexes. This mechanism demonstrates that the role of Lys<sup>365</sup> SUMOylation of PPAR $\gamma$ 1 is different from that of Lys<sup>107</sup> SUMOylation of PPAR $\gamma$ 2 in that Lys<sup>365</sup> SUMOylation of PPAR $\gamma$ 1 represses the expression of inflammatory genes in the presence of ligand.

## Ubiquitination

The ubiquitin-proteasome system (UPS) is responsible for the degradation of a variety of intracellular proteins including transcription factors.<sup>153,154</sup> Ubiquitin is well conserved between species, binding to target proteins in a sequential manner through the actions of three different cascading enzymes: an ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2), and an ubiquitin protein ligase (E3).<sup>155</sup> The polyubiquitinated proteins are recognized and degraded by the 26S proteasome.<sup>156</sup> The role of the UPS with respect to transcriptional regulation is well documented.<sup>157</sup> In the nucleus of adipocytes, the PPAR $\gamma$ 2 protein level is decreased by the action of TZDs.<sup>158</sup> Degradation occurs in a ubiquitin-dependent manner in the AF-2 domain of PPAR $\gamma$ .<sup>159</sup> However, the AF-1 domains of PPAR $\gamma$ 1 and PPAR $\gamma$ 2 are degraded by the REG $\gamma$  proteasome, a type of proteasome that degrades the target substrate in an ubiquitin and ATP-independent fashion.<sup>159-161</sup>

Degradation of PPAR $\gamma$  is also regulated by interferon- $\gamma$  (IFN- $\gamma$ ) in adipocytes. Transcription of PPAR $\gamma$  is decreased by IFN- $\gamma$ -activated STAT signaling.<sup>162</sup> When Ser<sup>112</sup> of PPAR $\gamma$ , which is known to be phosphorylated by ERK1/2, was replaced with Ala, degradation of the protein was decreased. In addition, U1026, an inhibitor of ERK1/2, decreased IFN- $\gamma$ -induced PPAR $\gamma$  degradation.<sup>163</sup> However, ERK1/2 is not known to be activated by IFN- $\gamma$  or TZDs; thus, it is assumed that there might be an indirect relationship between the phosphorylation and ubiquitination of PPAR $\gamma$ .<sup>163</sup>

TNF- $\alpha$  is well known for its role in insulin resistance.<sup>164</sup> Degradation of PPAR $\gamma$  is promoted by TNF- $\alpha$  in adipocytes. Treatment of adipocytes with TNF- $\alpha$  and cycloheximide yielded a 44-kDa sized fragment of PPAR $\gamma$ , which is also seen in the WAT or BAT of diabetic rats. However, the molecular link between this fragment and PPAR $\gamma$  degradation is not known.<sup>165</sup> Proteasome-dependent PPAR $\gamma$  degradation is increased by resveratrol, a potent activator of SIRT1; however, the mechanism of SIRT1 requires further investigation.<sup>84,166</sup>

## PERSPECTIVE

Regulation of PPAR $\gamma$  activity may be achieved through the interrelationship between agonists, PTM, and coregulators, rather than by the simple action of individual activators or inhibitors. Agonists can induce either coregulator exchange or PTM; the mechanisms of which require further study.

Understanding the mechanistic complexity underlying the interactions of these regulators may help accelerate the development of therapeutic drugs against obesity, T2DM, and metabolic syndromes.

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