The story so far: post-translational regulation of peroxisome proliferator-activated receptors by ubiquitination and SUMOylation

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Wadosky KM, Willis MS. The story so far: post-translational regulation of peroxisome proliferator-activated receptors by ubiquitination and SUMOylation. Am J Physiol Heart Circ Physiol 302: H515–H526, 2012. First published October 28, 2011; doi:10.1152/ajpheart.00703.2011.—Many studies have implicated the peroxisome proliferator-activated receptor (PPAR) family of nuclear receptor transcription factors in regulating cardiac substrate metabolism and ATP generation. Recently, evidence from a variety of cell culture and organ systems has implicated ubiquitin and small ubiquitin-like modifier (SUMO) conjugation as post-translational modifications that regulate the activity of PPAR transcription factors and their coreceptors/coactivators. Here we introduce the ubiquitin and SUMO conjugation systems and extensively review how they have been shown to regulate all three PPAR isoforms (PPARα, PPARβ/δ, and PPARγ) in addition to the retinoid X receptor and PPARγ coactivator-1α subunits of the larger PPAR transcription factor complex. We then present how the specific ubiquitin (E3) ligases have been implicated and review emerging evidence that post-translational modifications of PPARs with ubiquitin and/or SUMO may play a role in cardiac disease. Because PPAR activity is perturbed in a variety of forms of heart disease and specific proteins regulate this process (E3 ligases), this may be a fruitful area of investigation with respect to finding new therapeutic targets.

retinoid X receptor; small ubiquitin-like modifier; ubiquitin; phosphorylation

Introduction

Cardiac energy substrate utilization is transcriptionally controlled, in part, by the peroxisome proliferator-activated receptor (PPAR) family of transcription factors and their coreceptors/coactivators, including PPARα, PPARβ/δ, PPARγ, retinoid X receptor-α (RXRα), and PPARγ coactivator 1α (PGC-1α). Mechanistically, PPARs, RXR, PPARβ/δ, and PPARγ form heterodimers with the RXRα and coactivators (e.g., PGC-1α) and repressors [e.g., nuclear receptor corepressor (NCoR)] to regulate the transcription of genes involved in lipid metabolism and energy regulation (21, 48, 62). PPARs interact with their ligand(s), which include long chain fatty acids and their derivatives, whereas RXRα interacts with retinoic acid, both of which enhance the binding of PPAR complex to DNA binding elements in the promoter regions of genes, called PPAR response elements (PPREs) (59). Decreased cardiac PPAR activities have been implicated in the regulation of fatty acid utilization in mouse, rat, and dog models of heart failure (8, 64, 65, 74). The mechanisms by which PPAR is regulated are not entirely clear in the context of heart failure. However, recent studies have reported post-translational modifications of all three PPAR isoforms, the PPAR coactivator RXRα, or the PPAR coactivator PGC-1α, which may explain how PPAR isoform activities are regulated (52, 69, 71). PPARs and their coreceptors/coactivators also play nonmetabolic roles in cardiovascular disease, namely in the regulation of cardiac hypertrophy, myocardial inflammation, extracellular matrix remodeling, and oxidative stress. For example, PPAR activity regulates nuclear factor-kB (NF-kB) (19, 99), c-Jun/activator protein-1 (42), GATA (20, 33), and nuclear factor of activated T cells (5, 55) in response to hypertrophic and inflammatory stimuli, demonstrating that PPARs can play a protective role in cardiac disease independent of their ability to activate genes involved in fatty acid uptake and β-oxidation. PPARs may also play a role in the development of cardiac fibrosis and protection against cardiac oxidative stress (19, 37, 41, 85). In this review, we highlight recent studies implicating the post-translational regulation of PPAR transcription factors by ubiquitin and small ubiquitin-like modifier (SUMO) and then discuss how these modifications regulate PPAR function. While the studies presented in this review examine largely noncardiomyocyte systems, new studies have demonstrated their applicability to striated muscle in regulating fatty acid and glucose utilization and apoptosis. These findings offer insight into how the post-transcriptional regulation of PPARs may also be applicable to
an array of cardiac diseases characterized by altered PPAR activities.

Post-translational Modification by Ubiquitin and SUMO: Parallel Systems

The conjugation of ubiquitin or SUMO is unique among post-translational modifications in that it involves the attachment of another polypeptide (31, 60, 61, 96) instead of the addition of a functional group, such as a phosphate, acetate, lipid, or carbohydrate. The structures of ubiquitin, a 76-amino acid polypeptide, and SUMO, a 101-amino acid polypeptide, are very similar; however, they have only \( \sim 18\% \) sequence homology and differ greatly in their surface charge topology (88). The ubiquitin and SUMO conjugation pathways parallel each other in many aspects, only differing in the specific enzymes involved, as outlined in Fig. 1. In the first step, free ubiquitin or SUMO is covalently linked to the ubiquitin-activating enzyme (E1) in an ATP-dependent reaction (Fig. 1, A and B, far left) (70). Next, ubiquitin or SUMO is transferred from the E1 enzyme to the ubiquitin-conjugating enzyme (E2). Finally, the interaction between the E2 and the ubiquitin or SUMO ligase enzyme (E3) allows the E3 to initiate the transfer of ubiquitin or SUMO from the E2 to a lysine residue on the substrate (18, 32). The E3 is the pivotal component in both the ubiquitin and SUMO conjugation pathways since it confers specificity to the system by directly interacting with the substrate. Therefore, identifying ubiquitin ligase-substrate pairs is integral to understanding the cellular functions affected by ubiquitin or SUMO conjugation. Many E1, E2, and E3 enzymes in the ubiquitin pathway have been identified, whereas only a handful of these enzymes have been identified for the SUMO pathway (31, 43, 66). This distinction parallels our general understanding of the effects of ubiquitination and SUMOylation; i.e., much more is understood about the effects of ubiquitination compared with what is understood about the effects of SUMOylation.

The greatest difference between ubiquitination and SUMOylation lies in the effects that specific conjugation patterns foster on protein activity and cellular localization. The most commonly described ubiquitination pattern is polyubiquitination, where the initial ubiquitin molecule is conjugated to the substrate, followed by the addition of subsequent ubiquitin molecules linked by their lysine located in the 48th amino acid position (see Fig. 1C) (36). When the lysines are serially connected to each other by this canonical lysine number 48, it is referred to as a K48 (\( K = \) lysine) polyubiquitin chain. This canonical K48 ubiquitination pattern is recognized by the 26S proteasome, targeting the modified protein for degradation (Fig. 1C) (12, 36, 44). Other recognized ubiquitination patterns include monoubiquitination (the placement of only 1 ubiquitin on a substrate) (12) and polyubiquitination via atypical [non-K48 linked (K63)] ubiquitin chains (27). Monoubiquitination and polyubiquitination (Fig. 1C) via atypical ubiquitin chains have a wider effect on cellular function than polyubiquitination via K48, including translation, cell cycle regulation, signal transduction, protein trafficking, and protein-protein interactions (31, 39, 54, 96, 98). Similar to ubiquitination, SUMOylation patterns define the involvement of SUMO conjugation in cellular processes: polySUMOylation has been linked to the stress response, whereas monoSUMOylation has been primarily associated with transcription regulation, nuclear transport, and regulation of protein-protein interactions (43, 58, 82, 94).
Vertebrates have four SUMO isoforms, SUMO1–4, with the largest distinction between isoforms being the presence of a SUMOylation motif in SUMO-2/3 only. This SUMOylation motif allows for the formation of polySUMO chains with SUMO-2/3, but not with SUMO-1 (Fig. 1B) (28). The SUMO-4 isoform shares 87% amino acid homology with SUMO-2, but it lacks introns, suggesting it may be a pseudogene. Whereas endogenous SUMO-4 mRNA has been found in tissues, the protein has not been detected and it is unclear whether it can be conjugated to proteins based on its expected sequence and structure (11, 72, 97).

The post-translational modification of the PPAR complex with ubiquitin and SUMO has been described by a number of investigators. Figure 1D illustrates the many possible SUMO and ubiquitin modifications that may occur on the PPARα, PPARβ/δ, and PPARγ isoforms, the RXRα nuclear receptor, along with the PGC-1α coreceptor. Most of the studies described next investigate these post-translational modifications on individual subunits: the effects of SUMOylation and ubiquitination on PPAR activity likely reflect a composite of the post-translational modification on the individual subunits. Much work remains to be done to determine the molecular details of how specific ubiquitin and SUMO ligases affect the overall PPAR complex function.

Ubiquitination of PPARs

The regulation of PPARα by the ubiquitin proteasome system. Over the past 10 years, several lines of evidence have implicated the ubiquitin proteasome system (UPS) in the regulation of PPARα activity. In early studies, GFP- or hemagglutinin-tagged PPARα expression vectors were transfected into HeLa cells and analyzed by Western blot analysis for PPARα. These studies revealed that PPARα protein stability is greatly affected by the presence or absence of a selective PPARα ligand (40). Similar observations have been reported in HepG2 cells transfected with PPARα (10). In these cells, cotransfection with PPARα and hemagglutinin-tagged ubiquitin reveals that PPARα can be polyubiquitinated and that this post-translational modification is lost in the presence of multiple PPARα ligands. Likewise, increasing the expression of the RXRα and the CREB binding protein coactivator of PPARα (both PPARα coactivators) in HepG2 cells leads to a decrease in PPARα ubiquitination. Finally, when these cells are treated with MG132, a proteasome inhibitor, PPARα activity is increased, presumably because of the decrease in ubiquitin-mediated proteasomal degradation of PPARα (10).

Recently, additional molecular details surrounding PPARα ubiquitination and its effect on PPARα activity have been reported by Gopinathan et al. (34). In this study, an interesting relationship between PPARα activity and the ratio of PPARα to murine double minute 2 (MDM2, a ubiquitin ligase) was revealed. MDM2 associates with PPARα through the A/B domain of PPARα (Fig. 2A) and the coexpression of MDM2 increases PPARα ubiquitination (34). Increasing MDM2 levels relative to PPARα and PPARβ/δ, but not PPARγ, leads to a decrease in PPAR activity (34), whereas knocking down MDM2 expression levels with small interfering RNA in rat hepatoma cells inhibits mRNA expression of several PPARα targets (34). Increasing MDM2 levels in the presence of Wy-14643 (a PPARα selective agonist) leads to enhanced PPARα activity up to a ratio of MDM2 to PPARα <0.5 to 1; however, ratios of MDM2 to PPARα ≥1 inhibits PPARα activity (34). In summary, these studies demonstrate the ubiquitination of PPARα in a ligand-dependent manner and that effect of ubiquitination on PPARα activity depends on the systems studied (Table 1).

The regulation of PPARβ/δ by the UPS. Like PPARα, the mechanism by which ubiquitination affects PPARβ/δ activity and protein levels has been studied in different cell types. In U2OS human osteosarcoma cells expressing recombinant PPARβ/δ, PPARβ/δ is ubiquitinated and rapidly degraded in the absence of a ligand. However, the addition of PPARβ/δ-specific agonists such as L-165041, GW-501516, and the stable prostaglandin analog carboxaprostacyclin PGI2 completely inhibit PPARβ/δ degradation, a process that is reliant on the DNA binding domain of PPARβ/δ (Fig. 2B). When U2OS cells are treated with puromycin (an inhibitor of protein synthesis) and the proteasome inhibitor PS341, PPARβ/δ protein levels are stabilized to levels similar to what is seen with puromycin and L-165041 (a PPARβ/δ agonist) treatment, suggesting that L-165041 blocks ubiquitination and degradation of PPARβ/δ in U2OS cells (30) (Table 1). Independent of basal levels of PPARβ/δ, ligand binding of PPARβ/δ prevents its ubiquitination and subsequent degradation (30).

In contrast to the study described above, studies using mouse fibroblasts demonstrate that the ligand dependency of ubiquitination and degradation of PPARβ/δ is determined by PPARβ/δ protein levels. At low PPARβ/δ protein concentration, PPARβ/δ ubiquitination and degradation is not influenced by the synthetic agonist GW-501516 (77). However, at high PPARβ/δ levels, GW-501516 strongly inhibits the ubiquitination and degradation of PPARβ/δ (77). These findings have implications not only in the biological regulation of PPARβ/δ but also in the experimental design of overexpression systems used to determine the function and regulation of PPARβ/δ.

The regulation of PPARγ by the UPS. There are two forms of PPARγ that are generated from the same gene by alternative promoter usage, PPARγ1 and PPARγ2. PPARγ1 is found in most cell types, whereas PPARγ2 is found exclusively in adipose tissues (91). Ubiquitination of PPARγ has only been studied in adipocytes (26, 38, 46), and therefore our knowledge of the regulation of PPARγ by the UPS is limited to the PPARγ2 moiety. Like other PPAR family members, the rate at which PPARγ2 is degraded is dependent on its interaction with ligands and appears to be mediated by a ubiquitin-dependent mechanism. However, unlike PPARα and PPARβ/δ, ubiquitin-mediated regulation of PPARγ2 is enhanced rather than inhibited in the presence of PPARγ2-specific ligands. When differentiated adipocyte cells (3T3-F442A) are treated with the PPARγ2 ligand pioglitazone (or other thiazolidinediones), a dose-dependent increase in ubiquitination and a subsequent decrease in the PPARγ2 protein expression are observed (38). When the proteasome is inhibited in this adipocyte cell line, the degradation of PPARγ2 is also inhibited, indicating that the degradation of PPARγ2 in the presence of ligand occurs via proteasomal degradation. Interestingly, PPARγ2 constructs containing mutations in the activation function-1 domain, part of the ligand binding domain, are able to attenuate this ligand-dependent degradation of PPARγ2, supporting the theory that ligand binding to PPARγ2 is necessary for its ubiquitin-mediated proteasomal degradation (38) (Fig. 2C). In the con-
text of the previous two sections, these findings demonstrate that all three members of the PPAR family are degraded by the UPS, albeit by different mechanisms. Table 1 summarizes the studies demonstrating how degradation of PPARα and β/δ by the UPS is enhanced in the presence of ligand, whereas PPARγ degradation is inhibited, demonstrating the diverse regulation that the UPS has on PPAR isoforms (Table 1).

SUMOylation of PPARs

The regulation of PPARα by SUMOylation. Evidence from a number of cell studies suggests that SUMOylation of PPARα is a biochemical mechanism by which the cell regulates PPARα activity. In both COS-7 and human hepatoma cells (HuH-7), the SUMO E2 enzyme Ubc9 and the SUMO ligase PIASy SUMOylate PPARα with a SUMO-1 moiety at position K185 (75) (Fig. 2A), resulting in an inhibition of PPARα transcriptional activity. Conversely, substitution of K185 with an arginine (thereby preventing SUMOylation) increases the transcriptional activity of PPARα, indicating the importance of the SUMOylation at K185 in negatively regulating PPARα transcription (75). K185 SUMO-1 is also important for the recruitment of the corepressor protein NCoR (but not NOR2/NCoR2), which results in further repression of PPARα’s activity (75). Interestingly, similar to the case of PPARα ubiquitination, SUMOylation of PPARα is dependent on the presence or absence of specific ligands (75), demonstrating a parallel between the process of ubiquitination and SUMOylation of PPARα (Table 1).

SUMOylation of PPARα has also been described to occur in the liver where this post-translational modification results, once again, in the repression of PPARα activity. In studies using a mouse model designed to determine sex-specific PPARα-regulated gene repression, the repression of PPARα target hepatic genes involved in steroid metabolism and immunity were identified in female, but not male, mice (53). Further investigation revealed that SUMOylation of PPARα occurs (above each PPAR isoform) or, more broadly, that ubiquitination occurs (below each PPAR isomorph). The specific amino acids where SUMO modifications have been reported are indicated by the lysine (K) position in the PPAR amino acid sequence. Lysines in the 185, 358, 185, 77/107, and 365/395 positions have been reported as indicated above. For more experimental detail leading to these findings, see Table 1. N, NH2 terminus; C, COOH terminus.
Table 1. **Summary of ubiquitin and SUMO modification of PPARs and the resulting effects on transcription in the presence or absence of ligand**

<table>
<thead>
<tr>
<th>Nuclear Receptor</th>
<th>Post-translational Modification</th>
<th>Ubiquitin/SUMO Ligase (E3) Identified</th>
<th>Modification Type</th>
<th>Transcriptional Affect</th>
<th>Effect of Ligand</th>
<th>Cell type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα</td>
<td>Ubiquitination</td>
<td>MDM2</td>
<td>Polyubiquitination</td>
<td>MDM2-to-PPARα ratio &lt; 0.5: Activation MDM2-to-PPARα ratio ≥ 1.0: Inhibition</td>
<td>Wy14643 is required for MDM2-dependent modulation of PPARα activity</td>
<td>Rat hepatoma</td>
<td>(30)</td>
</tr>
<tr>
<td></td>
<td>SUMOylation</td>
<td>PIASy</td>
<td>SUMO-1, K185</td>
<td>Inhibition of PPARα activity by promoting its degradation</td>
<td>Wy14643 blocks PPARα polyubiquitination</td>
<td>HepG2</td>
<td>(11, 12, 36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SUMO-2/3, K358</td>
<td>SUMO-1, K358</td>
<td>Inhibition of PPARα activity by recruiting NCoR corepressor</td>
<td>GW-7647 blocks PPARα monoSUMOylation</td>
<td>COS-7; human hepatoma</td>
<td>(66)</td>
</tr>
<tr>
<td>PPARβ (also known as PPARδ)</td>
<td>Ubiquitination</td>
<td>Not determined</td>
<td>Polyubiquitination</td>
<td>Inhibition of PPARβ activity by promoting its degradation</td>
<td>PPARβ low and high: GW-501516, L-165041, and cPGI, block PPARβ polyubiquitination (HEK293; mouse fibroblasts)</td>
<td>U2OS; human lung cell lines</td>
<td>(28)</td>
</tr>
<tr>
<td></td>
<td>SUMOylation</td>
<td>PIAS1 &amp; PIASx</td>
<td>SUMO-1, K77/ K107 SUMO-1, K365/K395</td>
<td>Inhibition of PPARγ1 activity by promoting its degradation Inhibition of PPARγ1 activity by recruiting NCoR corepressor</td>
<td>Thiazolidinediones enhance PPARγ polyubiquitination</td>
<td>HEK293; mouse fibroblasts</td>
<td>(68)</td>
</tr>
<tr>
<td>PPARγ1/2</td>
<td>Ubiquitination</td>
<td>Not determined PIAS &amp; PIASβ SUMOylates SENP2 deSUMOylates</td>
<td>Polyubiquitination</td>
<td>Inhibition of PPARγ1 activity by promoting its degradation Inhibition of PPARγ1 activity by recruiting NCoR corepressor</td>
<td>Rosiglitazone enhances PPARγ1 SUMOylation</td>
<td>Differentiated adipocytes</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>SUMOylation</td>
<td>SUMO-1, K77/ K107 SUMO-1, K365/K395</td>
<td>SUMO-1, K365/K395</td>
<td>Inhibition of PPARγ1 activity by recruiting NCoR corepressor</td>
<td>Rosiglitazone has no affect on PPARγ1 deSUMOylation (C2C12)</td>
<td>HEK293; HepG2; NIH3T3; vascular smooth muscle cells; in vivo rat heart artery; C2C12</td>
<td>(15, 24, 49, 60, 64, 75, 89)</td>
</tr>
</tbody>
</table>

See main text for definition of abbreviations.
gene repression can be recapitulated in male mice treated with the PPARα ligand WY-14643, indicating that, in contrast to K185 SUMOylation of PPARα described above, ligand treatment initiates (rather than inhibits) PPARα SUMOylation on K358. This occurs as a result of a ligand-induced conformational change in the LBD that presents K358 at the surface of the protein (Fig. 2A). Interestingly, the ability of K358-SUMOylated PPARα to repress CYP7B1 expression is independent of PPARα’s ability to bind DNA, since the CYP7B1 promoter does not have a PPRE. Instead, K358 SUMOylation of PPARα promotes its interaction with the DNA binding subunit of GA binding protein (GABP), a heterodimeric transcription factor (Table 1). By binding GABP, SUMOylated PPARα promotes DNA methylation and histone acetylation of the CYP7B1 promoter, which leads to the repression of the gene (53). While considerable progress has been made in identifying how SUMOylation regulates PPARα activity, very little is known about the SUMOylation of PPARβ/δ and its effects on activity.

The regulation of PPARβ/δ by SUMOylation. One study has suggested a potential SUMOylation site of PPARβ/δ in the D region (K185), but evidence for this modification in any system has yet to be reported (Fig. 2B).

The regulation of PPARγ by SUMOylation. Experimental evidence has suggested that SUMOylation of PPARγ inhibits its activity. While both PPARγ isoforms are reported to be SUMOylated in their activation function-1 (AF-1) domain (Fig. 2C) (84), most SUMOylation studies have focused on PPARγ because it is ubiquitously expressed. Initial studies demonstrated that PPARγ can be covalently modified by SUMO-1 in HEK293 cells and that the SUMO E3 PIAS could enhance PPARγ’s SUMOylation (68). These studies also found that the lysine residue 107 (K107) within AF-1 region of PPARγ is modified by SUMO-1 (Fig. 2C). Other SUMO E3s in the PIAS family, including PIAS1 and PIASxβ, can also SUMOylate PPARγ in HEK293 cells (68). Mutating the K107 site enhances PPARγ transcriptional activity, suggesting that SUMOylation represses PPARγ activity (68). Similarly, PPARγ-dependent apoptosis is induced by rosiglitazone in HepG2 hepatoblastoma cells when the K107 SUMOylation of PPARγ mutant (K107R) is expressed, suggesting that PPARγ transactivation is modified by SUMO-1 to inhibit downstream PPARγ-mediated apoptosis (68).

Consistent with the study described above, reporter assays using the K107R mutant PPARγ have demonstrated a stronger transactivation of the PPARE promoter regions compared with wild-type in NIH3T3 cells (84, 100). Again, these results suggest that SUMO-1 modification negatively regulates PPARγ activity (84, 100). Studies by other groups, aimed at defining the mechanism of SUMOylation-dependent repression of PPARγ activity, found that SUMOylation of PPARγ does not affect the nuclear localization of PPARγ; instead, SUMOylation affects PPARγ’s stability and transcriptional activity (25). Interestingly, additional studies using a PPARγ phosphorylation mutant at S112 demonstrated that the lack of phosphorylation at this site promotes K107 SUMOylation, increasing the potency of the SUMOylation repressive effects (84, 100). These data demonstrate that SUMOylation of PPARγ may depend on the phosphorylation state of PPARγ, indicating multiple levels of post-translational regulation of PPARγ activity.

PPARγ agonists have been shown to inhibit the inflammatory response by blocking the activity of the proinflammatory NF-κB transcription factor in macrophages (73). These studies found that in the presence of ligand, PPARγ is SUMOylated at lysine 365 (K365) in the ligand-binding domain (Fig. 2C), targeting PPARγ to the NCoR-histone deacetylase-3 complex on inflammatory gene promoters (73). This recruits ubiquitination and degradation that mediates the removal of corepressor genes (73). PPARγ in addition can bind to PPREs in PPAR regulated genes to enhance their expression. This mechanism may explain how agonist-bound PPARγ can effectively inhibit NF-κB target genes. This mechanism contrasts to the PPARγ SUMOylation at K107 described above in HepG2 cells, whereby SUMOylation inhibits the transactivation (activation) of PPARγ target genes (14, 56, 68, 84, 100) and PPARγ SUMOylation at K365 activates the transrepression (inhibition) of PPARγ induction of target genes (73).

Post-translational Modifications of the PPAR Coreceptor RXRα

The regulation of RXRα by ubiquitination. Ubiquitination of the PPAR coreceptor RXRα has been reported, although there are a limited number of studies that address this particular post-translational modification (summarized in Table 2). Since PPAR transcription factors work by dimerizing with RXRα, these studies likely have relevance to the post-translational regulation of PPARs. Recent studies have demonstrated that RXR homologs are ubiquitinated by ubiquitin ligases. However, it has not been determined whether this leads to degradation of RXR, which would be expected to inhibit PPAR activity overall. A yeast two-hybrid screen of the Schistosoma mansoni (Sm) cDNA library using SmRXR1 and SmRXR2 as bait identified the RING finger protein Sm seven in absentia (SmSINA) as a potential ubiquitin ligase specific for SmRXRs (22). In vitro ubiquitination assays demonstrated that SmSINA has ubiquitin ligase activity and can polyubiquitinate both SmRXR1 and SmRXR2, targeting them for proteasomal degradation (22). The DNA binding domain of SmRXRs shares 80% homology with mammalian RXRα, but the ligand-binding E domain of SmRXRs shares only 22–25% homology with mammalian RXRα (17). Since SmSINA interacts with the E domains of SmRXR1 and SmRXR2, where S. mansoni and mammals share the least homology in RXR sequence, it is unclear whether SINA-dependent degradation of RXRα would occur in mammals. Another RING finger protein, RNF8, has also been identified as an RXRα-interacting protein using a yeast two-hybrid screen of a human liver cDNA library (90). Interestingly, increasing expression of RNF8 in COS7 cells has no effect on RXRα ubiquitination but increases its transactivation ability, a phenomena that is enhanced by retinoic acid treatment (90). Although this pathway does not lead to RXRα ubiquitination, it is an interesting example of how interaction with a ubiquitin ligase can affect a protein’s function in a ubiquitin-independent manner.

Further studies aimed at determining the physiological relevance of RXRα ubiquitination have revealed that RXRα is polyubiquitinated in smooth muscle cells (SMCs) derived from the myometrium and that this ubiquitination is significantly inhibited in cells isolated from leiomyomas, a benign smooth muscle neoplasm of the uterus. Protein lysates from tissue
Table 2. Summary of ubiquitin and SUMO modification of PPAR coreceptor RXRα and coactivator PGC-1α and the resulting effects on transcription

<table>
<thead>
<tr>
<th>PPAR Coreceptor/Coactivator</th>
<th>Post-translational Modification</th>
<th>Ubiquitin/SUMO Ligase (E3) Identified</th>
<th>Modification Type</th>
<th>Transcriptional Affect</th>
<th>Effect of Ligand</th>
<th>Cell Type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RXRα</td>
<td>Ubiquitination</td>
<td>SmSINA and RNFL (E3 ligase?) Not determined</td>
<td>Polyubiquitination targets Schistosoma mansoni RXR homologues (RXR1, RXR2) for degradation RNFL enhances RXRα activity independent of ubiquitination polyubiquitination K108 SUMO-1</td>
<td>Not determined Inhibition of RXRα by promoting its degradation</td>
<td>Not determined 9-cis-retinoic acid enhances RXRα polyubiquitination</td>
<td>COS-7 Smooth muscle cells derived from human myometrium and leiomyomas</td>
<td>(21)</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>Ubiquitination</td>
<td>Not determined</td>
<td>Polyubiquitination in nucleus (NH2-terminal pathway?) K183 SUMO-1 K183 SUMO-1</td>
<td>SUSP1 deSUMOylation of RXRα enhances its activity. Inhibition of PGC-1α by promoting its degradation</td>
<td>Not determined</td>
<td>HEK293</td>
<td>(14)</td>
</tr>
<tr>
<td>SUMOylation</td>
<td>SUSP1 (SEN6) SUMO protease (Removes SUMO)</td>
<td>K183 SUMO-1 K183 SUMO-1</td>
<td>SUMOylation of K183 activity</td>
<td>Not determined</td>
<td></td>
<td>COS-7; HL-1 (cardiac muscle cell line)</td>
<td>(2, 74)</td>
</tr>
<tr>
<td></td>
<td>PIAS1 and PIAS3 SUMOylates SUSP1 (SEN6) SUMO protease (Removes SUMO)</td>
<td></td>
<td>SUMOylation of K183 activity</td>
<td>Not determined</td>
<td></td>
<td>COS-1; HeLa</td>
<td>(71)</td>
</tr>
</tbody>
</table>

See main text for definition of abbreviations.
Post-translational Modifications of the PPAR Coactivator PGC-1α

In contrast to the PPAR coreceptor RXRα, the post-translational modifications of the coactivator PGC-1α have been thoroughly studied. As with other coactivators, when PGC-1α binds to the PPAR/RXRα coreceptors, it enhances their activity. Hence, post-translational modifications of PGC-1α have a direct bearing on PPAR activity. Known post-translational modifications of PGC-1α include phosphorylation, acetylation, methylation, O-linked-N-acetylgalactosylation, ubiquitination, and SUMOylation. These PGC-1α modifications and its modifiers have been recently reviewed by Fernandez-Marcos and Auwerx (23). For the purposes of this review, we will provide an overview in detail of those studies that have focused on the ubiquitination and SUMOylation of PGC-1α.

The regulation of PGC-1α by ubiquitination. The PPAR coactivator PGC-1α is ubiquitinated and targeted for degradation in the nucleus; however, the details of this mechanism are just starting to be understood. In response to oxidative stress, PGC-1α’s subcellular localization changes from a cytoplasmic distribution to a nuclear distribution, promoted by sirtuin-1 deacetylation (2). Moreover, PGC-1’s activity is regulated by glycogen synthase kinase-3β, which targets PGC-1α for intranuclear degradation (2). While the details of this process have not been delineated, the phosphorylated form of PGC-1α may make it a unique target for ubiquitination (i.e., the non-phosphorylated form is not ubiquitinated), leading to subsequent degradation and inhibition of its activity (2). Experimental evidence suggests that the COOH-terminal serine-arginine-rich and RNA recognition motif domains of PGC-1α are required for its polyubiquitination and subsequent proteasomal degradation in COS-7 cells (83). Interestingly, when the COOH-terminal region of PGC-1α is expressed alone, it is polyubiquitinated but resistant to proteasomal degradation. Instead, the COOH-terminal fragment forms intranuclear aggregates, tightly complexed with promyelocytic leukemia nuclear bodies, a phenomenon that does not occur with the full-length protein (83). This observation led to the report that proteasomal targeting of polyubiquitinated PGC-1α is dependent on its NH2 terminus, which contains two regions rich in proline (P), glutamic acid (E), serine (S), and threonine (T), termed PEST domains. PEST sequences have been found in proteins with a short intracellular half-life, suggesting they act as a signal for protein degradation, and the above observations would therefore suggest that PEST domains are essential for PGC-1α degradation (76, 83).

Trausch-Azar et al. (92), however, assert that the PEST regions do not play a role in PGC-1α turnover. They instead implicate the NH2-terminal region of PGC-1α as being targeted by the NH2 terminus-dependent ubiquitin proteasome pathway. In the NH2 terminus-dependent pathway, ubiquitin is transferred to the free NH2-terminal residue rather than the e-NH2 group of an internal lysine (16). Using a functional truncated form of PGC-1α, which is predominately nuclear and contains intact activation and the nuclear receptor domains, Trausch-Azar et al. (92) demonstrated that PGC-1α is degraded even when all of the four lysine residues within it are mutated to arginine. Furthermore, this study revealed that whereas the fragment they generated was nuclear and rapidly degraded, the endogenous PGC-1α splice variant, novel truncated (NT) -PGC-1α, is stable and cytoplasmic. This suggests that the cellular localization differences between these two NH2-terminal fragments of PGC-1α is dependent on their ability to interact with chaperones carriers, where NT-PGC-1α may not be able to interact with these chaperones, leading to its exclusion from the nucleus (92). While a post-translational modification of PGC-1α with ubiquitin appears to target it for degradation to inhibit its activity, other post-translational modifications inhibit it through other mechanisms. SUMOylation, for example, inhibits PGC-1α, inhibiting its transcriptional activity by affecting the activity of PGC-1α binding partners.

The regulation of PGC-1α by SUMOylation. The SUMO ligases PIAS1 and PIAS3 SUMOylate PGC-1α on the conserved lysine residue K183 in the NH2-terminal domain between the PEST motifs, resulting in the inhibition of its transcriptional activity (80). Acetylation, phosphorylation, and ubiquitination do not affect the ability of PGC-1α to be SUMOylated (80). Although SUMOylation does not affect subcellular localization or the stability of PGC-1α, it does attenuate its transcriptional activity, possibly by enhancing its interaction with RIP140, a corepressor (80). Interestingly, mutating the SUMOylation consensus sequence abolished PGC-1α-dependent PPARγ transcriptional activity, demonstrating that SUMOylation of PGC-1α constitutes an additional layer of regulating PPARγ activity in an indirect manner via PGC-1α (80).

The Significance of Post-translational Regulation of the PPAR Complex to Cardiac Disease

The role of PPARs in the heart. The pathophysiology of common cardiac diseases involves distinct changes in myocardial fuel utilization. The best examples of this include cardiac hypertrophy and heart failure, where the heart switches from the predominant use of fatty acids to glucose as its primary source of energy (93). In both humans and animal models alike, the development of cardiac hypertrophy results in decreases in PPARα expression that parallel a depression of fatty acid utilization (6, 45, 81). Experimental studies using transgenic and knockout animals confirm that PPARα does have a significant role in this metabolic shift. Conversely, the diabetic myocardium essentially does the opposite as the heart undergoing cardiac hypertrophy: it relies almost exclusively on fatty acids and uses glucose to a very small extent (9, 78, 87). While the cardiac changes in diabetic cardiomyopathy are caused by a number of complex mechanisms (89), fatty acid upregulation of PPARα activity has been proposed as one underlying mechanism (35). Overall, these dynamic shifts in myocardial fuel utilization by the regulation PPARα are characteristic in most common cardiac diseases.

A great deal of experimental evidence has placed the regulation of these changes in fatty acid and glucose utilization on the PPAR transcription factors (59). Alterations of all three PPARs in the heart results in derangements in fatty acid and glucose metabolism that result in a significant phenotype or increased susceptibility to insults (for a comprehensive review, see Madrazo and Kelly (59)). These findings are concrete enough to have evoked a number of therapeutic interventions focusing on partially restoring fatty acid utilization while inhibiting glucose utilization, including PPAR agonists (67). Despite the realization of the importance of PPAR regulation in
cardiac disease, the mechanisms by which they are regulated at the level of the cardiomyocyte have not been clearly delineated. Post-translational modifications of PPARs are emerging as one such way PPARs are regulated, including ubiquitination and SUMOylation. Evidence for their regulation in cardiomyocytes is just emerging in studies investigating other types of striated muscle.

Ubiquitination and SUMOylation of PPARs in the heart. To date, regulation of PPARα, PPARβ/δ, or PPARγ by ubiquitination or SUMOylation in the heart has not been reported. However, there are several examples whereby PPARs are regulated by these post-translational modifications in other closely related types of muscle cells. For example, recent studies have demonstrated the physiological relevance of PPARγ SUMOylation in striated muscle cells, suggesting that PPARγ SUMOylation may play a role in regulating the extent to which fatty acids are used in the production of ATP. In these studies, PPARγ was deSUMOylated by increasing the expression of SENP2 (a SUMO-specific protease/removes SUMO) in C2C12 myotubes (striated muscle cells). By deSUMOylating PPARγ, an enhanced PPARγ activity was detected by the identification of the enhanced expression of the PPARγ1 target genes fatty acid binding protein 3 (FABP3) and fatty acid translocate (CD36), in both the presence and absence of the PPARγ agonist rosiglitazone (15). Similarly, deSUMOylation of PPARγ1 by SENP2 increases the chromatin immunoprecipitation of PPREs of the endogenous PPARγ1 target genes CD36 and FABP3 (14, 15). These observations indicate an important role for both SUMO E3s as well as SUMO-specific proteases (deSUMOylases) in regulating PPARγ activity in skeletal muscle.

Other studies have identified that SUMOylation of PPARγ1 promotes proliferation and migration of vascular SMCs (VSMCs). This has been demonstrated using VSMCs transfected with a PPARγ1 construct in which K107R, the lysine on which SUMOylation occurs, is mutated, thereby inhibited SUMOylation. Inhibiting PPARγ1 SUMOylation in this manner results in a more potent transcriptional inhibition of inducible nitric oxide synthase compared with cells transfected with a wild-type construct (56). In addition, the PPARγ1 K107R mutant is more efficient at inhibiting proliferation and migration compared with wild-type PPARγ1, suggesting that PPARγ1 SUMOylation may play a role in promoting atherosclerosis, since VSMC proliferation and migration play a significant role in determining lesion severity. Indeed, adenoviral expression of the PPARγ1 K107R construct in the carotid arteries of rats after balloon injury results in a significantly decreased intima-to-media ratio compared with that in the controls (56). Consistent with this in vitro data, the PPARγ1 K107R-expressing arteries display a lower proliferation index and higher apoptotic index compared with those in wild-type PPARγ1 (56). These studies demonstrate a role for SUMOylation of PPARγ1 at K107 in VSMCs in the atherosclerotic response.

These examples of PPARγ SUMOylation in regulating the fatty acid oxidation response and apoptosis in striated muscle and vascular smooth muscle, respectively, provide support for the concept that PPARs could be regulated posttranslationally in the heart. This concept of post-translational regulation of PPAR activity in cardiomyocytes in not new. Specifically, PPARα phosphorylation by the MAPK p38 decreases PPARα transcriptional activity (7). Since the p38 pathway is activated in response to cardiac stress, such as that found in cardiac hypertrophy, heart failure, and diabetes, this study implicates PPARα activation as a mechanism by which the heart responds to adverse stimuli. The broader implications of these studies are that the fatty acid and glucose shifts seen in diseases such as cardiac hypertrophy, heart failure, and diabetic cardiomyopathy may be due to these regulatory mechanisms (67) (9, 78, 87). This concept has yet to be tested directly.

The role of PGC-1α in the heart. In addition to its coactivator function in the PPAR complex, PGC-1α is also a potent regulator of mitochondrial biogenesis. Increased expression of PGC-1α in cardiomyocytes activates mitochondrial biogenesis, oxidative phosphorylation, and respiration (50, 86). The mitochondrial biogenesis enhanced by increased constitutive PGC-1α expression can lead to such sarcomere displacement as to lead to heart failure (50). Even transient increases in PGC-1α expression leads to contractile dysfunction, which is reversible (79).

While excessive PGC-1α expression may have some detrimental effects, it generally is associated with directing beneficial adaptations in the heart and skeletal muscle. For example, long-term exercise leads to increased PGC-1α expression, increased mitochondrial content, and a resistance to fatigue (4). Pressure overload-induced cardiac hypertrophy induces enhanced PGC-1α expression and its target genes of fatty acid oxidation and oxidative phosphorylation (8, 29, 51). Conversely, when PGC-1α is knocked out of the heart and skeletal muscle, the gene expression of oxidative phosphorylation is blunted, leading to reduced mitochondrial activity and decreased ATP (3). Similarly, isolated hearts from PGC-1α−/− mice have decreased cardiac output in response to stimulation (3) and an enhanced mitochondrial susceptibility to apoptotic stimuli (1). These studies indicate a role of PGC-1α in the heart in response to cardiac stresses by supporting both metabolic adaptations (described above) and mitochondrial function (specifically oxidative phosphorylation) as well as the hearts susceptibility to apoptosis. The factors regulating PGC-1α in the heart have not yet been identified.

Ubiquitination and SUMOylation of PGC-1α in the heart. While regulation of PGC-1α by SUMO has not been identified in cardiomyocytes directly, recent studies have implicated the UPS in the degradation of PGC-1α (92). The steady-state levels of PGC-1α are controlled by dynamic changes in its synthesis and degradation. Its synthesis is regulated by a number of dietary and physiological factors, including exercise (24, 57). When full-length PGC-1α is expressed, it is rapidly degraded (half-life < 30 min). When the NH2-terminal splice variant of PGC-1α (NT-PGC-1α) is expressed, it remains in the cytoplasm and is stable (half-life > 7 h). In the presence of the proteasome inhibitor MG-132, ubiquitin PGC-1α conjugates accumulate, suggesting a role of the UPS in its degradation of PGC-1α (92). These findings suggest that PGC-1α is degraded by the ubiquitin proteasome, targeted by yet to be identified ubiquitin ligases. While much work is necessary to delineate the mechanisms by which the UPS regulates PGC-1α, it appears critical to the regulation of steady-state levels of PGC-1α and the truncated NT-PGC-1α isoforms. The functional consequences of UPS regulation of PGC-1α remain to be determined.
Summary

Results from the studies described above demonstrate several general principles surrounding PPAR post-translational modifications. First, ubiquitination and SUMOylation decrease the expression of PPAR target genes, either by promoting PPAR degradation (via ubiquitination) or recruiting additional proteins to PPAR promoter sequences that inhibit PPAR transactivation function or enhance PPAR transrepression function (SUMOylation) (Table 1). The one exception to this general statement is the case where PPARα-dependent transcription is actually activated by MDM2 at the MDM2-to-PPARα ratio of <0.5 (Table 1). The effect of ligand treatment on post-translational modifications of PPARs does not follow an observable pattern. Instead, ligand treatment can inhibit, enhance, or have no effect on the modification of PPARs depending on PPAR concentration and cell type (Table 1). Adding to the complexity of PPAR regulation by post-translational modifications is the fact that RXRs and PGC-1α, a coactivator and coregulator, respectively, are also able to be ubiquitinated and SUMOylated (Table 2) in ways that may affect PPAR activity, an end point that needs considerable more study. These studies have real relevance for the use of selective PPAR isotype-specific agonists, which have been shown to regulate cardiac structure and function. However, PPAR-directed therapies often have unexpected effects because of their pleiotropism and bodywide distribution. Understanding the interplay between post-translational modifications, ubiquitin ligase (E3) concentrations, and PPAR activity will help develop more rational means of treating cardiac disease. This may allow for the development of more targeted therapies that may include additional cardiogenic-specific regulators of PPARs, including ubiquitin and SUMO ligases (E3).

REFERENCES

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

K.M.W. and M.S.W. analyzed data; K.M.W. and M.S.W. interpreted results of experiments; K.M.W. and M.S.W. prepared figures; K.M.W. and M.S.W. drafted manuscript; K.M.W. and M.S.W. edited and revised manuscript; K.M.W. and M.S.W. approved final version of manuscript.

GRANTS

SUMO


