Review Article

Peroxisome Proliferator-Activated Receptors: Features, Functions, and Future

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Abstract. In this review, the history of the peroxisome proliferator-activated receptors (PPAR\(\alpha\), PPAR\(\beta/\delta\) and PPAR\(\gamma\)) discovery is briefly traced and major features of their structure and posttranslational modifications are presented. Furthermore, an overview of PPAR coactivators and corepressors as well as of endogenous and exogenous ligands is discussed. We have also summarized significant efforts underway to develop more effective and safer PPAR modulators as therapeutic agents to treat diseases such as diabetes, cancer, atherosclerosis, and inflammation. Finally, we share a hypothesis proposing how PPARs may control inflammatory events.

Keywords: PPARs; nuclear receptors; ligands; inflammation; endosalicylates

1. Introduction

In the course of attempting to delineate the mechanism(s) by which some chemicals induce peroxisome proliferation in rodents, a receptor, peroxisome proliferator-activated receptor (mouse PPAR\(\alpha\)), was discovered [1]. Shortly thereafter, two more PPAR subtypes, PPAR\(\beta/\delta\) and PPAR\(\gamma\), were identified. Although the three receptor subtypes share a high degree of homology, they differ in tissue distribution and level of expression as well as in ligand specificity [2].

As research in the field progressed, it was discovered that PPARs regulate a variety of biological processes in various tissues. Among other effects, PPAR\(\alpha\) control lipid metabolism and inflammatory processes, while PPAR\(\beta/\delta\) regulates glucose utilization, cell differentiation and inflammation. PPAR\(\gamma\) is involved in adipocyte differentiation, glucose metabolism and inflammatory pathways [3].

The receptor activity is modified posttranslationally by phosphorylation, sumoylation and ubiquitination as well as controlled by a myriad of coregulators [4–6]. Research in the field continues to reveal new roles for these receptors in a variety of normal and disease conditions. In this review we trace the history of the receptor discovery and describe their identified features and posttranslational modifications.
Furthermore, an overview of PPAR coactivators and corepressors as well as endogenous and exogenous ligands is presented. We also present a plausible hypothesis stipulating how PPARs may control inflammation.

2. Historical Perspective

It has been well documented that an increase in size and/or number of peroxisomes in the rodent liver is caused by a group of structurally diverse chemicals known as peroxisome proliferators [7]. However, despite dissimilarity in the structure of these chemicals (Table 1), a receptor-mediated mechanism for peroxisome proliferation was postulated [8]. A peroxisome proliferator-binding protein was later purified from rat liver cytosol and was identified as a dimer protein which has a molecular weight of 140,000–160,000 KDa [9]. This protein was capable of binding to peroxisome proliferators structurally related to clofibrate and was suggested to play an important role in the regulation of peroxisome proliferator-induced pleiotropic response [9]. Further analysis of the isolated protein revealed that it was homologous with the heat shock protein HSP70 [10]. Eventually, PPARα was discovered [1] and subsequent studies indicated that Hsp72 and PPAR form a complex in vivo, suggesting that this protein may play a role in the activity of PPARs [11].

The cloned receptor was found to possess structural similarities to the steroid hormone receptors. Since the identified receptor was thought to mediate the peroxisome proliferative response to peroxisome proliferating chemicals, the receptor was named peroxisome proliferator-activated receptor (PPAR).

Following the discovery of mouse PPARα, the receptor was identified in other species, including rat [12] and human [13]. In addition, three related Xenopus receptors were cloned, PPARα, PPARβ and PPARγ [14]. PPARδ was later identified in human and was found to be closely related to PPARβ described earlier in Xenopus. These receptors bind to and are activated by numerous ligands, including fatty acids, eicosanoids and numerous xenobiotics; some of which possess therapeutic value [15–17]. Structures of representative ligands are presented in Figures 1 and 2.

3. PPARs: Genomics and Proteomics

3.1. Genomics.

3.1.1. PPARα. Several genetic variants of PPARα have been identified (Table 2). A variant lacking exon 6, found in human tissues, is generated by alternative splicing [18, 19]. The corresponding protein of this variant is localized exclusively in the cytoplasm and inhibits wild type PPARα protein activity [18]. In addition, the presence of two other PPARα variants has been revealed; one with a mutation at codon 162 (L162V) and the other with a less frequent mutation at position 131 (R131Q) [20, 21]. These variants possibly account for species-related differences in the response to PPARα activators (Table 3). It has also been suggested that the PPARα L162 V polymorphism is involved in liver tumor progression in patients with hepatocellular carcinoma [22]. Another variant, PPARα V227A, is considered a major polymorphism in the Japanese population [23]. An association has been described between this polymorphism and the pathogenesis of non-alcoholic fatty liver disease as well as with a protective role against obesity [24].

3.1.2. PPARβ/δ. A 3′ splice variant of human PPARδ (PPARδ2) has been reported [25]. This variant is a potential repressor of the PPARδ wild type receptor. The existence of a PPARδ +294T/C polymorphism (Table 2) has also been demonstrated [26], and this polymorphism is associated, in humans, with elevated levels of LDL and apolipoprotein B, lower levels of HDL and higher risks of coronary heart disease [27–29]. The +294T/C polymorphism of this receptor may be linked to an increase in fasting glucose levels in women with polycystic ovary syndrome [30].

3.1.3. PPARγ. It has been reported that alternate transcription start sites and alternative splicing are responsible for the generation of four types of PPARγ mRNA. However, it is believed that mRNAs of PPARγ 1, 3, and 4 are translated into an identical protein [31, 32]. The presence of PPARγ2 Pro12Ala variant (Table 2) in humans has been reported [33]. An association of this polymorphism with type2 diabetes, insulin resistance and obesity is controversial where conflicting data, regarding its effect in different populations, are available [34–38]; with gender differences as well as genetic factors potentially contributing to the discrepancy of reported results [39–41]. A meta-analysis study shows that the Ala allele is associated with a lower risk of developing type2 diabetes in Caucasians and with improved insulin sensitivity in overweight individuals [42].

Studies have suggested that PPARγ Pro12Ala expression may increase the risk of cognitive impairment and dementia upon the development of diabetes [43], and this polymorphism may also play a role in the development of dementia at a younger age [44]. In addition, an association of this polymorphism with peripheral arterial disease has been reported [45]. Furthermore, involvement of the PPARγ Pro12Ala polymorphism has been mentioned in the development of gastric cancer [46–48] as well as endometriosis [49].

Another frequent PPARγ polymorphism (C1431T) was identified and found to be associated with higher plasma leptin levels [50]. While some studies reported no association between this polymorphism and body mass index (BMI) [50, 51], other investigations linked C1431T to higher BMI values [52]. Studies have also reported opposing effects of C1431T polymorphism and Pro12Ala polymorphism on BMI and diabetes [53, 54].

In addition to the above mutations, a PPARγ C190S mutation has been associated with partial lipodystrophy.
Table 1: Representative Peroxisome Proliferators.

<table>
<thead>
<tr>
<th>Fibrates</th>
<th>Nonfibrates</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bezafibrate</td>
<td>Phthalic acid esters</td>
<td>Aspirin</td>
</tr>
<tr>
<td>Ciprofibrate</td>
<td>Mono (2-ethylhexyl) Phthalate</td>
<td>Dehydroepiandrosterone</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>Di (2-ethylhexyl) Phthalate</td>
<td>Ethanol</td>
</tr>
<tr>
<td><strong>Perfluorinated fatty acids</strong></td>
<td></td>
<td>Valproic acid</td>
</tr>
<tr>
<td></td>
<td>Perfluorooctanoic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Perfluorodecanoic acid</td>
<td></td>
</tr>
</tbody>
</table>

![Clofibrate](image)

(a)

![MEHP](image)

Figure 1: Representative PPAR Agonists: A, exogenous; B, endogenous.

![Oleic acid](image)

![Arachidonic acid](image)

(b)

Table 2: Common PPAR Variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPARα</strong></td>
<td></td>
</tr>
<tr>
<td>L 162 V</td>
<td>[20, 22]</td>
</tr>
<tr>
<td>R 131 Q</td>
<td>[20, 21]</td>
</tr>
<tr>
<td>V 227 A</td>
<td>[23, 24]</td>
</tr>
<tr>
<td><strong>PPARβ/δ</strong></td>
<td></td>
</tr>
<tr>
<td>+294T/C</td>
<td>[26–30]</td>
</tr>
<tr>
<td><strong>PPARγ</strong></td>
<td></td>
</tr>
<tr>
<td>P 12 A</td>
<td>[33]</td>
</tr>
<tr>
<td>C 1431 T</td>
<td>[50]</td>
</tr>
<tr>
<td>C 190 S</td>
<td>[55]</td>
</tr>
<tr>
<td>R 166 W</td>
<td>[56]</td>
</tr>
<tr>
<td>R 194 W</td>
<td>[56]</td>
</tr>
</tbody>
</table>

Table 3: Species Differences in Hepatic Peroxisomal β-Oxidation in Response to PPARα Activators in vitro.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rat</th>
<th>Rhesus Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bezafibrateb</td>
<td>7.99</td>
<td>1.39</td>
</tr>
<tr>
<td>Ciprofibrateb</td>
<td>9.95</td>
<td>1.77</td>
</tr>
<tr>
<td>LY 17,1883c</td>
<td>5.44</td>
<td>1.44</td>
</tr>
</tbody>
</table>

*aFold increase in hepatic peroxisomal β-oxidation activity; b200 μM for 3 days; c100 μM for 3 days [300].

[55]. Other mutations, R166W in PPARγ1 and R194W in PPARγ2, are also associated with familial partial lipodystrophy and diminished transcriptional activity [56].

Screening for these mutations in various populations will undoubtedly facilitate the process of ascertaining susceptibilities to their linked diseases, aiding the determination of...
individual disease risk and the consequent development of preventive measures and/or personalized medical treatments.

3.2. Proteomics. PPARs are composed of several domains [4], and interactions of these different domains are vital for the receptor functions (Figure 3 and Table 4). These domains are: (1) an N-terminal region (A/B domain), (2) a DNA-binding domain (DBD, C domain); (3) a flexible hinge region (D domain) and (4) the C-terminal region (E/F domain).

3.2.1. N-terminal (A/B domain). This domain contains a weak ligand-independent transactivating function (AF-1); a structure that is responsible for the constitutive transcriptional activity of PPAR-responsive genes in the absence of a ligand [57]. The N-terminus is a key determinant of subtype-selective target gene expression [58], where investigations have demonstrated that the N-termini limit the transcriptional activity of PPARs to their particular target genes [59].

3.2.2. DNA-binding domain (C domain), (DBD). This region contains two zinc finger-binding motifs, as well as amino acid motifs which recognize PPAR response elements (PPREs) located in the promoter region of target genes. The DBD may also participate in the dimerization of PPARs with RXRs, a process necessary for transcriptional activation [60]. In addition, DBD is involved in co-activator binding [61] and ligand-induced stabilization of PPAR/β/δ [62]. The DBDs of PPARα and PPARγ contain phosphorylation sites which posttranslationally modulate the transcriptional activity of these receptors [63, 64].

3.2.3. Hinge region (D domain). This structure acts connects the DBD to the LBD, and acts as a docking domain for co-activators [65, 66]. Studies have suggested that the hinge region may contain a nuclear localization signal [18]. This region is also thought to modulate binding of the receptor to DNA as well as play an important suppressor role in PPARα function [67, 68]. The ribosomal protein rpl11 inhibits transcriptional activity of PPARα by associating to its hinge region; an interaction which is not seen with either PPAR/β/δ or PPARγ [67]. Also, heat shock protein 90 (HSP90) interacts with the hinge region and the LBD of the PPARs; a phenomenon which occurs to a greater extent with PPARα than with PPAR/β/δ or PPARγ. In addition, HSP90 acts as a repressor of both PPARα and PPAR/β/δ activity [69]. Mutation at potential phosphorylation sites within the hinge region of PPARα blocks its phosphorylation and prevents heterodimerization [63].

3.2.4. Carboxyl terminal (E/F domain). This is the largest domain in the receptor, and its overall structure is common to the three PPAR subtypes. It contains a ligand-binding domain (LBD) [70]. The LBD is a Y-shaped hydrophobic pocket to which ligands bind to either activate or repress the receptor transactivation [71].

Although the overall domain structure of the three receptor subtypes is similar, X-ray crystal structure analyses have revealed that the LBD has some markedly different features among the receptor subtypes [70]. The binding pockets of PPARα and PPARγ are significantly larger than that of PPAR/β/δ which may play a major role in determining ligand-binding selectivity [70]. Also, studies have revealed that the PPARα pocket is more lipophilic than either PPARγ or PPAR/β/δ pocket, which may explain its higher affinity for saturated fatty acids [70].

In addition to ligand binding, The LBD domain is required for heterodimerization and interaction with transcriptional cofactors [72]. Studies have suggested that the PPARγ LBD cooperates with the DBDs of both PPARγ and RXRα to enhance binding to the response element [73]. Studies have also suggested that the extreme carboxyl-terminal amino acids of PPARα are required for the formation of PPAR-RXR heterodimers [74, 75].

The receptor carboxyl termini also contain a segment, ligand-dependent activation function, AF-2, which is engaged in the recruitment of PPAR cofactors [1]. Deletion of a short segment from the carboxyl terminus of PPARγ abolishes transcription activation [76]. The AF-2 domain in the latter receptor also mediates ligand-induced receptor degradation [77].

3.2.5. Post-translational modification of PPARs. PPAR activity is modulated by several post-translational modifications including phosphorylation, sumoylation, ubiquitination and nitration. Furthermore, it has been reported in the literature that activity depends on intracellular localization of the receptor where nuclear localization leads to genomic effects.

**Figure 2:** Representative PPAR Subtype-Selective Agonists.
while cytosolic or cell membrane localization promotes non-genomic effects [5, 6].

Subtype-specific phosphorylation of PPARs is mediated by MAPKS as well as by Cdk5, MEK, PKA, PKC and GSK3 [4, 6]. However, while activity of PPARγ1 and PPARγ is modified by phosphorylation, that of PPARδ is not [4]. Furthermore, the phosphorylation site dictates the outcome of this effect. For example, while phosphorylation of serine 12 and 21 in the PPARγ A/B domain enhances the transcriptional activity, phosphorylation of serine 76 leads to increased receptor degradation [4]. In addition, phosphorylation of serine 112 of PPARγ2 enhances transcriptional activity of the receptor [4]. Conversely, another study has shown that dephosphorylation of serine 112 of PPARγ enhanced the transcription activity of the receptor [78]. Similarly, phosphorylation of serine 82 in the PPARγ1 A/B domain inhibits both ligand-dependent as well as ligand-independent transactivation [4]. In addition, phosphorylation of PPARγ at serine 273 has been linked to obesity and lack of sensitivity to insulin [79]. Inhibition of phosphorylation at the latter site, both in vitro and in vivo, by antidiabetic PPARγ ligands was accompanied by improvements in obesity and responsiveness to insulin [79]. Phosphorylation of serine 273 is believed to influence the ability of the receptor to recruit coactivators and corepressors [79].

Both, sumoylation as well as ubiquitination of PPARs have also been reported [4, 6]. Interestingly, phosphorylation may reduce or enhance PPARs ubiquitination, depending on the phosphorylation site [4], whereas sumoylation of PPARγ has been shown to repress activity of this receptor subtype [4].

In addition to the above modifications, nitration of tyrosine residues of PPARs occurs when levels of nitric oxide are increased, e.g., during inflammation [6]. This post-translational modification blocks ligand-induced nuclear translocation of PPAR, and consequently inhibits its activity [6].

Another level at which the activity of PPARs is post-translationally regulated relates to the intracellular distribution of the receptor. As the genomic effects of the receptor require its presence in the nucleus, a nuclear-cytosol shift would be expected to diminish the genomic activity, while potentially enhancing its nongenomic functions [80]. In this regard, it has been reported that phosphorylation or sumoylation of PPARγ promotes exporting the receptor from the nucleus to the cytosol [4]. Furthermore, PPARs have been detected in the plasma membrane, subjecting the receptor to the influence of extracellular signals [6].

3.2.6. PPAR mode of action. Various postulates have been proposed to explain the mechanisms through which PPARs mediate their ascribed effects. As explained above, while modulating the expression of specific genes through these receptors (genomic effect) has been well established, a role not involving gene expression (nongenomic effect) has also been promulgated.

a- Genomic pathways to PPAR effects.

The transcriptional activities of PPARs and other members of the nuclear receptor (NR) superfamily are controlled by a large group of proteins known as transcriptional coregulators. These coregulators modify chromatin structure or interact with the components of cellular transcription machinery to enhance or inhibit transcription of NR target genes [81].

Prior to ligand binding, PPARs heterodimerize with retinoid x receptor (RXR), forming a complex [2]. This complex is required for binding to specific DNA sequences, known as PPAR response elements (PPREs), in the promoter region of target genes. Upon binding to their ligands, PPARs undergo conformational changes allowing recruitment of co-activators and release of co-repressors, followed by the activation or repression of transcription [2, 82]. Coactivators recruited and/or corepressors released determine the direction of the expressed effect; activation or repression of transcription. In some cases, a “ligand-independent” repression of target genes takes place [6].

b- Transcriptional factor coregulators (Figure 4 and Tables 5 and 6).

Transcriptional coregulators of nuclear receptors are subdivided into coactivators which potentiate transcription and corepressors which silence or inhibit gene expression. In most NR-coregulator interactions, unliganded receptor remains associated with corepressor until conformational changes are imparted on the receptor by agonist binding. The chemical structure of a given ligand determines the conformational state of the receptor and its interaction with different coregulators [83]. As a result of these conformational changes corepressor dissociates from NR and coactivator binds to the receptor. However, some corepressors target agonist-bound receptors to reduce receptor-mediated gene transcription and in some cases coactivators may stimulate NR in the absence of a binding agonist. PPAR coregulators play key roles affecting physiological and pathological functions of the receptor and as such represent potential therapeutic targets for novel drug design.
3.3. PPAR coactivators (Figure 4 and Table 5). Several nuclear receptor coactivators have been identified as essential for PPAR transcriptional activity. Some of these coactivators are recruited to ligand-bound receptor and enhance transcription activation by modifying the chromatin organization via intrinsic histone acetyltransferase or methyltransferase activities [84]. Other coactivators possess no enzymatic activity but enhance PPAR transcriptional function by stabilizing transcriptional complexes [84]. The assembly of coactivator complexes with multiple possible configurations allows the liganded PPAR to activate the transcription of specific target genes in a tissue/cell-specific manner. Most coactivators possess one or more short amphipathic leucine rich motif (LXXLL; L: leucine and X: any amino acid) known as the NR box, some of which may interact with a coactivator-binding groove located within the ligand-binding domain of nuclear receptor [85].

3.3.1. PPARγ coactivator-1 (PGC-1). This family of coactivators includes PGC-1α and PGC-1β which share extensive sequence homology, but contain distinct binding sites for different transcription factors. Both PGC-1α and PGC-1β play important roles in the regulation of mitochondrial functions by enhancing transcriptional activity of nuclear receptors, including PPARs [86, 87].

Studies have shown that PGC-1α is induced in a tissue-specific manner under conditions such as cold, fasting and exercise [6] and regulates the expression of mitochondrial genes involved in adaptive thermogenesis [88, 89], as well as mitochondrial biogenesis during exercise and caloric restriction [90]. PGC-1α knockout mice have a reduced expression of mitochondrial genes in several tissues including brown adipose tissue (BAT), skeletal and cardiac muscles as well as in the brain. These animals exhibit an impaired metabolic response to cold exposure and starvation [91]. PGC-1β knockout mice display an impaired expression of a large number of genes involved in mitochondrial and metabolic functions in multiple tissues including BAT, liver, brain, skeletal and cardiac muscle. These animals develop abnormal hypothermia and morbidity after acute cold exposure and, unlike the hyperactive PGC-1α knockout mice, PGC-1β knockout mice show a greatly decreased activity during the dark cycle [92].

Binding of PGC-1α to nuclear receptors is usually ligand-dependent; as is the case with PPARα. This binding involves an interaction between PGC-1α LXXLL domain and the AF2 region of PPARα [93]. Evidence demonstrates that PGC-1α acts as a coactivator of PPARα in the transcriptional control of mitochondrial genes involved in fatty acid oxidation [93]. A study has shown PGC-1β to physically interact with and potently coactivate hepatic PPARα [87]. However, other studies have demonstrated that overexpression of hepatic PGC-1β in vivo, induced by increasing dietary fat, does not potentiate PPARα–mediated fatty acid oxidation. Indeed, PGC-1β overexpression blunted the effects of agonist-mediated PPARα activation on gene expression and fatty acid oxidation [94].
A strong physical interaction between PGC-1α and PPARβ/δ has been demonstrated [89]. Although this interaction is enhanced in the presence of PPARβ/δ agonists, PGC-1α acts as a powerful coactivator of PPARβ/δ even in the absence of an agonist. Studies have shown that cooperation between PGC-1α and PGC-1β appears essential for maximizing PPARβ/δ-mediated mitochondrial fatty acid oxidation [95].

Interaction of PGC-1α with PPARγ is ligand-independent and is mediated also through an LXXLL motif necessary for PGC-1α to coactivate PPARγ [96].

3.3.2. P 160 family of steroid receptor coactivators (SRCs). Three members of the p160/SRC family of coactivators have been identified, including (1) SRC-1/NCoA-1 (nuclear receptor coactivator A-1), (2) SRC-2/TIF2 (transcriptional intermediary factor 2)/GRIP1 (glucocorticoid receptor interacting protein 1), and (3) SRC-3/pCIP (CBP-interacting protein)/RAC3 (receptor-associated coactivator 3)/ACTR (activator of thyroid and retinoid receptors)/AIB1 (amplified in breast cancer-1)/TRAM-1 (thyroid hormone receptor activator molecule 1) [97].

SRC proteins are required for mediating the transcriptional function of nuclear receptors in a ligand-dependent manner. They possess intrinsic histone acetyltransferase (HAT) activity responsible for acetylation of histones and remodeling of chromatin structure resulting in increased levels of transcription [98]. SRC1, cloned and identified as the first authentic nuclear receptor coactivator, interacts with steroid receptors and markedly increases their transcriptional activity [99]. Subsequent studies revealed that SRC1 interacts with PPARγ in a ligand-dependent manner, potentiating the receptor transcriptional activity and playing a role in the PPARγ-mediated signaling pathway [100, 101]. The X-ray crystal structure of SRC1 and the liganded PPARγ complex revealed that binding occurs between highly conserved glutamate and lysine residues in the PPARγ ligand binding domain and the backbone atoms of the LXXLL helices of SRC1 [101].

Studies have shown that PPARγ function is impaired in SRC-1 null mice and this impairment is manifested as reduced energy expenditure with increased sensitivity to high fat diet and predisposition to obesity [102]. Conversely, disruption of SRC-2 gene in the mouse reduces PPARγ function in white adipose tissue (WAT) leading to a reduction in fat accumulation and protection against obesity [102]. The latter mice display enhanced adaptive thermogenesis and resistance to diet-induced obesity due to increased function and development of brown adipose tissue (BAT), promoting energy expenditure with improved glucose tolerance and insulin sensitivity [102]. PGC1α is up-regulated in SRC-2-depleted BAT and the interaction between SRC-1 and PGC1α is facilitated in SRC-2 null mice leading to an increase in the thermogenic activity of PGC1α in these animals [102]. It is suggested that SRC-1 and SRC-2 control the energy balance between WAT and BAT, whereby SRC-1 promotes energy expenditure via fatty acid oxidation in BAT, while SRC-2 represses this process by activating PPARγ in WAT [103].

Like SRC-1 and SRC-2, SRC-3 has an LXXLL motif through which it binds directly to PPARγ in a ligand-dependent manner [104]. SRC-3 null mice show reduced body weight and adipocyte tissue mass with a significant decrease in PPARγ expression, compared to wild type mice, indicating that SRC-3 plays an important role in adipocyte differentiation [105]. SRC-1 and SRC-3 double null mice are lean and resistant to high-fat diet induced obesity. These mice exhibit no BAT lipid storage with decreased uncoupling.

Table 4: PPAR Domain Functions.

<table>
<thead>
<tr>
<th>Domain Function</th>
<th>DNA Binding Domain</th>
<th>Hinge Region</th>
<th>Carboxyl terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transactivating function (AF-1)</td>
<td>Zinc finger binding motifs</td>
<td>Nuclear localization signals</td>
<td>Ligand – independent domain</td>
</tr>
<tr>
<td>Determined sub-type-selective gene expression</td>
<td>Phosphorylation sites (PPARα &amp; PPARγ)</td>
<td>Interacts with HSP90 (PPARα more than PPARβ/δ or PPARγ)</td>
<td>Interacts with HSP90 (PPARα more than PPARβ/δ or PPARγ)</td>
</tr>
<tr>
<td>Docking domain for coactivators</td>
<td>Ribosomal protein rpL11 inhibits activity</td>
<td>Ligand-induced stabilization (PPARβ/δ)</td>
<td>AF-2; for coactivators</td>
</tr>
</tbody>
</table>

Table 4: PPAR Domain Functions.
Table 5: PPAR Coactivators.

<table>
<thead>
<tr>
<th>Coactivator</th>
<th>Receptor</th>
<th>Condition</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGC1α</td>
<td>PPARα</td>
<td>– ligand dependent</td>
<td>– Stimulation of FA oxidation, promotion of mito biogenesis</td>
</tr>
<tr>
<td>PGC1β</td>
<td>PPARα</td>
<td>– ligand independent</td>
<td>– inhibition of PPARα transcriptional activity</td>
</tr>
<tr>
<td>SRC1</td>
<td>PPARα</td>
<td>– ligand independent</td>
<td>– promotion of mito FA oxidation</td>
</tr>
<tr>
<td>SRC2</td>
<td>PPARγ</td>
<td>– ligand dependent</td>
<td>– repression of energy expenditure, promotion of adipocyte differentiation</td>
</tr>
<tr>
<td>SRC3</td>
<td>PPARα</td>
<td>– ligand independent</td>
<td>– promotion of adipocyte differentiation</td>
</tr>
<tr>
<td>SRC3</td>
<td>PPARγ</td>
<td>– ligand dependent</td>
<td>– regulation of brown fat UCP-1</td>
</tr>
<tr>
<td>CBP/P300</td>
<td>PPARα</td>
<td>– ligand dependent</td>
<td>Fat-burning stimulation</td>
</tr>
<tr>
<td>CBP/P300</td>
<td>PPARβ/δ</td>
<td>– ligand dependent</td>
<td>– no effect on PPARγ transcriptional activity</td>
</tr>
<tr>
<td>CBP/P300</td>
<td>PPARγ</td>
<td>– ligand dependent</td>
<td>– fatty acid storage and adipogenesis</td>
</tr>
<tr>
<td>Med 1</td>
<td>PPARα</td>
<td>– ligand dependent</td>
<td>– hepatic cell and peroxisome proliferation</td>
</tr>
<tr>
<td>Med 16</td>
<td>PPARγ</td>
<td>– ligand dependent</td>
<td>– no effect on PPARα transcriptional activity</td>
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<tr>
<td>PRIP</td>
<td>PPARα</td>
<td>– Ligand dependent</td>
<td>– adipogenesis</td>
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<td>PRIC 285</td>
<td>PPARα</td>
<td>– ligand dependent</td>
<td>– FA oxidation and hepatic lipid metabolism</td>
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<td>PRIC 320</td>
<td>PPARα</td>
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<td>PRIC 295</td>
<td>PPARα</td>
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<tr>
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<td>PPARα</td>
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<td>– FA oxidation and hepatic lipid metabolism</td>
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<td>BAF60a</td>
<td>PPARα</td>
<td>– PGC1α dependent</td>
<td>– Mitochondrial and peroxisomal β-oxidation</td>
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<td>BAF60C</td>
<td>PPARγ</td>
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<td>– adipogenesis</td>
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<td>– adipocyte differentiation</td>
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<td>CARMI</td>
<td>PPARγ</td>
<td>– ligand independent</td>
<td>– adipocyte differentiation</td>
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</tbody>
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in vivo play an essential role in PPAR to same agonists, suggesting that these coactivators do not displayed similar responses as wild type animals exposed 1, SRC-2 or SRC-3 null mice treated with PPAR actions appear to be ligand- independent. However, SRC-α with full-length PPAR

Table 5: Continued.

<table>
<thead>
<tr>
<th>Coactivator</th>
<th>Receptor</th>
<th>Condition</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>NeoA4/ARA70</td>
<td>PPARα</td>
<td>– in absence of RXR</td>
<td>– activation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– in presence of RXR</td>
<td>– repression</td>
</tr>
<tr>
<td>PRDM 16</td>
<td>PPARγ</td>
<td>– ligand independent</td>
<td>– activation of BAT selective genes and suppression of WAT selective genes</td>
</tr>
<tr>
<td>TLE3</td>
<td>PPARγ</td>
<td>– ligand independent</td>
<td>– adipocyte differentiation blocks interaction with PRDM 16, promotes lipid storage, inhibits thermogenic gene expression</td>
</tr>
<tr>
<td>CCPG</td>
<td>PPARγ</td>
<td>– ligand independent</td>
<td>– adipogenesis</td>
</tr>
<tr>
<td>TRIP3</td>
<td>PPARγ</td>
<td>– ligand dependent</td>
<td>– adipogenesis</td>
</tr>
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</table>

protein 1 (UCP1) expression and defective adaptive thermogenesis due to a deficiency in the regulation of selective PPARγ target genes. The increased basal metabolic rates and enhanced physical activity in these animals result in a lean phenotype despite the higher food consumption [106].

SRC-2 and SRC-3 concomitantly promote human adipocyte differentiation and lipid accumulation by attenuating PPARγ phosphorylation at S114, an inhibitor of PPARγ transcriptional activity and adipogenesis. It is suggested that targeting the PPARγ–SRC interaction may present strategies for developing new therapeutics to prevent obesity associated with the treatment of type-2 diabetes [107].

In vitro studies have documented protein-protein interaction between PPARα and SRC-1 and SRC-3 and these interactions appear to be ligand- independent. However, SRC-1, SRC-2 or SRC-3 null mice treated with PPARα agonists displayed similar responses as wild type animals exposed to same agonists, suggesting that these coactivators do not play an essential role in PPARα-regulated transcriptional activation in vivo [98].

3.3.3. CREB binding protein (CBP) and p300. CBP and p300, proteins are universal coactivators that link transcriptional factors to the basal transcription apparatus and provide a platform to integrate multiple cofactors. A high degree of homology is shared between CBP and p300 that are usually referred to as CBP/p300 [108]. Both proteins possess HAT activity and have the ability to recruit other proteins having HAT activity to further enhance acetylation of the coactivator complex for efficient gene transcription [98].

CBP and p300 directly interact with the ligand-binding domain (LBD) of several nuclear receptors including PPARs. Studies have demonstrated that CBP is a component of PPARα-interacting cofactor (PRIC) complex that interacts with full-length PPARα in the presence of ciprofibrate and leukotriene B4 [109]. CBP also coactivates PPARα-dependent regulation of the brown fat UCP-1 gene promoter in the presence WY14643 [110]. In the intestinal cell line Caco-2, CBP and p300 interact with the ligand-binding domain of PPARα and PPARγ in the presence of their specific ligands [111].

In addition, both p300 and CBP interact with PPARγ. This interaction is described as complex and involves multiple domains in each protein. p300/CBP bind in a ligand-dependent manner to the DEF region of PPARγ in addition to ligand-independent direct binding to a region in the AB domain. Studies have shown that p300/CBP enhance the transcriptional activities of both AF-1 and AF-2 domains [112].

3.3.4. Mediator complex subunits. Several proteins participate in the formation of a multisubunit complex called TRAP (thyroid hormone receptor-associated protein) /DRIP (vitamin D3 receptor-interacting protein) /ARC (Activator-rerecruited cofactor)/Mediator complex. These subunits are thought to be lacking intrinsic enzymatic activity but the complex is involved in facilitating interaction of the RNA polymerase II machinery with other transcription coactivators containing chromatin remodeling enzymatic activities [113].

PPAR-binding protein is a component of the mediator complex and is alternatively referred to as PB (PPARBP)/TRAP 220/DRIP 205/Med1 (Mediator 1) [114]. This protein binds to several nuclear receptors including PPARα and PPARγ, in a ligand-dependent manner via two conserved LXXLL motifs [115]. Conditional deletion of Med1 gene in the liver results in the abrogation of PPARα ligand-induced pleiotropic effects, indicating that Med1 is essential for PPARα signaling. Studies have shown that Med1 deficiency in liver parenchymal cells results in near elimination of PPARα ligand-induced peroxisome proliferation, liver cell proliferation, and induction of PPARα-regulated genes. Moreover, mice deficient in Med1 gene exhibited a severe impairment of liver regeneration following partial hepatectomy. Studies have also suggested that Med1...
plays a key role in PPARα ligand-induced liver tumor development [116].

Although direct binding of Med 1 to PPARγ has been demonstrated, experiments utilizing cells depleted of different Mediator subunits indicate that Med1 depletion does not affect PPARγ-dependent activation of target genes or PPARγ-dependent recruitment of Mediator to target promoters [117]. In the same study, another Mediator subunit, Med 14, was identified as a critical component for PPARγ-dependent transactivation and Mediator recruitment. It was shown that Med14 interacts with the N-terminal domain of PPARγ in a ligand-independent manner both in vitro. The same study also demonstrated that knockdown of Med14 results in reduced PPARγ-mediated activation of target genes involved in fatty acid storage and impairment of adipogenesis in 3T3-L1 cells [117].

3.3.5. PPAR-interacting protein (PRIP/NCoA6). PRIP is also referred to as activating signal cointegrator-2 (ASC-2)/nuclear receptor activating protein 250 (RAP250)/nuclear receptor coregulator (NRC)/thyroid hormone receptor (TR)-binding protein (TRBP), (PIMT/NCoA6IP) and serves as a linker between the initial HAT complex of CBP/p300 and other coactivators and the downstream mediator complex [97].

Although it was shown that PRIP binds to PPARα and this binding is increased in the presence of specific ligands [118], subsequent studies have demonstrated that targeted deletion of PRIP gene in liver does not affect the induction of PPARα-regulated pleiotropic responses, including hepatomegaly, hepatic peroxisome proliferation, and induction of genes involved in fatty acid oxidation, indicating that PRIP may not be essential for PPARα-mediated transcriptional activity [119]. Alternatively, absence of PRIP may trigger the recruitment and binding of other cofactors to PPARα.

Studies have shown that PRIP potentiates the transcriptional activities of PPARγ in a ligand-dependent way and a truncated form of PRIP acts as a dominant-negative repressor [118]. It has been demonstrated that coactivation of PPARγ by PRIP is required for adipogenesis [120].

3.3.6. PPAR alpha-interacting cofactor 285 (PRIC285). PRIC285 is a component in the PRIC complex isolated from rat liver nuclear extracts. This complex interacts with full-length PPARα in the presence of synthetic and natural ligands and acts as a coactivator by moderately stimulating PPARα-mediated transcription in transfected cells [109]. PRIC285 binds to the DBD-hinge of the PPARs through its C-terminal region [121]. Human PRIC285 has been shown to enhance PPARγ-mediated transactivation. PRIC285 also coactivates PPARβ/δ [121]. Assessment of PRIC285 function in vivo suggests a functional redundancy of this coregulator in the general transcriptional machinery of PPARα and PPARγ. These results imply that loss of a single component of a multisubunit protein complex could be compensated in vivo by other members of this complex [97].

3.3.7. PRIC320. PRIC320 possesses a chromodomain helicase DNA (CHD)-binding function and is also known as CHD9 or CReMM (Chromatin Related Mesenchymal Modulator). Members of the CHD family of proteins interact with nucleosomes and modulate chromatin remodeling to control transcription [122]. Studies indicate that two isoforms of PRIC320, PRIC320-1 and PRIC320-2, interact with PPARα in a ligand-dependent manner [123]. PRIC320 also binds to PPARγ but to a much lesser degree than to PPARα suggesting a differential role for the cofactor in the regulation of downstream target genes [123].

3.3.8. PRIC295. PRIC295 has been identified and characterized as a novel coactivator protein that interacts with the Med1 and Med24 subunits of the mediator complex [124]. It binds to PPARα, PPARγ and other members of the nuclear receptor superfamily in a ligand-dependent manner and enhances the transactivation function in vitro [124]. However, the role of this cofactor as a regulator of nuclear receptor signaling in vivo is yet to be determined.

3.3.9. Switch/sucrose non-fermenting (SWI/SNF) ATP-dependent chromatin-remodeling complex. Chromatin remodeling complexes mobilize nucleosomes and function as important regulators of transcription factor function [125]. In mammals SWI/SNF complexes are present in multiple forms made up of several proteins referred to as BRG1-associated factors (BAFs) [126]. Studies have identified SWI/SNF as a regulator of genes involved in fatty acid oxidation and hepatic lipid metabolism through an interaction with PPARα mediated by BAF60a [127]. SWI/SNF complex is also recognized as a regulator of genes involved in adipocyte differentiation. Experimental evidence indicate that the SWI/SNF complex is recruited on the promoter of PPARγ to transactivate PPARγ during adipogenesis [128].

3.3.10. BAF(s) Family. The BAF(s) family represents a molecular link between transcription factors and SWI/SNF complexes [94]. In vivo, BAF60α induces expression of genes involved in both peroxisomal and mitochondrial fatty acid oxidation and ameliorates hepatic steatosis suggesting the existence of a cross talk between BAF60α and PPARα. This has been confirmed by experimental findings revealing a significant decline in of BAF60α transcriptional function in PPARα-null hepatocytes. In addition, activation of PPARα by WY14643 was found to increase the induction of fatty acid oxidation genes by BAF60α providing more evidence for the existence of a connection between BAF60α and the PPARα pathway [127]. PGC-1α was found necessary for the function of BAF60α as a regulator of fatty acid oxidation genes by mediating the recruitment of BAF60α to PPARα-binding sites, leading to transcriptional activation of peroxisomal
and mitochondrial lipid oxidation genes [127]. Studies have shown that PGC-1α interacts with PPARα and BAFl60a through different domains suggesting the formation of a transcrip-
tional complex, involving these three factors, in the vicinity of fatty acid oxidation genes promoters [127].

Another member of the BAF family, BAF60c binds to PPARγ and enhances its transcriptional activity in a ligand-independent manner [129]. Two isoforms, BAF60c1 and BAF60c2, are localized primarily in the cell nucleus to help recruit SWI/SNF complex to nuclear receptors and other transcription factors [129].

3.3.11. PIMT. PIMT (PRIP-interacting protein with methyltransferase domain), enhances the nuclear receptor transcriptional activity and its methyltransferase property is involved in the formation of the 2, 2,7-trimethylguanosine cap of non-coding small RNAs [130]. In addition to its interaction with PRIP, PIMT has been shown to interact with transcriptional coactivators, CBP, p300, and PBP under in vitro and in vivo conditions [131]. Studies have shown that while PIMT enhances PBP-mediated transcriptional activity of PPARγ, it represses CBP/p300-mediated transactivation of this receptor [131].

3.3.12. CARM1 (coactivator-associated arginine methyltransferase 1). CARM1 functions as a coactivator for many nuclear receptors and acts synergistically with the p160 family of SRCs [132]. Studies have shown that CARM1 promotes adipocyte differentiation by coactivating PPARγ [132].

3.3.13. Nuclear receptor co-activator 4 (NcoA4)/ ARA70. NcoA4 was initially identified as androgen receptor (AR)-associated protein 70 (ARA70) and was thought to be AR-specific, but subsequent investigations revealed that it interacts with a variety of nuclear receptors, including PPARα and PPARγ [133, 134]. Studies have shown that NcoA4 acts as a PPARα coactivator in the human prostate cancer cell line DU145 but functions as a PPARα repressor in adrenal Y1 cells [133]. It appears that the availability of PPARα heterodimer partner retinoic X receptor (RXR) determines the activity of NcoA4 toward PPARα, thus by acting as a coactivator in the absence of RXR and as a repressor in the presence of RXR [133]. It is suggested that an additional protein or complex of proteins in Y1 cells may interact with the PPARα:RXR-NcoA4 complex to mediate repression of PPARα transcription [130]. It is also proposed that phosphorylation of NcoA4 may contribute to its repressing activity in Y1 cells [133].

Studies have demonstrated that PPARγ interacts with NcoA4 in the absence of PPARγ ligands, but with enhanced transactivation in the presence of a ligand [134]. The antinflammatory effect of PPARγ in the colon is thought to be mediated by NCOA4 [135].

3.3.14. PRDM16. PRDM16 is a zinc finger protein required for development and function of BAT and beige adipocytes [136]. It is also considered as an effective regulator of muscle cell metabolism [137]. Studies have shown that direct interaction of PRDM16 with PPARγ is critical for activation of brown fat-selective transcription program and promoting BAT-like phenotype in WAT [138]. Studies have also demonstrated that PPARγ agonists induce browning of WAT by stabilizing PRDM16 protein [139]. It has also been shown that PRDM16 suppresses the expression of certain WAT-selective genes [140].

Studies employing transgenic mice overexpressing PPARγ2 suggest that this receptor induces conversion of myogenic cells into adipocytes and PRDM16 mediates the browning of such adipocytes and thus may represent a potential target for prevention and treatment of obesity [141].

3.3.15. Transducin-like enhancer of split 3 (TLE3). TLE3 is a member of the Groucho/TLE family of corepressors and acts as an activator of adipogenesis as well as a suppressor of osteoblast differentiation [142]. Studies have demonstrated that TLE3 is a white fat selective PPARγ cofactor enhancing adipocyte differentiation by stimulating transcriptional activity of PPARγ [142, 143]. Experimental evidence indicates that TLE3 is a constituent of PPARγ containing transcriptional complexes but direct interaction between these two proteins is not apparent [143]. However, TLE3 is able to bind PRDM16 and compete for its interaction with PPARγ thereby inhibiting the co-occupancy of PRDM16 and PPARγ on adipocyte promotors [144]. Blocking the interaction between PRDM16 and PPARγ by TLE3 promotes lipid storage and inhibits thermogenic gene expression [144].

Transgenic mice lacking TLE3 gene exhibit enhanced expression of thermogenic genes in both BAT and WAT and have improved tolerance to cold [144]. It has been suggested that in cell types where both TLE3 and PRDM16 exist, the ratio between PPARγ-TLE3 and PPARγ-PRDM16 complexes may present an important determining factor of the extent of activation of particular promotors and these complexes may exist in equilibrium in beige adipocytes existing in WAT depots [144].

3.3.16. CCPG (Constitutive coactivator of PPARγ). CCPG interacts with the hinge region of PPARγ in a ligand-independent manner and enhances the receptor’s transactivation function [145]. None of the four LXXLL motifs of CCPG were found to be essential for interaction with PPARγ. Studies have shown that CCPG is involved in the process of adipogenesis [145].

3.3.17. TRIP3 (Thyroid interacting protein 3). TRIP3 interacts with PPARγ in a ligand-dependent manner via an LXXLL motif [104]. Impaired adipocyte differentiation
3.4. PPAR corepressors (Figure 4 and Table 6). The corepressor proteins possess or recruit histone deacetylases (HDACs) and other enzymatic activities that participate in down regulating gene transcription by enforcing a tight chromatin structure [146]. Corepressors interact with unliganded nuclear receptors through amphipathic leucine-rich helices known as the corepressor/ nuclear receptor (CoRNR) boxes located within the corepressor [147]. A distinct group of corepressors target the agonist-bound receptor’s activating function-(AF) 2 region to reduce receptor-mediated gene transcription. The latter group of corepressors also utilizes CoRNR boxes and may function to mediate a submaximal response during exposure to excess ligand [148].

3.4.1. Nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptor (SMRT). NCoR and SMRT proteins share a high degree of homology and interact with unliganded NRs through (CoRNR) boxes [147]. NCoR and SMRT possess three and two CoRNR boxes, respectively, and the release of the corepressor molecules upon ligand binding is due to reduced affinity of the receptor for the CoRNR box motif [147]. Studies have shown that NCoR and SMRT form multisubunit complexes that contain HDAC activities mediating repressive reactions [149]. Experimental evidence indicates that NCoR and SMRT act as repressors of PPARs and that the unliganded PPAR-RXR heterodimer remains bound to NCoR and SMRT mostly present in the corepressor complex [150].

Studies have also demonstrated that SUMOylation of human PPARα on lysine 185 downregulates its transcriptional activity [103]. Further research in the area of PPAR transcriptional coactivators may indeed prove valuable to the task of identifying new coactivators and/or pinpointing the exact role of each of the already discovered ones, with the ultimate goal of targeting these molecules/complexes as a therapeutic means to combat diseases. In addition, determining coactivator-specific involvement in the regulation of the excretion, secretion and effect of adipokines on energy expenditure is expected to be invaluable to the task of designing strategies to control the epidemic of obesity and related metabolic diseases.

<table>
<thead>
<tr>
<th>Corepressor</th>
<th>Receptor</th>
<th>Condition</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>NCoR/SMRT</td>
<td>PPARα</td>
<td>In absence of ligands SUMOylation leads to recruitment of NCoR, but not SMRT</td>
<td>Downregulation of transcriptional activity</td>
</tr>
<tr>
<td></td>
<td>PPARβ/δ</td>
<td>Dependent of coactivator/Corepressor ratio</td>
<td>Decreased exercise endurance</td>
</tr>
<tr>
<td></td>
<td>PPARγ</td>
<td>In absence of ligand Binding increases by SUMOylation of PPARγ</td>
<td>Inhibition of adipogenesis, increase of inflammation, decreased insulin activity</td>
</tr>
<tr>
<td>HMGA 1</td>
<td>PPARγ</td>
<td>Ligand-dependent</td>
<td>Loss of vascular protection</td>
</tr>
<tr>
<td>TRB3</td>
<td>PPARγ</td>
<td>Ligand-dependent</td>
<td>Inhibition of adipocyte differentiation</td>
</tr>
<tr>
<td>LCoR</td>
<td>PPARγ</td>
<td>Ligand-dependent Acts as depressor at low levels</td>
<td>Inhibition of splenic macrophages</td>
</tr>
<tr>
<td>RIP 140</td>
<td>PPARα</td>
<td>Ligand dependent activator and repressor depending on the relative level of RTP 140 in comparison to other cofactors</td>
<td>Inhibition of mRNA expression of PPARα and PPARβ/δ and their target genes. Suppression of BAT-specific genes in WAT</td>
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<tr>
<td></td>
<td>PPARβ/δ</td>
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<td></td>
<td>PPARγ</td>
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<tr>
<td>TNIP 1</td>
<td>PPARα</td>
<td>Ligand dependent</td>
<td>Partial repression</td>
</tr>
<tr>
<td>TAZ</td>
<td>PPARγ</td>
<td>Ligand dependent</td>
<td>Attenuation of adipogenic gene expression and adipocyte differentiation</td>
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<tr>
<td>CoPR 1</td>
<td>PPARs</td>
<td>Ligand dependent</td>
<td>Partial repression</td>
</tr>
<tr>
<td>CoPR 2</td>
<td>PPARs</td>
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<td>Partial repression</td>
</tr>
<tr>
<td>Brd 2</td>
<td>PPARγ</td>
<td></td>
<td>Inhibition of adipogenesis</td>
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via the recruitment of NCoR, but not SMRT, leading to the differential expression of various PPARα target genes [151].

NCoR has been shown to act as a negative regulator of both muscle mass and mitochondrial oxidative metabolism. Enhanced exercise endurance in genetically modified mice with muscle-specific loss of NCoR is attributed, in part, to increased transcription activity of PPARγ [152] suggesting that NCoR acts as a repressor for this receptor [152].

Experimental evidence indicates that PPARγ recruits SMRT and NCoR in the absence of ligand resulting in down-regulation of the receptor-mediated transcriptional activity. The PPARγ-corepressor complex was dissociated when the agonist pioglitazone was introduced. Furthermore, 3T3-L1 cells deficient in SMRT or NCoR exhibit increased expression of adipocyte-specific genes and increased production of lipid droplets, as compared with control cells [153]. Further studies have demonstrated that NCoR promotes PPARγ-corepressor-273 phosphorylation in adipocytes by recruiting cyclin-dependent kinase 5 (Cdk5/CDK5). NCoR deletion led to adipogenesis, reduced inflammation, and enhanced systemic insulin sensitivity [154]. Genetically modified mice with a mutation in the nuclear receptor interacting domain of SMRT exhibit a number of metabolic changes which were attributed to enhanced PPARγ activity resulting from lack of association with the mutated SMRT [155].

3.4.2. High-mobility group (HMG). High-mobility group (HMG) proteins are ubiquitous chromatin-binding proteins consisting of three family members, HMGA, HMGB, and HMGN. These proteins induce structural changes in the chromatin fiber and regulate gene transcription. A carboxy terminus rich in acidic amino acids is a feature in all HMGs but each member is characterized by a unique functional motif and participates in distinct cellular functions [156].

Studies have shown that HMGA1 is involved in vascular smooth muscle cell PPARγ-mediated transrepression, by facilitating PPARγ SUMOylation through the rate-limiting SUMO E2 ligase Ubc9. Glitazone-mediated vascular protection through PPARγ activation is lost in HMGA1-deficient mice undergoing arterial injury [157].

3.4.3. TRB3 (Tribbles homolog 3). TRB3 was identified as a mammalian homolog of Drosophila tribbles. It is a 354-amino acid protein involved in multiple cellular pathways. Studies have shown that TRB3 downregulates PPARγ transcriptional activities through protein-protein interaction [158]. Expression of TRB3 in 3T3-L1 adipocytes results in decreased levels of PPARγ-target gene products and knockdown of TRB3 increased expression of these genes [158]. TRB3 was also found to inhibit PPARγ-dependent adipocyte differentiation [158]. These studies have provided evidence that TRB3 acts as a corepressor of PPARγ.

3.4.4. Ligand-dependent corepressor (LCoR). LCoR is an NR box-containing protein that interacts with the ligand binding domains of agonist-bound receptors and represses hormone-dependent transactivation [159]. It is widely expressed in human tissues and in addition to its histone deacetylase activity; it functions by recruiting a variety of proteins that mediate transcriptional repression [159, 160]. The type of repressor recruited by LCoR depends on the nuclear receptor with which it interacts or on the kind of tissue involved, allowing for a differential control of gene expression [160]. Studies have demonstrated that LCoR acts as a repressor of PPARγ in splenic macrophages [161].

3.4.5. Receptor interacting protein 140 (RIP140). Also known as NRIP1 (nuclear receptor interacting protein 1), RIP140 is another ligand dependent coregulator involved in both activation and repression of NR-mediated transcription [162]. The activity of RIP140 depends on the relative level of RIP140 expression in comparison with other cofactors. Post-translational modifications and interactions with other transcription regulators are additional factors controlling the outcome of RIP140 binding [162]. RIP140 contains nine LXXLL motifs and four repression domains and acts primarily as a platform that links NRs to chromatin remodelling enzymes [163].

RIP140 recruits HDAC and represses the activity of various nuclear receptors including PPARs by competing with their coactivators [163]. Studies have suggested that RIP140 acts as a negative counterpart of the coactivator PGC-1α, by directly interacting with PGC-1α and blocking its binding to the nuclear receptors [164]. SUMOylation of PGC-1α enhanced its sensitivity to repression by RIP140 and disruption of SUMOylation increased the capacity of PGC-1α as a coactivator for PPARγ-dependent transcription [165]. Real-time PCR analyses revealed that mRNA expressions of PPARα, PPARγ and their target genes were repressed by RIP140 and induced by PGC-1α in a dose-dependent manner in neonatal rat cardiomyocytes [166]. Additional studies have reported that RIP140 interacts with PPARγ and suppresses brown adipose specific gene transcription in white adipose tissue [103].

3.4.5. TNIP1 (TNFα-induced protein 3-interacting protein 1). In addition to its NR repressing activity, TNIP1 functions as an NF-κB inhibitor [167]. TNIP1 contains transcriptional activation and repression domains and is widely distributed suggesting that it may play an important regulatory role in multiple tissues [167]. The regulatory effect of TNIP1 depends on its expression level and the expression of other regulators in NR and/or NF-κB signaling pathways [167]. Studies have shown that TNIP1 interacts with agonist-bound PPARs and partially represses the receptor transcriptional activity without total loss of receptor function [168]. TNIP1 does not interact with the PPAR heterodimer partner retinoid X receptor (RXR) even in the presence of that receptor’s ligand [169].
3.4.6. TAZ (transcriptional coactivator with PDZ-binding motif). TAZ acts as a transcriptional coactivator or corepressor and modulates the differentiation of mesenchymal stem cell (MSCs) into osteoblasts or adipocytes by stimulating the transcription factor RUNX2 (runt-related transcription factor 2) and repressing PPARγ-dependent transcription [170]. Investigating mechanisms of antiadipogenic activity of PPARγ ligand, KR62980 has indicated that this compound increases the nuclear localization of TAZ and augments the interaction between PPARγ and TAZ resulting in suppression of PPARγ activity and attenuation of adipogenic gene expression. Furthermore, KR62980 failed to suppress PPARγ-mediated adipogenic gene expression and adipocyte differentiation in TAZ knockdown 3T3-L1 cells suggesting that TAZ is an important mediator of KR62980 antiadipogenic effects [171].

The dietary flavonoid kaempferol (KMP) also has been found to enhance the association of TAZ with PPARγ resulting in suppression of PPARγ target gene expression and diminishing adipocyte differentiation. The effect of KMP on PPARγ activity was impaired in TAZ-null mouse embryonic fibroblasts and was regained by restoration of TAZ expression [172]. Another compound, TM-25659, a TAZ modulator enhanced nuclear TAZ localization and attenuated PPARγ-mediated adipocyte differentiation by facilitating PPARγ suppression activity of TAZ [173]. These studies suggest that TAZ modulators may prove to be beneficial in the control of obesity.

3.4.7. Co-Modulators of PPAR and RXRα (COPR1 and COPR2). COPR1 and its longer variant COPR2 are apparently derived by variant splicing of the same transcript resulting in a 50 amino acid difference between the two proteins. COPR1 and COPR2 utilize the LXXLL box and target the AF-2 domains of nuclear receptors to decrease, but not to completely silence, receptor function. A proline-rich autonomous activation domain (AAD) distinguishes COPR1 and COPR2 from other ligand dependent corepressors. Studies have reported that the COPR1 isoform has greater functional interaction with PPARs than does COPR2 [174].

3.4.8. Brd2. The double bromodomain protein (Brd2) can function as either a transcriptional coactivator or corepressor and regulates gene expression by controlling the activity of transcription complexes, interpreting the histone code and remodelling chromatin structure [175]. Studies suggest that Brd2 acts as a PPARγ corepressor; since Brd2 knockdown increases PPARγ activity and facilitates adipogenesis [176].

In light of the above brief presentation on coregulators of transcription, more research is required to identify the exact physiological function(s) of each of these molecules and their complexes and to characterize structural features involved in differential regulation of PPAR target genes. Such studies are expected to yield significant information essential for understanding the roles of gene regulation in various diseases and for designing preventive and palliative approaches for these diseases.

3.5. Nongenomic pathways to PPAR effects. Nongenomic PPAR-mediated effects occur within a much shorter time frame (minutes) compared with those effects mediated through the genomic pathway (hours). The nongenomic pathways involve the interactions of PPARs with extranuclear, fast-acting second messengers, e.g., kinases [5, 6, 80, 176]. In addition to kinases, evidence has been presented showing that PPARγ may also affect the activity of phosphatases, NADH cytochrome c reductase, as well as PKCα [6]. Furthermore, the existence of extranuclear, plasma membrane PPAR receptors has been advanced to support a nongenomic effects mediated by these receptors [178].

In support of a nongenomic effects, a study has shown that PPARγ agonists of various chemical structures rapidly diminished ERK phosphorylation in human microvascular endothelial cells in parallel to their known receptor binding affinity [178]. Similarly, our recent findings showing a fast, PPARα-mediated, enhanced cardiac muscle contractility and blood vessel relaxation is in support of the existence of such nongenomic receptor pathways [179]. Furthermore, a most recent study has demonstrated that pioglitazone, a PPARγ agonist, rapidly reduced neuropathic pain through non-genomic PPARγ mechanisms [180]. In addition, another recent study utilizing rat cardiac ventricular myocytes has shown that the PPARβ/δ specific agonist GW0742 attenuated ERK1/2 and Akt phosphorylation, an effect which was not blocked by the specific PPARβ/δ antagonist GSK0660 but was mitigated by the tyrosine phosphatase inhibitor vanadate [181]. The latter study suggests, however, that the effect of GW0742 maybe the result of an off-target action by the ligand.

Thus, effects such as inhibition of the production of reactive oxygen species and protein phosphorylation [178, 181] may represent nongenomic pathways through which PPAR agonists exert their beneficial effects. Whether these effects involve the PPAR receptor and/or other receptor(s) or are indeed totally receptor-independent are questions that await answers at this time.

4. PPAR Ligands

All three PPARs are activated by a variety of polyunsaturated long chain fatty acids and arachidonic acid derivatives [182, 183]. In addition to these physiological substances, numerous synthetic ligands of varying selectivity bind to and activate the various PPAR subtypes.

4.1. Endogenous ligands. These ligands are naturally occurring physiologically molecules that would be expected to have binding affinity to the receptor in the nanomolar
range under physiological conditions and modulate receptor activity (Table 7).

It is imperative, however, to state that experiments designed to identify endogenous ligands should consider the fact that results obtained in ex-vivo testing may prove physiologically irrelevant, unless evidence is presented showing that the intracellular concentration and location of the presumed endogenous agonist is consistent with physiological conditions. Therefore, whether many of these compounds satisfy the criteria of actual endogenous PPAR ligands remains to be determined.

4.1.1. PPARα. An example of the proposed endogenous ligands for this receptor is 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine; a FAS-dependent phosphatidylcholine [184]. While this molecule activates hepatic PPARα, arachidonic acid derivatives, e.g., Leukotriene B4 have strong PPARα-binding in immune cells [185].

Furthermore, oxidized phospholipids exert a phospholipase A2-dependent activation of PPARα in endothelial cells, suggesting that these phospholipids may be precursors of endogenous ligands [186]. In addition to the above physiological ligands, endocannabinoids are natural lipids included among endogenous PPARα ligands. Some endocannabinoids bind with relatively high affinity to PPARα and regulate lipid and glucose metabolism, as well as inflammatory responses [187]. In vitro experiments have identified other PPARα ligands including (85)-hydroxyeicosatetraenoic acids, carbaprostacyclin and unsaturated fatty acids [188]. The physiological relevance of these findings is yet to be elucidated.

4.1.2. PPARβ/δ. Studies have demonstrated that PPARβ/δ is activated by prostacyclin (PGI2) [189–191]. In addition, experiments have demonstrated that although the vitamin A metabolite all-trans-retinoic acid (RA) binds to PPARα and PPARγ with a low affinity, this compound has a high affinity to PPARβ/δ [192]. C16 and C18 fatty acids have also been suggested as potential endogenous ligands for PPARβ/δ [193]. 4-hydroxynonenal (4-HNE), a lipid peroxidation product has also been reported as an endogenous ligand for PPARβ/δ [194].

4.1.3. PPARγ. Although PPARγ can be activated by prostaglandinJ2 (PGJ2) as well as by polyunsaturated and oxidized fatty acids, these compounds bind the receptor with low affinity [16]. Other PPARγ endogenous ligands have been described in more recent studies, including nitro-derivatives of unsaturated fatty acids [195], serotonin metabolites [196], farnesyl pyrophosphate [197] and some endocannabinoids [187]. Cyclic phosphatidic acid has been identified as an endogenous PPARγ antagonist [198].

4.2. Exogenous PPAR modulators.

4.2.1. Fibrates. Clofibrate was approved in the United States for the treatment of hyperlipidemia in 1967 [17]. Later it was noted that individuals with type 2 diabetes taking clofibrate for treatment of hyperlipidemia showed reduction in fasting blood glucose concentration [199]. Intensive research resulted in the discovery of fenofibrate [200]; demonstrated to be superior to clofibrate as a hypolipidemic agent. In the late 1970s and early 1980s, other fibrates, gemfibrozil, bezafibrate and ciprofibrate, were also introduced, and the beneficial cardiovascular effects of these drugs lead to their wide clinical use.

4.2.2. Thiazolidinediones, also referred to as glitazones were discovered in the 1980s [201, 202]. Beside its hypolipidemic effect, ciglitazone was shown to normalize hyperglycemia and hyperinsulinemia in animal models of type 2 diabetes [203, 204]. The antidiabetic effect of ciglitazone, however, was too weak for clinical application. In addition, edema was found to be a significant problem associated with this agent.

In search for more potent insulin sensitizers with less serious side effects, two other glitazones, troglitazone [205] and rosiglitazone [206], were developed. However, troglitazone was rapidly withdrawn from the market because of its liver toxicity [207]. Rosiglitazone which normalizes blood glucose levels and improves tissue sensitivity to insulin with more potency and more selectivity than ciglitazone [208] was approved for clinical use in the United States in 1999 [17]. Unfortunately, due to association with significant side effects including weight gain, congestive heart failure and fluid retention, rosiglitazone was banned from the European market in 2010, while in the United State, the Food and Drug Administration limited its use to situations in which other medications are not effective, a decision that was later repealed in late 2013.

Pioglitazone was synthesized and evaluated for hypolipidemic and hypoglycemic activities. Clinical studies revealed that pioglitazone ameliorates the glucose and lipid profile of patients with type-2 diabetes. Although this drug is orally active, well tolerated and provides beneficial effects on insulin resistance [17], association with an increased incidence of bladder cancer has been raised.

4.2.3. Allosteric ligands. Interaction between a receptor and its ligands is traditionally considered to occur at the endogenous agonist-binding site in the receptor, the orthosteric site [209, 210]. However, there has been a marked increase in the discovery of ligands that interact with alternate sites on receptors, allosteric sites [209, 211–213]. Binding of ligands to these allosteric sites results in modifying the conformation of the receptor, with significant consequences related to its activity [209].
Although in comparison with other classes of receptors, developing allosteric ligands for nuclear receptors is lagging behind, efforts are in earnest to develop such ligands for various members of this receptor superfamily, including PPARs. In 2000, Camp et al., [214] observed that while only troglitazone was a partial agonist in transfected muscle and kidney cells, rosiglitazone, pioglitazone and ciglitazone exerted full agonistic effects. Furthermore, troglitazone and rosiglitazone produced different states of receptor conformation to a degree that allowed troglitazone to antagonize rosiglitazone-stimulated PPARγ receptor conformation to a degree that allowed troglitazone to antagonize rosiglitazone-stimulated PPARγ transfectional activity [214]. This effect was confirmed by limited protease digestion studies of PPARγ bound to either ligand as well as by in vitro coactivator association assays [214]. Although these surprising results were explained by possible agonist-induced variations in the affinity of cofactors to the receptor, the potential presence of PPARγ isoforms and/or undefined cell transfection-associate events, the possibility of different troglitazone and rosiglitazone binding sites was not raised by these authors [214]. More recently, however, the fact that agonists of both PPARα and RXRα exerted a synergistic effect on the activity of the PPARα/RXRα heterodimer lead investigators to speculate that there may be an allosteric communication between these two obligatory partner receptors in the heterodimer [215]. Indeed, molecular dynamic simulations as well as experimental evidence have revealed that RXRα ligands stabilize the PPARα coactivator binding site, via an allosteric effect that impacts the PPARα/RXRα heterodimer activity [215].

Subsequently, a novel alternate PPARγ ligand binding site was most recently identified [216]. These authors [216] have shown that, at pharmacologically relevant concentrations, synthetic PPARγ ligands bind to an allosteric receptor site, leading to unique receptor conformational changes that influence the receptor transactivation and target gene expression [216]. The reported ligand-receptor binding was not blocked by either antagonists or by endogenous agonists, indicating that binding was not occurring at the orthosteric site [216].

Since various known PPARγ agonists have shown varying potencies and/or efficacies in binding to the described receptor allosteric site [216], it is likely that these agonists will exert a corresponding range of different receptor conformation states and subsequent transcriptional activity. Thus, this relatively new allosteric binding and the documented subsequent alteration in receptor function have substantial promising implications for future drug discovery in the field of PPARs.

It is noteworthy in this regards to refer to a reported evidence pointing to the fact that a PPARγ ligand with minimal known agonistic activity exerted an antidiabetic effect comparable to, or better than, that produced by a full agonist [79]. This effect could not therefore be explained based on the classical concept of agonist-receptor interaction, but was explained by the ability of these ligands to inhibit the receptor-protein-phosphorylation at serine 273 by cdk5, through altering the receptor conformation making serine 273 inaccessible to the kinase [79]. The involvement of an alternate receptor binding site in the effect of the PPARγ ligand with minimal agonistic activity remains a possibility in attempting to explain its superior effect to the full agonist. This is particularly plausible in light of the fact that this agonist did induce receptor conformational changes that are different from those produced by the full agonist [79].

5. The Future of PPARs

5.1. Challenges facing PPAR research. Because PPARs control a plethora of cell functions in various organs, a variety of adverse effects may arise as a consequence of the activation or inhibition of these receptors. Indeed, significant therapeutic as well as toxicological profile differences have been observed with various PPAR agonists, presenting major challenges to researchers in the field.

5.1.1. PPARα. The PPARα agonists, fibrates, represent a valuable class of drugs for the treatment of dyslipidemia [217]. These drugs are generally well tolerated, and infrequently associated with major safety concerns [218]. To highlight a few untoward effects encountered with fibrates, one would have to refer, albeit rarely, to myopathy as the most serious safety risk associated with these drugs [219]. Furthermore, although some clinical trials have demonstrated a significant reduction in nonfatal myocardial infarction in
fibrate users, others have noted increases in cardiovascular and total mortality; a finding that has resulted in FDA-mandated warning about mortality in the fibrate package insert [218]. In addition, a small, but statistically significant, increased risk for pulmonary embolism and deep venous thrombosis due to fenofibrate has been reported [219]. Treatment with fibrates is also known to cause an increase in plasma homocysteine levels that increases the risk for hypercoagulability and coronary, cerebral, or peripheral vascular disease [218]. In addition, a higher incidence of pancreatitis has also been observed in patients treated with fenofibrate [200], and an increase in creatinine has been noted with other fibrates [218]. Furthermore, all fibrates appear to have the propensity to cause gallbladder disease [219].

5.1.2. PPARγ. Increased rates of bone fractures in women taking the PPARγ agonist, rosiglitazone has been reported and this effect was not observed in men [220]. Also, an increase in myocardial infarction with rosiglitazone versus placebo or other antidiabetic drugs, with no increase in mortality, has been observed [221]. The concern about the cardiovascular safety of rosiglitazone was confirmed by an internal FDA meta-analysis as well as in other studies [222–230]. Conversely, the RECORD study (rosiglitazone evaluated for cardiac outcomes and regulation of glycemia in diabetes) trial [231], one of the most important studies on the rosiglitazone cardiovascular safety issue [232], showed no increase in the primary endpoint of hospitalization or death from cardiovascular causes with rosiglitazone. Other studies [233–235] failed to observe this risk with rosiglitazone. However, these studies were also criticized for several limitations [232].

In July 2010, the FDA re-evaluated rosiglitazone cardiovascular safety data and a new internal FDA meta-analysis showed that total myocardial ischemia was significantly increased in patients receiving rosiglitazone [232]. A repeat meta-analysis by Nissen and Wolski also showed a similar trend [236] and other meta- as well as observational studies provided more evidence for increased cardiovascular events with rosiglitazone [225, 232]. Based on these discoveries, the FDA announced the restricted availability of rosiglitazone a decision that was rescinded in late 2013, based on the reevaluation of the RECORD trial [237]. In contrast, the European Medicines Agency has completely withdrawn rosiglitazone from the market [232], an action that remains in effect to date.

Weight gain is also a known side effect of glitazone treatment [238–240], an effect that is due to increased adipogenesis in the subcutaneous fat depot [241, 242] as well as due to fluid retention [243].

Clinical trials have shown that pioglitazone increases rates of congestive heart failure (CHF). This effect is further increased when pioglitazone is combined with insulin [232]. This untoward effect has prompted The American Diabetes Association and the American Heart Association to recommend against the use of any glitazone in patients with known class III or IV CHF [232]. It is generally accepted, however, that pioglitazone does not cause an increase in myocardial infarction and may actually reduce the number of myocardial infarction and strokes [244].

A nonsignificant trend towards increased bladder cancer in patients treated with pioglitazone has been reported [232]. As a precaution, The FDA has recommended that pioglitazone should not be used in patients with active bladder cancer. It is also recommended that caution should be exercised in prescribing pioglitazone to patients with a history of bladder cancer, and that patients on a pioglitazone regimen should be informed of the signs and symptoms of bladder cancer [232].

Pioglitazone has also been associated with increased bone fracture risk, but to a lesser extent than rosiglitazone. Thus, despite the lack of guidelines limiting pioglitazone use as a result of this effect, restricting the use of pioglitazone in patients with low bone density has been suggested [232].

Although data from clinical trials have not shown liver toxicity in patients using pioglitazone, some cases of liver failure associated with the use of this drug have been reported [232]. Consequently and as a precaution, the pioglitazone package contains a recommendation to examine hepatic functions prior to the initiation of treatment [232].

Pioglitazone may also contribute to increased diabetic macular edema probably due to fluid overload [245]. In addition, mild but statistically significant decreases in hemoglobin have been observed after extended treatment with pioglitazone [246]. The decreases in hematologic parameters were not sufficient, however, to impose a limitation on the use of this drug [232].

PPARα/γ dual agonists, muraaglitazar, ragaglitazar, and tesaglitazar, were initially considered promising, but alarming side effects observed during their development have diminished enthusiasm for these drugs [247]. In addition, a meta-analysis of phase II and phase III clinical trials has shown that muraaglitazar increased the composite risk of nonfatal myocardial infarction, nonfatal stroke, or all-cause mortality in diabetic patients compared with pioglitazone [248]. Furthermore, ragaglitazar showed significant carcinogenic effects in rodent bladders and is no longer under development for human use [249]. Similarly, tesaglitazar’s development was discontinued because it severely increased serum creatinine in diabetic patients [250].

5.1.3. PPARδ. Preclinical and short-term clinical studies have demonstrated that PPAR3/δ agonists were beneficial in the treatment of type 2 diabetes mellitus and other conditions. However, issues concerning the connection between PPAR3/δ agonists and carcinogenesis, observed in animal models, necessitate further preclinical and when deemed appropriate, long-term clinical trials to determine the suitability of these agonists for human use [251].
Based on the above findings, it is feasible to conclude that drugs modulating PPAR activity require careful examination, through extensive preclinical and clinical studies, in order to determine benefit to risk ratios that are expected as a result of using these drugs in disease management.

5.2. The promise of PPAR modulators. PPARs have been identified as key regulators in a number of important diseases, physiological and pathological conditions such as diabetes [253, 254], inflammation [254–256], immunity [257–259], pain [260], regulation of male and female fertility [261, 262], obesity [263–265], senescence and senescence-related diseases [266–269], as well as various types of cancer [270–272]. Furthermore, novel PPAR functions are still being discovered and significant new elements in their signal transduction pathways continue to be identified. Consequently, these receptors remain as important potential pharmacological targets for the treatment of a variety of diseases and conditions such as epilepsy [273], drug addiction [274] and Alzheimer’s disease [275].

5.2.1. Selective PPAR modulators (SPPARMs). There are efforts aimed at identifying selective PPAR modulators (SPPARMs) to optimize benefit and minimize the toxic effects imparted by full PPAR agonists [276]. Since adverse effects encountered in patients treated with full PPAR agonists may be attributed to the wide spectrum of affected genes, the use of partial PPAR agonism may be a plausible approach to provide a desirable therapeutic outcome [276]. It is reported that a new generation of highly potent and selective PPAR modulators is being developed with the goal of separating their benefits from their unwanted side effects. Among these modulators is aleglitazar [277].

Aleglitazar is a dual PPARα/γ agonist (Figure 5); thus possesses the potential to treat diabetes and dyslipidemia simultaneously. Although preliminary data had shown improvement in hyperglycemia, dyslipidemia and blood pressure in type 2 diabetic patients treated with this drug [247], it is unfortunate that a large study, AleCardio, has recently concluded that this drug did not reduce cardiovascular risk in patients suffering from type 2 diabetes and acute coronary syndrome [278]. Additional studies are needed to ascertain the clinical promise as well as safety of similar drugs [279].

In addition to aleglitazar, a short-term study has shown that INT131, a SPPARγM, did not cause fluid retention or weight gain, but reduced fasting plasma glucose to level comparable to that reached with rosiglitazone [277]. Furthermore, GFT505, a dual PPARα/δ agonist, has also recently entered late-phase development [277]. In experimental studies, PPARγ/δ agonists have shown beneficial effects in managing dyslipidemia. These agonists may also cause less weight gain than rosiglitazone [280].

Balaglitazone, a PPARγ partial agonist developed in India, is reported to currently be undergoing a phase III clinical trial [281]. In comparison to full PPARγ agonists, preclinical studies have shown that balaglitazone caused no reduction in bone density and caused less fluid retention and heart enlargement [281, 282]. While these findings are an encouraging indicator of a better safety profile for balaglitazone, further testing is required before ascertaining risks associated with long-term use of this drug.

Recently, two selective PPARγ ligands, INT131, and F12016, have been identified [283, 284]. While INT131 has been found to possess an improved hemodynamic and less cardiovascular adverse effects, F12016 exhibited a suppressed ability to cause weight gain, compared to the full agonist rosiglitazone [283, 284]. Both of these ligands induce a distinct pattern of coregulator recruitment by the receptor, and their binding to the PPARγ receptor does not involve Tyr473; a characteristic which is essential for the rosiglitazone-induced receptor activation [283, 284].

Further clinical studies are still needed before these drugs can gain approval for the treatment of diabetes associated with vascular complications.

With the advent of the relatively recent discovery of PPAR receptor allosteric and allosteric ligands, the field is now poised for a renewed surge in enthusiasm and effort to develop novel PPAR-centered therapies for many diseases. This discovery is hoped to lead to the development of a new class of PPAR modulators, allosteric ligands, which possess more targeted pharmacological effects, combined with less toxicity, compared with the canonical ligands.

5.2.2. Other potential therapeutic values of PPAR agonists. The wide spectrum and large number of proposed beneficial uses and various clinical trials utilizing PPAR modulators provide a strong evidence in support of the promise these receptors hold as crucial targets of future novel effective therapies against numerous diseases. For example, it has been shown that endurance exercise training increases the proportion of oxidative fibers in the muscle and enhances the capacity for exercise [285]. While this effect is reported to be mediated by PPARβ/δ through enhanced receptor expression in response to exercise [286–288], studies have also demonstrated that activation of PPARβ/δ can reprogram muscle metabolism and increase endurance in sedentary animals, leading to the suggestion that PPARβ/δ agonists as potential exercise mimetic drugs [289]. However, while the use of PPARβ/δ agonists may provide some benefits in treating certain muscle as well as metabolic diseases, when physical exercise is not an option, caution must be practiced before using these drugs as a routine alternative to exercise in light of their potential other known undesirable effects.

In addition, there are several clinical trials looking into expanding the utilization of PPAR agonists in treating more diseases and conditions. For example, current trials are examining the outcome of treatment with various PPAR agonists on dementia, mental disorders, and cognition [290]. In addition, there is a clinical trial examining the effect of CS7017, an experimental PPARγ agonist, in subjects with...
stage IIIb/IV non-small cell lung cancer [290]. Another trial, currently in phase II, is investigating the impact of the same agonist in subjects with advanced anaplastic thyroid cancer [290].

Beyond the aforementioned, there are numerous trials evaluating the potential therapeutic benefits of the antidiabetic, PPARγ agonist pioglitazone, in diseases other than diabetes. For example, there is a phase III clinical trial evaluating the benefit of this PPARγ agonist in the treatment of Alzheimer’s disease [275]. It has been recently reported that pioglitazone restored memory deficit and brain BDNF levels in animals treated with β-amyloid [291], suggesting an antioxidant, anti-inflammatory, ant apoptotic, as well as a neurogenesis-like effect by this agonist.

Another trial is looking also into the effect of pioglitazone in rheumatoid arthritis [290]. In another trial, the effect of the same PPARγ agonist is being evaluated against asthma [292], while in a fourth trial it is being evaluated in patients addicted to heroin and nicotine [290]. Additionally, the effects and safety of pioglitazone on non-alcoholic fatty liver disease are currently undergoing evaluation in patients with impaired glucose regulation or type 2 diabetes mellitus [283]. Furthermore, a phase III trial is exploring the effects of pioglitazone on neurological functions in Friedrich’s ataxia patients [290], and the effect of this PPARγ agonist on insulin and glucose metabolism in women with polycystic ovary syndrome is also under evaluation [290].

5.2.3. Beyond diabetes control: Endosalicylates: a hypothesis to explain PPAR-mediated anti-inflammatory effects. Aside from the well-established clinical benefit of PPAR agonists as hypolipidemics and insulin sensitizers in treating type II diabetes, there are numerous trials exploring the promise of these drugs in treating a myriad of additional diseases and conditions. For example, experimental and clinical findings over the last decade have provided evidence demonstrating that PPARs modulate both acute and chronic inflammation [292]. However, the mechanism through which PPARs control inflammatory events remains unclear. In an attempt to evoke a conversation among interested scientists to ultimately reach a rational, scientifically sound understanding, we present below a hypothesis of how these receptors may control a myriad of inflammatory diseases.

When we compared the profile of the anti-inflammatory effect produced by PPARα or PPARγ agonists with those exhibited by dexamethasone or aspirin, only PPAR agonists and aspirin were found to diminish inflammation when given after the inflammatory insult [293]. Furthermore, in contrast to aspirin, only dexamethasone produced an additive anti-inflammatory effect when administered in combination with an agonist of either PPARα or PPARγ [294]. Based on these findings we hypothesize that PPARα and PPARγ regulate inflammation through a mechanism similar to that ascribed to salicylates, while different from that ascribed to steroids.

The literature reveals that while salicylates alleviate peripheral inflammation and hyperalgesia via an action in the
central nervous system, dexamethasone does not. Considering the fact that PPARs are abundant in the central nervous system [294, 295] and that we have previously documented that, like aspirin, PPAR agonists act centrally to produce their peripheral anti-inflammatory and analgesic effects [293–296], an interplay between salicylates and PPAR-mediated anti-inflammatory effect is hereby postulated. Since salicylates do not activate PPARs [297], the possibility that PPARs may mediate the aspirin effect is thus excluded. Alternatively, a salicylate-like effect by PPAR agonists presents itself at the forefront of plausible hypotheses. This salicylate-like effect may be through a direct effect on the cyclooxygenase enzyme by PPAR agonists, or through invoking a PPAR-dependent release of a salicylate-like compound, an endosalicylate, which in turn, would exert the observed anti-inflammatory effect (Figure 6). A direct or indirect role for the cyclooxygenase enzyme is highly unlikely since PPAR agonists failed to affect inflammation when administered at the inflammation site [293]. Thus, since intracerebroventricular administration of salicylates has been reported to elicit the release of physiological anti-inflammatory mediators (e.g., α-melanocyte-stimulating hormone) from the central nervous system, which in turn may exert a peripheral anti-inflammatory effect [298, 299], we hypothesize that: (1) activating PPARs in the central nervous system may elicit the release of a salicylate-like compound, an endosalicylate, (2) the released endosalicylate compound may subsequently cause the release of a physiological anti-inflammatory substance, e.g., α-melanocyte-stimulating hormone, or other substances, in the central nervous system and (3) The central anti-inflammatory

Figure 6: A Schematic Depiction of the Endosalicylate Hypothesis.
mediator may then reach the peripheral inflammation site where it would act to resolve inflammation (Figure 6). Validation of this hypothesis, including the identification of the proposed endosalicylate as well as localizing its exact site in the central nervous system, obviously requires extensive experimental testing.

### 6. Concluding Remarks

In this review, we attempted to give the reader a quick glance at the field of PPAR research, starting with a historic perspective looking at the events that lead to their discovery. Also, we briefly sketched the structural traits of these receptors and how they exert their multifaceted functions. In addition, we presented a summary of the known receptor endogenous as well as exogenous ligands and coregulators. Finally, we shared a provocative hypothesis stipulating that PPAR agonists cause a PPAR-mediated release of putative substance we termed endosalicylates, which is responsible for the known anti-inflammatory effects of these agonists.

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