Epigenetic Codes of PPAR γ in Metabolic Disease

Shigeki Sugii^{1,2} and Ronald M. Evans^{1,*}

¹ Howard Hughes Medical Institute and Gene Expression Laboratory, The Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd, La Jolla, CA 92037, USA

² Singapore Bioimaging Consortium and Duke-NUS Graduate Medical School, 11 Biopolis Way #02-02, Singapore 138667

Abstract

Peroxisome proliferator-activated receptor gamma (PPAR γ), a ligand-regulated nuclear hormone receptor, plays critical roles in metabolism and adipogenesis. PPAR γ ligands such as thiazolidinediones (TZDs) exert insulin sensitizing and anti-inflammatory effects primarily through action on adipocytes, and are thus widely used to treat metabolic syndrome, especially type II diabetes. A number of PPAR γ interacting partners have been identified, many of which are known epigenetic regulators, including enzymes for histone acetylation/deacetylation and histone methylation/demethylation. However, their functional roles in the PPAR γ transcriptional pathway are not well defined. Recent advances in ChIP-based and deep sequencing technology are revealing previously underappreciated epigenomic mechanisms and therapeutic potentials of this nuclear receptor pathway.

Keywords

Obesity-induced insulin resistance; Adipose tissue; Epigenome; histone acetyltransferases/deacetylases; histone methyltransferases/demethylases

1. Introduction

PPAR γ , a member of the nuclear receptor superfamily, is a ligand-regulated transcription factor that is a key metabolic regulator. It forms a heterodimer with retinoid X receptors (RXRs) and binds to a specific DR-1 motif (direct hexa-nucleotide repeats, separated by a single nucleotide) of PPAR response elements (PPREs). There are two splice variants, PPAR γ 1, which shows relatively ubiquitous expression, and PPAR γ 2, whose expression is restricted to mature adipocytes [1]. These variants are driven by distinct promoters, but differ only in their N-terminal sequence and have similar transcriptional activities [1,2]. It is well established that PPAR γ is a master regulator of adipogenesis. PPAR γ targets hundreds, possibly thousands, of downstream genes in adipocytes, and is the only known factor that is necessary and sufficient for induction of adipocyte differentiation [3,4]. The PPAR γ 2 isoform is induced earlier and more strongly than PPAR γ 1 during adipogenesis. The receptor is also required for maintaining the proper functions of differentiated adipocytes

[5,6]. While PPAR γ is most highly expressed in adipocytes and adipose tissue, it is present in numerous other cell types and tissues including macrophages, osteocytes, endothelial cells and placenta. PPAR γ in macrophages is implicated in anti-inflammation, uptake and reverse transport of cholesterol, and subtype specification [7,8]. The receptor was shown to drive macrophage differentiation into the alternatively activated, anti-inflammatory population (M2), rather than the classically activated, proinflammatory population (M1) [9]. PPAR γ influences distinct target genes in adipocytes versus macrophages, but how these different genetic networks are established is not well understood. It is likely that the network is in part established by the distinctive chromatin pattern in each cell type to produce broad patterns of eu- and hetero-chromatin. In addition, PPAR γ function is dependent on the availability of coregulator proteins that modify chromatin states and as a result, may differentially regulate the transcriptional activities of PPAR γ and its target genes.

Many known coactivators and corepressors of PPAR γ and other nuclear receptors have intrinsic histone modifying activities [10]. A nucleosome, the fundamental unit of chromatin structure, consists of two of each core histone, H2A, H2B, H3 and H4, and DNA wrapped around the octameric core. The H3 and H4 histones possess N-terminal tails protruding from the nucleosome, and are particularly susceptible to post-translational modifications by specific enzymes. Modifications of chromatin, such as histone acetylation, methylation and phosphorylation, are important mechanisms of epigenetic regulation. It is thought that combinations of these modifications alter the local chromatin structure or physical properties of histone tails, which can facilitate or block the transcriptional machinery to bind to the promoter and initiate its activities. Whereas histone acetylation and phosphorylation may use charge neutralization to affect chromatin structure, this may not be true for other modifications. Furthermore, it is also likely that some of the modifications are merely a read-out of activities rather than a primary mediator. In addition, it was demonstrated that a number of these enzymes can modify not only histones but non-histone proteins to influence transcription [10]. Furthermore, direct methylation of DNA, usually at CpG dinucleotides, and transcriptional modulation by small non-coding RNA are often considered additional epigenetic mechanisms. Epigenetic enzymes that are known to play pivotal transcriptional roles include histone methyltransferases (HMTs), histone demethylases (HDMs), histone acetyltransferases (HATs), histone deacetylases (HDACs), DNA methyltransferases and DNA demethylases. For example, di- and tri-methylation of lysine 9 or 27 of H3 (designated H3K9me₂, H3K9me₃, H3K27me₂ or H3K27me₃) are considered repressive chromatin marks, whereas mono-methylation of either lysine of H3 or lysine 20 of H4 (H3K9me₁, H3K27me₁, or H4K20me₁) and acetylation of lysine 9 (H3K9ac) are marks of active chromatin [10,11]. Histone acetylation exemplified by H3K9ac facilitates the access of RNA polymerase II to promoter regions just upstream of transcriptional start sites and thus stimulates transcription [11]. It is reasonably expected that like other transcription factors, epigenetic regulators underlie many activities of the nuclear receptor superfamily including PPAR γ . Therefore, studying epigenetic control by PPAR γ is key to understanding ligand-mediated transcriptional regulation of metabolic pathways. However, few studies have investigated the specific contribution and association of epigenetics changes that might be directed by this receptor. Recent technical advances involving chromatin immunoprecipitation (ChIP) such as ChIP-chip, ChIP-PET, and ChIP-Seq now make it possible to uncover potential epigenomic processes that regulate or are regulated by PPAR γ under different conditions. Here we review the current understanding of PPAR γ biology in the context of epigenetics and present its perspectives in novel therapeutic approaches against metabolic disorders.

2. PPAR γ in metabolic diseases

Thiazolidinediones (TZDs), a class of drugs that act as insulin sensitizers, are high affinity ligands for PPAR γ [12]. Rosiglitazone and pioglitazone, are the most common anti-diabetic TZDs used clinically. While systemic, TZD treatment is effective in reducing circulating glucose, insulin, triglyceride, and free fatty acid levels in diabetic subjects, although the molecular basis of this effect is incompletely understood [13]. In order to gain insight into the site and mechanism of action of the insulin-sensitizing pathway, various tissue-specific knockout models of PPAR γ were created, overcoming the obstacle of embryonic lethality in the whole body knockouts [3]. Major tissues or cell types that affect systemic glucose homeostasis include pancreas (e.g. β cells), muscle, liver (hepatocytes) and adipose tissue (adipocytes and macrophages). Deletion of PPAR γ in adipose tissue leads to insulin resistance in mice fed a high fat diet [14,15]. On the other hand, muscle-specific PPAR knockout mice develop insulin resistance γ under a normal chow diet [16,17]. Macrophage-restricted deletion of PPAR γ also results in systemic insulin resistance and glucose intolerance [9,18]. In contrast, absence of PPAR γ in hepatocytes or β cells confers only mild effects on whole body glucose metabolism [19,20]. While these studies suggested that adipose and non-adipose PPAR γ contribute to insulin resistance, it remains unclear how and where TZDs act as insulin sensitizers in the diabetic condition. *Pparg* expression in non-adipose tissues is generally very low. It was reported, however, that when adipose PPAR γ is deleted, compensation occurs by increased gene expression in muscle and liver, further complicating the use of tissue-specific knockouts to delineate the site of the TZD action [21,22]. To avoid these complications, we created gain-of-function PPAR γ models where the receptor is constitutively activated through the fusion of a VP16 transactivation domain controlled by tissue-specific promoters [6]. These animals allowed us to study the contribution of PPAR γ in individual tissues or cell types under diet-induced insulin resistant states. Unexpectedly, we found that selective activation of PPAR γ in adipocytes is sufficient to improve whole body insulin resistance to a degree similar to systemic TZD treatment. Adipocyte-specific PPAR γ activation leads to improved adipokine and lipid profiles, lowered serum insulin levels, and suppression of macrophage inflammatory responses in a paracrine manner. These results support an “adipocentric” model in which adipose activation of PPAR γ accounts for the systemic insulin sensitizing effect of TZDs in the diabetic state. Consistent with this idea, the non-TZD compound AG035029 exhibits more adipose-selective and greater insulin sensitizing effects on Zucker fatty rats than any other drugs tested, including TZDs such as pioglitazone, rosiglitazone and troglitazone [6]. TZDs are known to have adverse side effects of osteoporosis, edema and heart failure, presumably by activating PPAR γ in other cell types such as osteoclasts, collecting duct cells and cardiomyocytes, respectively [23–26]. By developing fat-specific PPAR γ modulators, it should be possible to lower the risk of side effects while achieving maximum efficacy in diabetic therapy.

3. Transcriptional mechanisms of PPAR γ in adipocytes and macrophages

PPAR γ binds to PPREs of its target gene promoters as a heterodimer with RXR. It is generally believed that in the absence of ligands, PPAR γ associates with corepressor proteins including SMRT and NCoR on PPREs. When agonists bind the receptor, these corepressors are dissociated from the complex, and coactivator proteins such as p300/CREB-binding protein (CBP) and SRC/p160 family proteins are recruited to the PPAR γ complex, resulting in transcriptional activation. It was reported that this exchange of corepressors and coactivators is mediated by TBL1 (transducin β -like 1) and the related protein TBLR1 [27]. This suggests that the transcriptional activities of PPAR γ are dynamic, yet highly coordinated processes. However, the repertoire of its interacting partners can be significantly different from one cell type to another or from one gene locus to another.

Pparg expression is high in adipocytes from both white adipose tissue (WAT) and brown adipose tissue (BAT). White adipocytes store energy primarily in the form of triglycerides and cholesterol esters in a morphologically large lipid droplet, whereas brown adipocytes contain abundant mitochondria, have more lobular fat droplets and are specialized in burning energy. While PPAR γ is critical in the development of both WAT and BAT *in vivo* [5,14], many of its target genes are distinct at least in part due to the presence of different sets of interacting coregulators. Before full adipogenesis takes place in white adipocytes, corepressors such as NCoR and SMRT are associated through their receptor-interacting domains on PPAR γ 2 and other specific target gene promoters and maintain the repressive states of adipogenic genes [28,29]. In addition, it was reported that PPAR γ forms a repressive complex that contains the retinoblastoma protein (pRb) and histone deacetylase HDAC3 [30]. As HDAC3 is found in the complex with NCoR and SMRT [31], the authors speculated that the presence of these corepressors facilitates pRb/HDAC3's repressive activity, although this hypothesis was not tested. Other corepressor proteins that are reported to interact with PPAR γ and have repressive roles in adipocytes are RIP140, SIRT1, TRB3 and TAZ (Fig. 1) [32]. During differentiation into white adipocytes, phosphorylation of pRb dislodges the pRb-HDAC3 repressive complex from the receptor [28]. This may allow coactivator proteins such as p300/CBP to associate with PPAR γ and assist with its adipogenic activity. PPAR γ activity also depends on components of a chromatin remodeling SWI/SNF complex, ATPase BRG1 and BAF60c subunits [33]. Other coactivators reported to be involved in PPAR γ -mediated adipogenesis are subunits of the Mediator complex (PBP/MED1/TRAP220 and MED14), CARM1 (coactivator-associated arginine methyltransferase 1), MEN1/Menin, CCPG (constitutive coactivator of PPAR γ), ASC-2/PRIP/NCOA6, and PTIP (Fig. 1) [32,34]. In addition to PPAR γ , the CCAAT/enhancer binding protein (C/EBP) family of transcription factors play an important role in adipogenesis. At the initial phase of differentiation, C/EBP β and C/EBP δ are induced and stimulate expression of C/EBP α and PPAR γ . C/EBP α and PPAR γ then act reciprocally to induce a number of adipogenic target genes (e.g. aP2/FABP4, CD36, PEPCK, LPL, GLUT4, and adiponectin) and sustain their own expression by a positive feedback loop, resulting in terminal differentiation [13]. The majority of PPAR-binding γ sites have been identified near C/EBP α -binding regions [35,36]. However, PPAR γ seems to be the principal driver of the adipogenic program. Thus, while overexpression of PPAR γ can induce adipogenesis in C/EBP α -deficient mouse embryonic fibroblasts (MEFs), exogenous C/EBP α expression is unable to do so in PPAR γ -deficient MEFs [13]. Accordingly, C/EBP α is most likely an associate factor that helps establish a poised or "adipogenic" state by remodeling chromatin but is dependent on PPAR γ to actually drive the program.

Despite its essentiality, the contribution of PPAR γ to brown adipocytes is less well understood. Also, C/EBP β appears to substitute for C/EBP α , though the rationale for this is also not clear [37]. A special feature of BAT is that its function of consuming or burning fat to create heat is highly conditional. Induction of PPAR γ coactivator-1 alpha (PGC-1 α) is one key way to promote this process [38]. PGC-1 α mRNA and protein levels are induced by beta-adrenergic stimulation triggered by cold exposure in BAT. In turn this increases expression of mitochondrial oxidative metabolism and adaptive thermogenic genes. One critical BAT-specific gene product that helps to generate heat is UCP-1, which is activated by PGC1 α in a PPAR γ -dependent manner [13]. In contrast, PGC-1 α does not induce the classical PPAR γ target *aP2/Fabp4* gene, indicating gene selective coactivator functions of PGC-1 α . PGC-1 α also coactivates a number of other transcription factors including other nuclear receptors such as PPAR α , ERR α , ERR γ , ER α and ER β [13]. Another BAT-specific transcription factor PRDM16 (PR domain containing 16) was recently identified, and shown to bind PPAR γ in a ligand-independent manner and enhance the PPAR γ transcriptional activity [39]. Overexpression of PRDM16 in white preadipocytes leads to induction of some brown adipogenic programs albeit at lower levels of expression than in BAT itself. This

“browning” may increase energy expenditure of WAT without actually converting it to BAT. Other coregulators that are suggested to contribute to brown adipogenesis are RIP140, SRC-1, lipin-1, and CtBP1/2 (C-terminal binding protein 1 and 2) [32,40].

The macrophage PPAR γ program is also distinct from both adipocyte populations. It was proposed that when TZD binds the receptor in macrophages, a small fraction of PPAR γ is SUMOylated, a modification that converts the RXR:PPAR heterodimer to a monomer, creating a novel interaction surface with the NCoR and SMRT corepressor complex. This SUMOylated monomer then associates, via a DNA binding independent process, to the promoters of inflammatory genes to inhibit their transcriptional activity [8,41]. This active “transrepression” mechanism maintains a repressive state of inflammatory transcription in a ligand-dependent manner without involvement of classical PPREs. The SUMOylated PPAR γ -NCoR complex has been shown to be recruited to AP1 sites through interaction with c-Jun, and represses TLR4-inducible genes. However, the extent to which ligand-dependent repression is due to this SUMOylated transrepression mechanism is not yet resolved. Furthermore, indirect mechanisms such as induction of transcriptional repressors or inhibitory micro-RNAs are likely. In regards to direct ligand dependent effects, the ChIP-based whole genome analysis shows that a majority of PPAR γ binding sites identified in macrophages are classical DR-1 elements and adjacent to those of C/EBP β and/or hematopoietic-specific transcription factor PU.1, as opposed to C/EBP α in adipocytes [42]. While little is known about the identity of PPAR γ -interacting partners associated on the DR-1 sites in this cell type, the proximity with distinct transcriptional machinery (i.e. PU.1) and possible differences in epigenetic marks may be key determinants to achieve specificity of macrophage target genes.

4. Genome-wide studies of PPAR γ in adipocytes and macrophages

Recent reports employing genome-wide ChIP analyses identified the collection of cis-acting PPAR γ genomic targets (termed “cistrome”) and suggested new aspects as to how chromatin remodeling helps to direct both adipogenesis [35,36,43–46] and macrophage activation [42]. Microarray-based platforms (ChIP-on-chip [35,44,45]) and direct DNA sequencing-based methods (ChIP-Seq [36,42,46], ChIP-PET [43]) have been used. As expected, after adipocyte differentiation of 3T3-L1 cell lines, the vast majority of PPAR γ binding sites overlap with those of its obligate heterodimer RXR α . PPAR γ -binding regions are enriched in acetylation of lysine 9 of H3 (H3K9ac) [35], consistent with the idea that receptor binding correlates with active histone marks in mature adipocytes. Importantly, a significant proportion of PPAR γ binding regions are shared by C/EBP α binding sites (> 60%), the majority of which are associated with up-regulated genes [35]. Interestingly, C/EBP β , another C/EBP family protein implicated in early adipogenesis, had a profile very similar to C/EBP α even in mature adipocytes, suggesting possible redundancy of the two isoforms. The motif analyses of surrounding sequences reveals that most of the PPAR γ DR-1 sites are adjacent to C/EBP binding elements [36]. This suggests that PPAR γ and C/EBP family proteins functionally cooperate, in the context of short distances, to induce the adipogenic transcriptional program (Fig. 1).

More recently, Mikkelsen *et al.* constructed genome-wide chromatin state and PPAR γ localization maps during adipogenesis [46]. Using murine 3T3-L1 cells and human adipose-derived mesenchymal stem cells (ASCs) as models, they presented the extensive list of preadipocyte- and mature adipocyte-specific cis-regulatory elements. Transcriptionally active histone marks H3K4me2/me3 and H3K27ac were enriched near *Pparg1* and *Pparg2* promoters in adipocytes from both species, while repressive H3K27me3 was not. H3K36me3, which is associated with transcriptional elongation, was increased throughout the *Pparg* loci during adipogenesis. PPAR γ -binding sites were also mapped genome-wide

and localized largely to distal regions enriched for the open chromatin mark H3K27ac [46]. Although those overlapping in both models were likely to have their genes upregulated, the majority of PPAR γ -binding regions were not shared between 3T3-L1 and hASCs (total of approximately 7,000 and 40,000 sites, respectively). It was reasoned that this discrepancy is due to genetic divergence between mouse and human. However, it is worth noting that the cell lines they used are derived from different tissues and ages. Therefore, their cell identities are likely to be different, and mechanisms of adipogenic differentiation and phenotypes of mature adipocytes may not be similar. Within adult adipose tissue, at least two populations of cell types are capable of becoming mature adipocytes – preadipocytes and ASCs [47]. 3T3-L1 cells are a derivative line originally subcloned from embryonic fibroblasts. While it is not clear whether 3T3-L1 cells are identical or close to the preadipocyte lineage, this line is widely considered to be the best representative of this cell type. ASCs are multipotent and can be differentiated into mesodermal cell types, osteoblasts and chondrocytes, in addition to adipocytes, whereas preadipocytes are committed to become adipocytes. Furthermore, compared to preadipocytes, ASCs can be more effectively ‘transformed’ to induced pluripotent stem cells (iPSCs) by introducing pluripotency-reprogramming factors [48]. It will be of interest to study genome-wide epigenomic changes at PPAR γ target gene loci in adipocytes, preadipocytes, ASCs and iPSCs to delineate molecular signatures and mechanisms that correspond to cell-specific PPAR γ activity states.

One study investigated the cistrome of PPAR γ in a non-adipogenic cell type, thioglycollate-activated peritoneal macrophages, and compared these results to that found in adipocytes [42]. As was the case for adipocytes, PPAR γ -specific DR-1 elements are found in the majority (>75%) of identified binding sites in macrophages. Surprisingly, however, only 4% of PPAR γ binding regions in adipocytes are also shared in macrophages. It was demonstrated that macrophage PPAR γ colocalizes with PU.1, a hematopoietic transcription factor that is required for monocyte development and is not present in adipocytes. In addition, PPAR γ binding regions are also adjacent to those of C/EBP β . Whether C/EBP α also shares C/EBP β binding sites was not addressed. Macrophage-unique PPAR γ regions show increased repressive histone marks, H3K9me2 and H3K27me3, in adipocytes. In contrast, these regions exhibit open chromatin states and increased active histone mark H3K9ac in macrophages [42]. These data implicate that cell type specificity of cistromes is achieved by unique transcriptional components and distinct epigenetic regulation. Collectively, ChIP-based cistromic studies revealed that proximity of PPAR γ binding sites to C/EBP and PU.1 response elements helps to establish the macrophage specific PPAR γ response network. However, many questions remain such as, what other proteins including coactivators, corepressors and transcription factors are involved in determining cellular specificity in addition to C/EBPs and PU.1? How do epigenetic changes and chromatin remodeling contribute to the transcriptional activities of PPAR γ in adipogenesis and inflammation? The effect of PPAR γ ligands on the unliganded cistromes in adipocytes and macrophages is also not clear. More extensive cistromic analyses investigating other proteins and enzymes are likely to answer some of these questions.

5. Potential epigenetic players in PPAR γ signaling

Specific chromatin and DNA modifications that affect the PPAR γ transcriptional pathway are considered in detail below. The histone modifying enzymes and their possible functions described in the text are listed in Table 1.

5.1. Histone acetyltransferases (HATs)

While we have limited knowledge about the effect of histone acetylation and deacetylation on PPAR γ transcriptional activity, several relevant enzymes are reported to regulate this

pathway. Two classes of common PPAR γ interacting coactivators, SRC/p160 family proteins and p300/CBP, are known to possess intrinsic HAT activities. SRC family coactivators shown to interact with PPAR γ are SRC-1, GRIP1/TIF2 (SRC-2), and ACTR/AIB1 (SRC-3). While *in vitro* differentiation experiments point to important contributions for each SRC in adipogenesis, studies using knockout mice suggest much wider roles of each isoform in controlling energy balance between BAT and WAT *in vivo* [49–51]. Upon ligand binding, PPAR γ undergoes conformational changes which provide a contact site for LXXLL motifs in p300/CBP and SRC-1 and other co-activators, through ‘charge clamp’ in its ligand binding domain (LBD) [52]. In addition to ligand induced binding, p300/CBP proteins can interact with the N-terminal AF-1 domain of PPAR γ in a ligand-independent fashion [53]. Homozygous deletion of either p300 or CBP is embryonic lethal. Although p300 and CBP are indispensable for adipogenesis *in vitro*, their roles *in vivo* are not clear. Studies in CBP heterozygotes imply a WAT-specific function of CBP as this tissue shows significant reduction in weight in these animals [54]. Very recently, it was found that at the initial stage of adipogenesis another nuclear receptor, glucocorticoid receptor (GR), is transiently recruited along with C/EBP β to a complex consisting of PBP/MED1/TRAP220 and p300 to enhancer regions of *Pparg2* isoform [55]. In response to glucocorticoids, this results in a transient increase in H3K9 acetylation and enhances the induction of PPAR γ 2, which becomes the principal driver of adipogenesis. Cyclin D1 was shown to physically interact with p300 where it inhibits HAT activity and thus represses PPAR γ activity [56]. PGC-1 α activation of PPAR γ also enhances interactions with SRC-1 or p300/CBP both *in vitro* and *in vivo* [57]. However, how HAT activities of the SRC and p300 family proteins contribute to the PPAR γ transcriptional program remains obscure. It is presumed that recruitment of these proteins promotes histone acetylation near transcriptional start sites, allowing the RNA polymerase to initiate transcription. As was described earlier, increased H3K9ac marks were found to increase at PPAR γ binding sites during adipogenesis [35,58]. It would be important to test if the enzymatic activities of SRC or p300 coactivators are correlated with histone acetylation. Other acetylation-dependent mechanisms are also possible. One possibility is that SRC or p300 family members can directly acetylate other interacting proteins in a complex or even PPAR γ itself. Such non-histone acetylation may induce their conformational changes and alter protein functions, as was demonstrated for other nuclear receptor pathways. For instance, it was shown that p300 is capable of acetylating androgen receptor (AR) and estrogen receptor alpha (ER α), to control both transcriptional activity and ligand sensitivity [59,60].

5.2. Histone deacetylases (HDACs)

In contrast to the gene activating functions of HATs, HDACs are known to repress gene expression by deacetylating histones and condensing chromatin, thereby making the regions inaccessible to the transcriptional machinery. The 18 known human HDACs are classified by sequence and domain organization into four classes, class I (HDAC1, –2, –3, and –8), class IIa (HDAC4, –5, –7 and –9), class IIb (HDAC6, and –10), class III (sirtuin family including SIRT1 through –7), and class IV (HDAC11). Various HDAC inhibitors are effective in causing cell cycle arrest, differentiation and apoptosis of tumor cells *in vivo*, and thus considered promising candidates for cancer therapy [61]. Several HDACs, notably HDAC1, –2, –3 and –5, are significantly downregulated during adipogenesis *in vitro* [62]. However, there are conflicting results regarding the effects of knocking down HDACs on adipogenesis [62,63]. For example, treatment with HDAC inhibitors such as valproic acid and trichostatin A, reduces PPAR γ levels and blocks adipogenesis in 3T3-L1 cells [64]. Cyclin D1 inhibits PPAR γ -dependent transcription and adipogenesis. It was reported that cyclin D1 recruits HDAC1, HDAC3 and histone methyltransferase SUV39H1 to PPRES, decreases total H3 or H3K9 acetylation, and thus inhibits the PPAR γ activity [65]. As described above, HDAC3 associates with pRb as a PPAR γ repressive complex, leading to

inhibition of adipocyte differentiation, which is reversed by phosphorylation of pRb [30]. TNF γ also is known to block PPAR γ function. It appears that I κ B α -dependent nuclear translocation of HDAC3 accounts for the inhibitory action of TNF α [66]. In addition, HDAC3 mediates the transrepression mechanism of PPAR γ in macrophages by associating with the NCoR complex on inflammatory gene promoters in a SUMOylation-dependent manner as discussed above [41]. Adipose (ADP) protein represses adipogenesis by inhibiting PPAR γ transcriptional activity [67]. It was found that ADP associates with histones and HDAC3, implicating a potential chromatin modification role for ADP. Consistent with this idea, inhibition of HDAC activities effectively reverses ADP-mediated blockage of adipogenesis [67].

Among seven mammalian sirtuins, class III NAD-dependent HDACs, SIRT1 plays an essential role in stress resistance and calorie restriction-related extension of lifespan (in yeast and worms), and thus has been most extensively studied. It was reported that SIRT1 is expressed in WAT and represses *Pparg* [68]. SIRT1 overexpression blocks adipogenesis, whereas inhibition of the endogenous protein increases it *in vitro* in 3T3-L1 cells. *In vivo*, fasting induces SIRT1 to bind to PPAR γ binding sites of fat-specific genes along with corepressors NCoR and SMRT, enhancing repressive functions. As a result, SIRT1 mediates fasting-induced lipolysis of triglycerides into free fatty acids, which is compromised in heterozygous SIRT1 mutant animals [68]. It was demonstrated more recently that a subset of PPAR γ target genes, including *Ero1-L α* , *FGF21*, *SCD3* and *ELOVL3*, but not classical targets, such as *aP2*, adiponectin and *C/EBP α* , are repressed by SIRT1 *in vitro* [69]. Activation of these genes was observed by inhibition of SIRT1, to a degree similar to TZD treatment. How SIRT1 acquires binding specificity to this subgroup of PPAR γ targets remains to be determined.

5.3. Histone methyltransferases (HMTs)

Histone methylation on lysine or arginine residues also plays a key role in regulating transcriptional activities. In contrast to histone acetylation that is mostly indicative of activation marks, methylation marks are associated with both active and inactive transcription. Modifications at specific residues of histones and most likely combinations of these changes determine active or repressive status of gene expression. The finding that macrophage-specific PPAR γ regions exhibit dramatically increased repressive marks H3K9me2 and H3K27me3 in adipocytes indicates the importance of histone methylation in achieving cell specificity of its target gene expression [42]. The best-studied HMTs for PPAR γ regulation are SET domain family proteins including SETDB1 (SET domain bifurcated 1) and SETD8/PR-Set7 (PR/SET domain-containing protein 7). SETDB1 forms a corepressor complex that includes NLK (Nemo-like kinase) and represses PPAR γ transactivation via H3K9 methylation [45,70]. Wnt-5a, a known inhibitory factor of adipogenesis, activates NLK, which phosphorylates SETDB1. This leads to formation of the corepressor complex and inhibits adipogenesis, which in turn promotes osteogenesis of multipotent mesenchymal stem cells [70]. ChIP-chip experiments identified significant PPAR γ binding to 10 of over 45 SET domain family genes [45]. Among these, SETDB1 and SETD5 are downregulated during adipogenesis, and knockdown of SETDB1 enhances differentiation of 3T3-L1 cells. In contrast, SETD8 is a direct target of PPAR γ , is induced during adipocyte differentiation, and its knockdown leads to inhibition of adipogenesis [45]. Importantly, SETD8 mono-methylates H4K20, and regulates PPAR γ -dependent activation of many target genes during adipogenesis through an increase in H4K20 monomethylation. Thus, H4K20me1 is a novel epigenetic mark for adipogenesis at least *in vitro*. SUV39H1 is another SET domain family protein and as described earlier, it cooperates with deacetylases, HDAC1 and HDAC3 in cyclin D1-mediated inhibition of PPAR γ -dependent adipogenesis, although its exact repressive function as a HMT is not clear [65].

MLL3 and MLL4, H3K4 methyltransferases, form a complex including the ASC-2/PRIP/NCOA6 protein, termed ASCOM (ASC-2 complex). ASC-2 is known to play an essential role in adipogenesis directed by PPAR γ . Using knockout models, both MLL3 and MLL4 proteins were demonstrated to play redundant but critical roles in PPAR γ -dependent adipogenesis *in vitro* and *in vivo* [71]. MLL3 and MLL4 interact with PPAR γ /RXR α in a TZD ligand-dependent manner, tri-methylate H3K4 at the *aP2* promoter, and concomitantly increase H3 and H4 acetylation, possibly through the HAT activity of p300/CBP. PTIP (Pax transactivation domain-interacting protein) is also capable of forming a complex with ASC2-MLL3-MLL4 and modulating HMT activity. PTIP is required for expression of *Pparg* and *Cebpa* by increasing the active mark H3K4me3 on these promoters [72]. Consequently, PTIP was shown to be essential for adipogenesis both *in vitro* and *in vivo*. Other members of H3K4 methyltransferases, MLL1 and MLL2, establish an integral complex with the MEN1/menin tumor suppressor, whose human mutations are associated with various tumors including lipomas. It was demonstrated that MEN1 interacts with PPAR γ , enhances its target gene expression through increased H3K4 trimethylation, and is required for adipogenesis [73]. Hepatic stellate cells (HSCs) undergo myofibroblastic transdifferentiation by suppressing the PPAR γ -dependent adipogenic program. Recent studies showed that methyl-CpG binding protein 2 (MeCP2) is recruited to *Pparg* promoter along with transcriptional repressor heterochromatin protein 1 alpha (HP1 α), and increases its H3K9 methylation in HSCs [74,75]. It is not certain which HMT is responsible for HP1 α -mediated H3K9 methylation. MeCP2 also stimulates expression of HMT EZH2 and enhances H3K27 methylation in HSCs, further remodeling chromatin into the repressive structure surrounding *Pparg*.

In contrast to lysine methylation, very little is known about the potential role of arginine methylation in adipogenesis. Protein arginine methyltransferases (PRMTs) are comprised of 10 members (PRMT1 through -9 and FBXO11). PRMTs can methylate a diverse group of transcription factors and coregulators as well as histones [76]. PRMT4/CARM1 was originally identified as a binding partner for the p160/SRC protein GRIP1/TIF2/SRC-2 and acts as a coactivator for several nuclear receptors. In addition to H3 histone at H3R2, H3R17 and H3R26, CARM1 also methylates other coactivators, p300/CBP and SRC-3, indirectly affecting the activities of these HATs [76]. Only one study has investigated the role of PRMTs, specifically CARM1, in the PPAR γ pathway [77]. CARM1 was found to associate with PPAR γ on the *aP2* promoter, and enhance transcriptional activity of the receptor in a ligand-dependent manner. CARM1 knockdown blocks adipogenesis *in vitro*, and CARM1-null embryos exhibit reduced BAT [77]. Although CARM1 recruitment is coincident with increased H3R17 methylation at the *aP2* promoter, it is not yet clear how CARM1 augments PPAR γ activity and adipogenesis. For example, CARM1 has been shown to methylate the nuclear receptor co-factor CBP and might thus act independently of histone modification. In addition to CARM1, PRMT2 was reported to interact with PPAR γ as well as other nuclear receptors, but its function is unknown [78]. The observation that PRMT2 coactivates the transcriptional activity of ER α predicts that this may also be the case for PPAR γ .

5.4. Histone demethylases (HDMs)

Direct evidence for HDMs to participate in the PPAR γ pathway is scarce as well. One report investigated the function of H3K9-specific HDM, JHDM2A (a.k.a. JMJD1A/KDM3A), by knocking out this protein [79]. JHDM2A is highly expressed in BAT, and animals lacking the protein have defects in β -adrenergic responses in BAT and display obesity and dyslipidemia. Upon β -adrenergic stimulation, JHDM2A is recruited along with PPAR γ , RXR α , PGC-1 α , p300/CBP and SRC-1 to the PPRE of UCP1 gene, and reduces H3K9me2 levels at the PPRE [79]. This result unveils dynamic involvement of the HDM in a coactivator complex-mediated metabolic gene regulation. The HMT PTIP-ASC2-MLL3-

MLL4 complex, which was described earlier, also contains UTX, a H3K27 demethylase, though the functional significance for its presence in the complex is unknown [72]. It is possible that many HMT complexes also possess components with counteracting HDM activities. Important roles of HDMs in other nuclear receptors have been noted. For example, LSD1/KDM1 associates with and is important for transcriptional activation of androgen receptor and ER α target genes by demethylating H3K9 [80,81]. Similar mechanisms may exist for the PPAR γ cascade.

5.5. DNA methylation

DNA methylation is the only known regulatory modification of DNA itself, and involves addition of a methyl group to the carbon-5 position of cytosine typically in CpG dinucleotides. Although it is associated with gene silencing it is believed that this is dependent on the recruitment of relevant repressive complexes. DNA methylation is catalyzed by DNA methyltransferases (DNMTs). As with HDAC inhibitors, DNMT inhibitors (such as 5-aza-cytidine) have attracted great interest as epigenetic therapeutic agents for cancer [82,83]. 5-azacytidine is the first epigenetic drug approved by FDA and used for treatment of myelodysplastic syndrome. Combinations of HDAC and DNMT inhibitors also appear to have synergistic effects and are currently explored for treating acute myeloid leukemia and other tumors. On the other hand, the identities of DNA demethylases have been controversial, although several proteins have been proposed to possess such activities [82,83]. There have been no studies that clearly define the molecular mechanisms of DNA methylation in *Pparg* regulation. In the case of the PPAR γ coactivator PGC-1 α , it was demonstrated that DNMT3B is involved in TNF α - or free fatty acid-induced hypermethylation at non-CpG nucleotides and reduction of mitochondrial content in skeletal muscle from diabetic subjects [84]. In one study, hypomethylation of adipogenic loci including those from *Pparg2*, *Leptin*, *aP2* and *Lpl* genes, was found in ASCs, which have a high adipogenic potential [85]. In contrast, myogenic or endothelial loci were hypermethylated in ASCs, and adipogenic loci were more methylated in non-adipogenic, hematopoietic cells. However, no change in hypomethylation was observed after adipogenesis, implying that DNA methylation serves as a molecular signature for a differentiation potential, but does not determine gene expression for driving adipogenesis [85]. Nevertheless, another study reported that the *Pparg2* promoter is hypermethylated in the 3T3-L1 preadipocyte model and that upon induction of differentiation the region is gradually demethylated [86]. Methyl CpG-specific binding protein MeCP2 is associated with the promoter in preadipocytes, but is dismissed after differentiation. PPAR γ expression is increased in non-adipogenic NIH3T3 cells by treatment of the DNMT inhibitor, 5-azacytidine. Moreover, increased methylation of the *Pparg2* promoter and concomitant reduction of its expression was found in visceral WAT of obese animals [86]. As described above, MeCP2 is recruited to the PPAR γ site, leading to HP1 α -dependent H3K9 methylation and EZH2-dependent H3K27 methylation to repress its expression in HSCs [74,75]. This result demonstrates an epigenomic relay pathway, which connects DNA methylation to histone methylation for gene regulation. Recently, it has been shown that the Tet1 (Ten-eleven translocation) protein can convert 5-methylcytosine to 5-hydroxymethylcytosine (5hmC) raising the possibility that this represents a new epigenetic mark [82,83]. More work will be necessary to explore and delineate additional roles of DNA methylation and associated enzymes in transcriptional regulations of PPAR γ .

6. Conclusion and perspectives

Despite the powerful influences on gene expression and phenotype, studies to define epigenetic molecular players and mechanisms in the PPAR γ signaling have just begun. Specific PPAR γ agonists exhibit adipogenic, lipogenic, insulin sensitizing and anti-

inflammatory effects. It is thought that these properties originate from adipose tissue and account for the anti-diabetic action of the TZD compounds in insulin resistance. However, TZDs cause unwanted side effects, presumably by targeting non-adipose cell types such as cardiomyocytes, collecting duct cells and osteoclasts. Development of selective PPAR γ modulators or activators that are specific to adipocytes should preserve the drug efficacy while reducing side effects. Another possible means to achieve adipose selectivity could be a combination therapy of the existing TZDs with epigenetic modulators. For example, by identifying HDAC or DNMT inhibitors that are specifically effective in epigenetically activating adipogenic gene promoters, it may be possible to combine them with a lower dose of TZD yet achieve a strong anti-diabetic action. Anti-inflammatory aspects of the PPAR γ ligands may also offer enhanced therapeutic power of existing epigenetic drugs for cancer or inflammatory diseases when used in combination. While use of HDAC and DNMT inhibitors has been successful in cancer therapy and may be a promising target for metabolic diseases, potential mechanisms of their action in metabolic pathways are largely unknown. Identification of additional molecular factors that mediate the PPAR γ transcriptional pathway is likely to contribute to our understanding and designing of future therapeutic approaches in the metabolic disorders.

Acknowledgments

Due to the space restriction, we could not cite many primary research papers, but instead cited review articles in many parts of the current review. Readers are encouraged to visit the Nuclear Receptor Signaling Atlas (NURSA) webpage at <http://www.nursa.org/> for updated database covering PPAR γ regulators. We thank Ruth Yu and Michael Downes for critical reading and helpful editing of the manuscript.

References

1. Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, Rao MS, Reddy JK. Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. *Proc Natl Acad Sci U S A*. 1995; 92:7921–5. [PubMed: 7644514]
2. Mueller E, et al. Genetic analysis of adipogenesis through peroxisome proliferator-activated receptor gamma isoforms. *J Biol Chem*. 2002; 277:41925–30. [PubMed: 12200443]
3. Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, Koder A, Evans RM. PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol Cell*. 1999; 4:585–95. [PubMed: 10549290]
4. Rosen ED, Spiegelman BM. Molecular regulation of adipogenesis. *Annu Rev Cell Dev Biol*. 2000; 16:145–71. [PubMed: 11031233]
5. Imai T, et al. Peroxisome proliferator-activated receptor gamma is required in mature white and brown adipocytes for their survival in the mouse. *Proc Natl Acad Sci U S A*. 2004; 101:4543–7. [PubMed: 15070754]
6. Sugii S, et al. PPARgamma activation in adipocytes is sufficient for systemic insulin sensitization. *Proc Natl Acad Sci U S A*. 2009; 106:22504–9. [PubMed: 20018750]
7. Moore KJ, Fitzgerald ML, Freeman MW. Peroxisome proliferator-activated receptors in macrophage biology: friend or foe? *Curr Opin Lipidol*. 2001; 12:519–27. [PubMed: 11561171]
8. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol*. 2010; 72:219–46. [PubMed: 20148674]
9. Odegaard JI, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature*. 2007; 447:1116–20. [PubMed: 17515919]
10. Wu SC, Zhang Y. Minireview: role of protein methylation and demethylation in nuclear hormone signaling. *Mol Endocrinol*. 2009; 23:1323–34. [PubMed: 19407220]
11. Wang Z, Schones DE, Zhao K. Characterization of human epigenomes. *Curr Opin Genet Dev*. 2009; 19:127–34. [PubMed: 19299119]

12. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem.* 1995; 270:12953–6. [PubMed: 7768881]
13. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem.* 2008; 77:289–312. [PubMed: 18518822]
14. He W, et al. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc Natl Acad Sci U S A.* 2003; 100:15712–7. [PubMed: 14660788]
15. Medina-Gomez G, et al. PPAR gamma 2 prevents lipotoxicity by controlling adipose tissue expandability and peripheral lipid metabolism. *PLoS Genet.* 2007; 3:e64. [PubMed: 17465682]
16. Hevener AL, et al. Muscle-specific Pparg deletion causes insulin resistance. *Nat Med.* 2003; 9:1491–7. [PubMed: 14625542]
17. Norris AW, et al. Muscle-specific PPARgamma-deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones. *J Clin Invest.* 2003; 112:608–18. [PubMed: 12925701]
18. Hevener AL, et al. Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. *J Clin Invest.* 2007; 117:1658–69. [PubMed: 17525798]
19. Gavrilova O, et al. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. *J Biol Chem.* 2003; 278:34268–76. [PubMed: 12805374]
20. Rosen ED, et al. Targeted elimination of peroxisome proliferator-activated receptor gamma in beta cells leads to abnormalities in islet mass without compromising glucose homeostasis. *Mol Cell Biol.* 2003; 23:7222–9. [PubMed: 14517292]
21. Koutnikova H, Cock TA, Watanabe M, Houten SM, Champy MF, Dierich A, Auwerx J. Compensation by the muscle limits the metabolic consequences of lipodystrophy in PPAR gamma hypomorphic mice. *Proc Natl Acad Sci U S A.* 2003; 100:14457–62. [PubMed: 14603033]
22. Chao L, Marcus-Samuels B, Mason MM, Moitra J, Vinson C, Arioglu E, Gavrilova O, Reitman ML. Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. *J Clin Invest.* 2000; 106:1221–8. [PubMed: 11086023]
23. Wan Y, Chong LW, Evans RM. PPAR-gamma regulates osteoclastogenesis in mice. *Nat Med.* 2007; 13:1496–503. [PubMed: 18059282]
24. Guan Y, et al. Thiazolidinediones expand body fluid volume through PPARgamma stimulation of ENaC-mediated renal salt absorption. *Nat Med.* 2005; 11:861–6. [PubMed: 16007095]
25. Zhang H, Zhang A, Kohan DE, Nelson RD, Gonzalez FJ, Yang T. Collecting duct-specific deletion of peroxisome proliferator-activated receptor gamma blocks thiazolidinedione-induced fluid retention. *Proc Natl Acad Sci U S A.* 2005; 102:9406–11. [PubMed: 15956187]
26. Son NH, et al. Cardiomyocyte expression of PPARgamma leads to cardiac dysfunction in mice. *J Clin Invest.* 2007; 117:2791–801. [PubMed: 17823655]
27. Perissi V, Aggarwal A, Glass CK, Rose DW, Rosenfeld MG. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. *Cell.* 2004; 116:511–26. [PubMed: 14980219]
28. Yu C, Markan K, Temple K, Deplewski D, Brady M, Cohen R. The nuclear receptor corepressors NCoR and SMRT decrease peroxisome proliferator-activated receptor gamma transcriptional activity and repress 3T3-L1 adipogenesis. *J Biol Chem.* 2005; 280:13600–5. [PubMed: 15691842]
29. Nofsinger RR, et al. SMRT repression of nuclear receptors controls the adipogenic set point and metabolic homeostasis. *Proc Natl Acad Sci U S A.* 2008; 105:20021–6. [PubMed: 19066220]
30. Fajas L, Egler V, Reiter R, Hansen J, Kristiansen K, Debril M, Miard S, Auwerx J. The retinoblastoma-histone deacetylase 3 complex inhibits PPARgamma and adipocyte differentiation. *Dev Cell.* 2002; 3:903–10. [PubMed: 12479814]
31. Guenther MG, Barak O, Lazar MA. The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. *Mol Cell Biol.* 2001; 21:6091–101. [PubMed: 11509652]
32. Koppen A, Kalkhoven E. Brown vs white adipocytes: the PPARgamma coregulator story. *FEBS Lett.* 2010; 584:3250–9. [PubMed: 20600006]

33. Debril MB, Gelman L, Fayard E, Annicotte JS, Rocchi S, Auwerx J. Transcription factors and nuclear receptors interact with the SWI/SNF complex through the BAF60c subunit. *J Biol Chem*. 2004; 279:16677–86. [PubMed: 14701856]
34. Musri M, Gomis R, Párrizas M. A chromatin perspective of adipogenesis. *Organogenesis*. 2010; 6:15–23. [PubMed: 20592861]
35. Lefterova MI, et al. PPARgamma and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. *Genes Dev*. 2008; 22:2941–52. [PubMed: 18981473]
36. Nielsen R, et al. Genome-wide profiling of PPARgamma:RXR and RNA polymerase II occupancy reveals temporal activation of distinct metabolic pathways and changes in RXR dimer composition during adipogenesis. *Genes Dev*. 2008; 22:2953–67. [PubMed: 18981474]
37. Kajimura S, Seale P, Kubota K, Lunsford E, Frangioni JV, Gygi SP, Spiegelman BM. Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex. *Nature*. 2009; 460:1154–8. [PubMed: 19641492]
38. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell*. 1998; 92:829–39. [PubMed: 9529258]
39. Seale P, et al. Transcriptional control of brown fat determination by PRDM16. *Cell Metab*. 2007; 6:38–54. [PubMed: 17618855]
40. Vernochet C, Peres SB, Davis KE, McDonald ME, Qiang L, Wang H, Scherer PE, Farmer SR. C/EBPalpha and the corepressors CtBP1 and CtBP2 regulate repression of select visceral white adipose genes during induction of the brown phenotype in white adipocytes by peroxisome proliferator-activated receptor gamma agonists. *Mol Cell Biol*. 2009; 29:4714–28. [PubMed: 19564408]
41. Pascual G, et al. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature*. 2005; 437:759–63. [PubMed: 16127449]
42. Lefterova M, et al. Cell-specific determinants of peroxisome proliferator-activated receptor gamma function in adipocytes and macrophages. *Mol Cell Biol*. 2010; 30:2078–89. [PubMed: 20176806]
43. Hamza MS, et al. De-novo identification of PPARgamma/RXR binding sites and direct targets during adipogenesis. *PLoS One*. 2009; 4:e4907. [PubMed: 19300518]
44. Nakachi Y, Yagi K, Nikaio I, Bono H, Tonouchi M, Schonbach C, Okazaki Y. Identification of novel PPARgamma target genes by integrated analysis of ChIP-on-chip and microarray expression data during adipocyte differentiation. *Biochem Biophys Res Commun*. 2008; 372:362–6. [PubMed: 18489901]
45. Wakabayashi K, et al. The peroxisome proliferator-activated receptor gamma/retinoid X receptor alpha heterodimer targets the histone modification enzyme PR-Set7/Setd8 gene and regulates adipogenesis through a positive feedback loop. *Mol Cell Biol*. 2009; 29:3544–55. [PubMed: 19414603]
46. Mikkelsen TS, Xu Z, Zhang X, Wang L, Gimble JM, Lander ES, Rosen ED. Comparative epigenomic analysis of murine and human adipogenesis. *Cell*. 2010; 143:156–69. [PubMed: 20887899]
47. Zeve D, Tang W, Graff J. Fighting fat with fat: the expanding field of adipose stem cells. *Cell Stem Cell*. 2009; 5:472–81. [PubMed: 19896439]
48. Sugii S, et al. Human and mouse adipose-derived cells support feeder-independent induction of pluripotent stem cells. *Proc Natl Acad Sci U S A*. 2010; 107:3558–63. [PubMed: 20133714]
49. Picard F, Géhin M, Annicotte J, Rocchi S, Champy M, O'Malley B, Chambon P, Auwerx J. SRC-1 and TIF2 control energy balance between white and brown adipose tissues. *Cell*. 2002; 111:931–41. [PubMed: 12507421]
50. Louet J, et al. Oncogenic steroid receptor coactivator-3 is a key regulator of the white adipogenic program. *Proc Natl Acad Sci U S A*. 2006; 103:17868–73. [PubMed: 17098861]
51. Wang Z, et al. Critical roles of the p160 transcriptional coactivators p/CIP and SRC-1 in energy balance. *Cell Metab*. 2006; 3:111–22. [PubMed: 16459312]
52. Nolte R, et al. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. *Nature*. 1998; 395:137–43. [PubMed: 9744270]

53. Gelman L, Zhou G, Fajas L, Raspe E, Fruchart JC, Auwerx J. p300 interacts with the N- and C-terminal part of PPARgamma2 in a ligand-independent and -dependent manner, respectively. *J Biol Chem.* 1999; 274:7681–8. [PubMed: 10075656]
54. Yamauchi T, et al. Increased insulin sensitivity despite lipodystrophy in Crebbp heterozygous mice. *Nat Genet.* 2002; 30:221–6. [PubMed: 11818964]
55. Steger DJ, et al. Propagation of adipogenic signals through an epigenomic transition state. *Genes Dev.* 2010; 24:1035–44. [PubMed: 20478996]
56. Fu M, et al. Cyclin D1 represses p300 transactivation through a cyclin-dependent kinase-independent mechanism. *J Biol Chem.* 2005; 280:29728–42. [PubMed: 15951563]
57. Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'Malley B, Spiegelman B. Activation of PPARgamma coactivator-1 through transcription factor docking. *Science.* 1999; 286:1368–71. [PubMed: 10558993]
58. Musri MM, Corominola H, Casamitjana R, Gomis R, Parrizas M. Histone H3 lysine 4 dimethylation signals the transcriptional competence of the adiponectin promoter in preadipocytes. *J Biol Chem.* 2006; 281:17180–8. [PubMed: 16613853]
59. Fu M, et al. p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. *J Biol Chem.* 2000; 275:20853–60. [PubMed: 10779504]
60. Wang C, et al. Direct acetylation of the estrogen receptor alpha hinge region by p300 regulates transactivation and hormone sensitivity. *J Biol Chem.* 2001; 276:18375–83. [PubMed: 11279135]
61. Carew JS, Giles FJ, Nawrocki ST. Histone deacetylase inhibitors: mechanisms of cell death and promise in combination cancer therapy. *Cancer Lett.* 2008; 269:7–17. [PubMed: 18462867]
62. Yoo E, Chung J, Choe S, Kim K, Kim J. Down-regulation of histone deacetylases stimulates adipocyte differentiation. *J Biol Chem.* 2006; 281:6608–15. [PubMed: 16407282]
63. Haberland M, Carrer M, Mokalled M, Montgomery R, Olson E. Redundant control of adipogenesis by histone deacetylases 1 and 2. *J Biol Chem.* 2010; 285:14663–70. [PubMed: 20190228]
64. Lagace D, Nachtigal M. Inhibition of histone deacetylase activity by valproic acid blocks adipogenesis. *J Biol Chem.* 2004; 279:18851–60. [PubMed: 14985358]
65. Fu M, et al. Cyclin D1 inhibits peroxisome proliferator-activated receptor gamma-mediated adipogenesis through histone deacetylase recruitment. *J Biol Chem.* 2005; 280:16934–41. [PubMed: 15713663]
66. Gao Z, He Q, Peng B, Chiao P, Ye J. Regulation of nuclear translocation of HDAC3 by IkappaBalpha is required for tumor necrosis factor inhibition of peroxisome proliferator-activated receptor gamma function. *J Biol Chem.* 2006; 281:4540–7. [PubMed: 16371367]
67. Suh JM, Zeve D, McKay R, Seo J, Salo Z, Li R, Wang M, Graff JM. Adipose is a conserved dosage-sensitive antiobesity gene. *Cell Metab.* 2007; 6:195–207. [PubMed: 17767906]
68. Picard F, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature.* 2004; 429:771–6. [PubMed: 15175761]
69. Wang H, Qiang L, Farmer SR. Identification of a domain within peroxisome proliferator-activated receptor gamma regulating expression of a group of genes containing fibroblast growth factor 21 that are selectively repressed by SIRT1 in adipocytes. *Mol Cell Biol.* 2008; 28:188–200. [PubMed: 17954559]
70. Takada I, et al. A histone lysine methyltransferase activated by non-canonical Wnt signalling suppresses PPAR-gamma transactivation. *Nat Cell Biol.* 2007; 9:1273–85. [PubMed: 17952062]
71. Lee J, et al. Targeted inactivation of MLL3 histone H3-Lys-4 methyltransferase activity in the mouse reveals vital roles for MLL3 in adipogenesis. *Proc Natl Acad Sci U S A.* 2008; 105:19229–34. [PubMed: 19047629]
72. Cho Y, Hong S, Jin Q, Wang L, Lee J, Gavrilova O, Ge K. Histone methylation regulator PTIP is required for PPARgamma and C/EBPalpha expression and adipogenesis. *Cell Metab.* 2009; 10:27–39. [PubMed: 19583951]
73. Dreijerink K, et al. The multiple endocrine neoplasia type 1 (MEN1) tumor suppressor regulates peroxisome proliferator-activated receptor gamma-dependent adipocyte differentiation. *Mol Cell Biol.* 2009; 29:5060–9. [PubMed: 19596783]

74. Mann J, Chu DC, Maxwell A, Oakley F, Zhu NL, Tsukamoto H, Mann DA. MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. *Gastroenterology*. 2010; 138:705–14. 714, e1–4. [PubMed: 19843474]
75. Zhu NL, Wang J, Tsukamoto H. Necdin-Wnt pathway causes epigenetic PPAR{gamma} repression in hepatic stellate cells. *J Biol Chem*. 2010 In Press.
76. Lee YH, Stallcup MR. Minireview: protein arginine methylation of nonhistone proteins in transcriptional regulation. *Mol Endocrinol*. 2009; 23:425–33. [PubMed: 19164444]
77. Yadav N, Cheng D, Richard S, Morel M, Iyer VR, Aldaz CM, Bedford MT. CARM1 promotes adipocyte differentiation by coactivating PPARgamma. *EMBO Rep*. 2008; 9:193–8. [PubMed: 18188184]
78. Qi C, Chang J, Zhu Y, Yeldandi AV, Rao SM, Zhu YJ. Identification of protein arginine methyltransferase 2 as a coactivator for estrogen receptor alpha. *J Biol Chem*. 2002; 277:28624–30. [PubMed: 12039952]
79. Tateishi K, Okada Y, Kallin E, Zhang Y. Role of Jhdm2a in regulating metabolic gene expression and obesity resistance. *Nature*. 2009; 458:757–61. [PubMed: 19194461]
80. Metzger E, et al. LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature*. 2005; 437:436–9. [PubMed: 16079795]
81. Garcia-Bassets I, et al. Histone methylation-dependent mechanisms impose ligand dependency for gene activation by nuclear receptors. *Cell*. 2007; 128:505–18. [PubMed: 17289570]
82. Szyf M. Epigenetics, DNA methylation, and chromatin modifying drugs. *Annu Rev Pharmacol Toxicol*. 2009; 49:243–63. [PubMed: 18851683]
83. Wu SC, Zhang Y. Active DNA demethylation: many roads lead to Rome. *Nat Rev Mol Cell Biol*. 2010; 11:607–20. [PubMed: 20683471]
84. Barrès R, Osler M, Yan J, Rune A, Fritz T, Caidahl K, Krook A, Zierath J. Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density. *Cell Metab*. 2009; 10:189–98. [PubMed: 19723495]
85. Noer A, Sorensen AL, Boquest AC, Collas P. Stable CpG hypomethylation of adipogenic promoters in freshly isolated, cultured, and differentiated mesenchymal stem cells from adipose tissue. *Mol Biol Cell*. 2006; 17:3543–56. [PubMed: 16760426]
86. Fujiki K, Kano F, Shiota K, Murata M. Expression of the peroxisome proliferator activated receptor gamma gene is repressed by DNA methylation in visceral adipose tissue of mouse models of diabetes. *BMC Biol*. 2009; 7:38. [PubMed: 19589179]

Figure 1.

Schematic representation of PPAR γ transcriptional cascades and chromatin modifications in the repressive (upper diagram) and active (lower diagram) states in adipocytes. Proteins shown in red are known to possess epigenetic enzyme activities, and possibly affect methylation (me) and acetylation (ac) of histones surrounding PPAR γ binding regions. Repressive histone marks shown to be associated with PPAR γ include H3K9me2, H3K9me3, H3K27me2, and H3K27me3, whereas activating marks include H3K4me2, H3K4me3, H4K20me1, H3K9ac and H3K27ac. The repressed state is characterized by corepressor binding and repressive methylation marks, whereas the activated state is indicated by coactivator binding and active methylation and acetylation marks. The active state is also characterized by PPAR γ agonist binding and C/EBP protein recruitment in proximity, leading to initiation of transcription by RNA polymerase II and general transcription factors (GTFs). Some associations in the diagram need further validating *in vivo* evidence and still remain hypothetical.

Table 1 Epigenetic enzymes involved in PPAR γ transcription

Enzyme	Histone substrate ¹	Cofactors or TFs affecting PPAR γ	Effects ²
Histone Acetyltransferases (HATs)			
SRC-1	H3, H4	PGC-1 α	Ligand-dependent PPAR γ coactivation
p300/CBP	H2A, H2B, H3, H4	(i) Cyclin D1 (ii) PGC-1 α (iii) GR, C/EBP β , MED1	(i) Inhibits PPAR γ activity (ii) PPAR γ coactivation (iii) Increases <i>Pparg2</i> expression
Histone deacetylases (HDACs)			
HDAC1	H2A, H2B, H3, H4	Cyclin D1, HDAC3, SUV39H1	Inhibits PPAR γ activity
HDAC3	H2A, H2B, H3, H4	(i) pRb, NCoR, SMRT (ii) I κ B α (iii) NCoR, c-Jun (iv) Adipose/ADP	(i) Corepresses PPAR γ (ii) TNF α -mediated inhibition of PPAR γ activity (iii) Ligand- and SUMOylation-dependent PPAR γ transrepression in macrophages (iv) Inhibits PPAR γ activity
SIRT1	H3K9, H4K16?	NCoR, SMRT	Corepresses PPAR γ activity
Histone methyltransferases (HMTs)			
SETDB1	H3K9	Wnt5a, NLK, CHD7	Corepresses PPAR γ activity
SETD8/Set7	H4K20	?	Induced by PPAR γ ; Coactivates <i>Pparg</i> and target gene expression
SUV39H1	H3K9	Cyclin D1, HDAC1, HDAC3	Inhibits PPAR γ activity
MLL3/MLL4	H3K4	(i) ASC-2/PRIP/NCOA6 (ii) PTIP	(i) Ligand-dependent PPAR γ /RXR α coactivation (ii) Increases <i>Pparg</i> and <i>Cebpa</i> expression
MLL1/MLL2	H3K4	MEN1/menin	Coactivates PPAR γ activity
?	H3K9	MeCP2, HP1 α	HP1 α -dependent corepression of <i>Pparg</i> expression in HSCs
EZH2	H3K27	MeCP2	Induced by MeCP2; represses <i>Pparg</i> expression in HSCs
CARM1	H3R2, H3R17, H3R26	?	Ligand-dependent PPAR γ coactivation of <i>aP2</i> gene
PRMT2	H4?	?	Interacts with PPAR γ ; coactivates PPAR γ activity?
Histone demethylases (HDMs)			
JHDM2A	H3K9	PGC-1 α , p300/CBP, SRC-1	Coactivates PPAR γ RXR α in β -adrenergic induction of UCP1 gene
UTX	H3K27	ASC-2/PRIP, MLL3, MLL4	Counteracts with HMT activities?

TF: transcription factor

¹ These do not necessarily affect PPAR γ activity; other substrates (histones or non-histones) are possible.² Unless noted, these effects are in adipocytes.