FEBS Letters 584 (2010) 3242–3249

journal homepage: www.FEBSLetters.org

Review

PPARγ in adipocyte differentiation and metabolism – Novel insights from genome-wide studies

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ARTICLE INFO

Article history:
Received 2 May 2010
Revised 3 June 2010
Accepted 7 June 2010
Available online 11 June 2010

Edited by Wilhelm Just

Keywords:
Adipogenesis
Genome-wide
Chromatin immunoprecipitation
PPARγ
C/EBP

ABSTRACT

Adipocyte differentiation is controlled by a tightly regulated transcriptional cascade in which PPARγ and members of the C/EBP family are key players. Here we review the roles of PPARγ and C/EBPs in adipocyte differentiation with emphasis on the recently published genome-wide binding profiles for PPARγ and C/EBPα. Interestingly, these analyses show that PPARγ and C/EBPα binding sites are associated with most genes that are induced during adipogenesis suggesting direct activation of many more adipocyte genes than previously anticipated. Furthermore, an extensive overlap between the C/EBPα and PPARγ cistromes indicate a hitherto unrecognized direct crosstalk between these transcription factors. As more genome-wide data emerge in the future, this crosstalk will likely be found to include several other adipogenic transcription factors.

1. Obesity and type 2 diabetes mellitus

During the last two decades the incidence of obesity and type 2 diabetes mellitus (T2DM) has increased rapidly worldwide. Consequently, huge efforts are being made to elucidate the mechanisms underlying adipose tissue development and the pathology of obesity. In a normal healthy person, white adipose tissue stores excess energy in the form of fat. When energy intake is scarce, this fat can be released as fatty acids into the blood stream, where it can be taken up by other tissues (e.g. liver and skeletal muscle) and used as an alternative energy source. When energy intake consistently exceeds energy expenditure, the adipose tissue expands due to hypertrophy in particular but also due to hyperplasia of the adipocytes. The hypertrophied adipocytes secrete adipokines which may directly interfere with insulin signaling and which have also been shown to recruit macrophages that induce an inflamed state in the adipose tissue. The macrophages secrete high amounts of pro-inflammatory cytokines, which further exacerbates insulin resistance of the adipocytes, and causes a decrease in the net flux of fatty acids into the adipocytes. This results in an increased deposition of fatty acids as triacylglycerides in non-adipose tissues such as the liver, skeletal muscle, and pancreas. A large body of literature has demonstrated that such ectopic deposition of fatty acids causes metabolic overload and stress that through various signaling pathways lead to insulin resistance in these tissues. Systemic insulin resistance increases the demand for insulin production, and T2DM may develop if the demand exceeds the secretory capacity of the β-cells. In addition to T2DM, obesity is often associated with cardiovascular diseases such as atherosclerosis, which is characterized by cholesterol accumulation in macrophages leading to the formation of foam cells and plaques in the arterial wall. These physiological and metabolic responses to obesity have been extensively reviewed elsewhere [1–4].

In keeping with the central role of adipocytes in maintaining whole body insulin sensitivity, drugs that target the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ), which is an important transcription factor in the development and function of the adipose tissue (see below), have been shown to function as potent insulin sensitizers. Activation of PPARγ in the adipose tissue by compounds such as thiazolidinediones (TZDs) leads to increased storage capacity of fatty acids in the adipocytes and thereby a decrease in the amount of circulating fatty acids [3,5]. In addition, PPARγ activates the expression of adiponectin, which is involved in the sensitization of liver and muscle to insulin [6]. Furthermore, liganded PPARγ antagonizes the function of pro-inflammatory transcription factors such as nuclear factor-κB (NF-κB), thereby decreasing the expression of pro-inflammatory cytokines and diminishing the inflamed state in the adipose tissue [3]. Finally, activation of PPARγ has been shown to lead to increased de novo

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0014-5793/$36.00
doi:10.1016/j.febslet.2010.06.010

differentiation of preadipocytes, thereby increasing the number of smaller and more insulin sensitive adipocytes [7]. Interestingly, recent data indicate that PPARγ agonists may also promote a more brown adipocyte-like phenotype with increased mitochondrial capacity and increased uncoupling potential [8–10]. Thus, the adipose tissue is an important regulator of whole body metabolism and a central target tissue in the treatment of insulin resistance and T2DM.

2. Roles of PPARγ and C/EBPs during adipogenesis

Adipogenesis is a tightly controlled process that involves an intricate network of transcription factors acting at different time points during differentiation [11,12]. Several studies have clearly established PPARγ as a key regulator of adipocyte development both in vitro and in vivo. This receptor is known to be obligate for adipocyte differentiation [13,14] and in many cases sufficient to convert non-adipose cells to adipocyte-like cells [15,16]. PPARs are members of the nuclear receptor superfamily and characterized as adopted orphan receptors, which are activated by a variety of fatty acids and their derivatives such as prostaglandins [17,18]. This receptor family is comprised of three different subtypes (PPARα, β/δ, γ), all of which are important regulators of lipid and glucose metabolism in many different tissues including skeletal muscle, liver, adipose tissue, and gut [19,20]. PPARγ is found in two different isoforms termed PPARγ1 and PPARγ2. The latter is almost exclusively expressed in adipose tissue, whereas PPARγ1 is more ubiquitously expressed. PPARs, like other nuclear receptors, are comprised of a non-conserved N-terminal A/B domain containing the activating function 1 (AF-1), a highly conserved DNA-binding domain (DBD), a hinge region (D domain), and a C-terminal ligand-binding domain (LBD) containing the activating function 2 (AF-2). The DBD anchors PPARs to their binding sites on the DNA template through interactions between the two zinc fingers in this domain and the major groove of the DNA double helix [21,22]. PPARs bind as obligate heterodimers with the retinoic X receptor (RXR) to direct hexanucleotide repeats spaced by 1 nucleotide (DR-1) termed PPAR response elements (PPREs) [23]. AF-2 of the PPARγ LBD interacts directly with various coactivators containing LXXLL motifs such as transcriptional intermediary factor-2 (TIF2) [24] and Mediator subunit 1 (MED1)/thyroid hormone receptor-associated protein 220 (TRAP220) [25,26] in a ligand-dependent manner. In addition, AF-1 in the N-terminal A/B domain has also been shown to interact directly with some coactivators including p300 [27], HIV-1-Tat interactive protein 60 (Tip60) [28], and MED14 [29] in a ligand-independent manner, and we have recently shown that this domain is important for PPARγ transactivation of a subset of PPARγ target genes [30]. Further emphasizing the important regulatory function of the A/B domain, phosphorylation of PPARγ2 on serine 112 in this domain has been shown to both inhibit and increase the transcriptional activity of the receptor depending on the cellular context [31–33]. Furthermore, it has recently been demonstrated that the phosphorylation status of serine 16 and 21 regulates the intracellular localization of PPARγ [34].

Members of the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors also play an important role in promoting adipocyte differentiation. The C/EBPs belong to the basic leucine zipper family and is comprised of six members, and three family members (C/EBPα, -β, and -δ) have a well established role in adipogenesis. C/EBPα is expressed late in adipogenesis and is a key regulator of adipocyte differentiation as evidenced by the lack of white adipose tissue in C/EBPα$^{-/-}$ mice [35,36]. There are two different isoforms of C/EBPα termed p30 and p42, respectively, with the latter isoform being the most potent transactivator [37,38]. C/EBPβ exists in three different isoforms (liver-enriched activating protein (LAP), LAP*, and liver-enriched inhibiting protein (LIP)). LAP* is the most potent transactivator followed by LAP, whereas LIP lacks the transactivation domains of LAP and LAP* and thus may function, at least in some cases, as a dominant negative C/EBP isoform [39,40]. C/EBPβ and C/EBPδ single knockout mice have only slightly smaller white fat pads compared to wild type [41]. However, the size of the adipose tissue in double knockout mice is significantly reduced [41], thereby indicating important but partially redundant roles of these two C/EBPs in adipogenesis. Interestingly however, whereas C/EBPα is antimitotic [42–44] C/EBPβ is required for mitotic clonal expansion [45]. Furthermore, whereas C/EBPα is required for proper development of white adipose tissue, C/EBPβ and C/EBPδ appear to be more important for brown adipocyte differentiation [36,41,46].

Most of our knowledge about the mechanisms of adipocyte differentiation has been established by studying immortalized fibroblast cell lines (e.g. 3T3-L1 and 3T3-F442A) that can be induced to differentiate into adipocytes in vitro by the administration of a hormonal cocktail [47,48]. From studies in these differentiation systems, the central transcriptional network controlling adipogenesis has been elucidated. Within few hours after induction of differentiation, the expression and transcriptional activity of C/EBPβ and C/EBPδ are induced and these transcription factors subsequently induce the expression of C/EBPα and PPARγ [12,49]. Furthermore, the expression of C/EBPα and PPARγ is sustained by a positive feedback loop, which serves to maintain the phenotype of the mature adipocyte [12,15,50]. In addition to these important transcription factors, which constitute the central transcriptional axis controlling adipogenesis, several other transcription factors such as Krox20 [51], Krüppel-like factors [52–54], sterol-regulatory element-binding protein (SREBP)-1c [55–57], and Stat5 [58–60] have been shown to be important for adipocyte differentiation. A common characteristic of all these factors is that they seem to regulate adipogenesis by regulating the expression or activity of C/EBPβ, C/EBPδ, C/EBPα, and PPARγ, thereby further highlighting the central role of these key transcription factors in the transcriptional cascade controlling adipocyte differentiation.

The relative importance of PPARγ and C/EBPα in adipogenesis has been investigated by elegant studies in mouse embryonic fibroblasts (MEFs) by Spiegelman and colleagues. Their results showed that PPARγ can induce adipogenesis in C/EBPα$^{-/-}$ MEFs in vitro [15], whereas C/EBPα is unable to do the same in PPARγ$^{-/-}$ MEFs [50]. These results confirm the central role of PPARγ in adipocyte differentiation and indicate that C/EBPα is not obligate for activation of adipocyte specific genes, provided PPARγ is ectopically expressed [50]. However, it remains to be investigated whether other members of the C/EBP family, like C/EBPβ and δ, may be able to partially compensate for the lack of C/EBPα – i.e. whether the C/EBPs as a family are obligate for adipocyte differentiation.

3. Novel Insights from genome-wide profiling of PPARγ binding during adipogenesis

Fixation of living cells with formaldehyde followed by immunoprecipitation using specific antibodies was developed more than 20 years ago as a technique to investigate protein–DNA interactions in a cellular context [61]. This technique, which is now termed chromatin immunoprecipitation (ChIP), has since been used extensively to determine transcription factor binding to the chromatin template within cells. Recently, ChIP has been combined with high-throughput techniques such as tiled oligo arrays (ChIP-chip) and deep sequencing (ChIP-seq) to get a global map of transcription factor binding and distribution of histone marks (see Fig. 1 for further explanations) [62–64]. The development of these

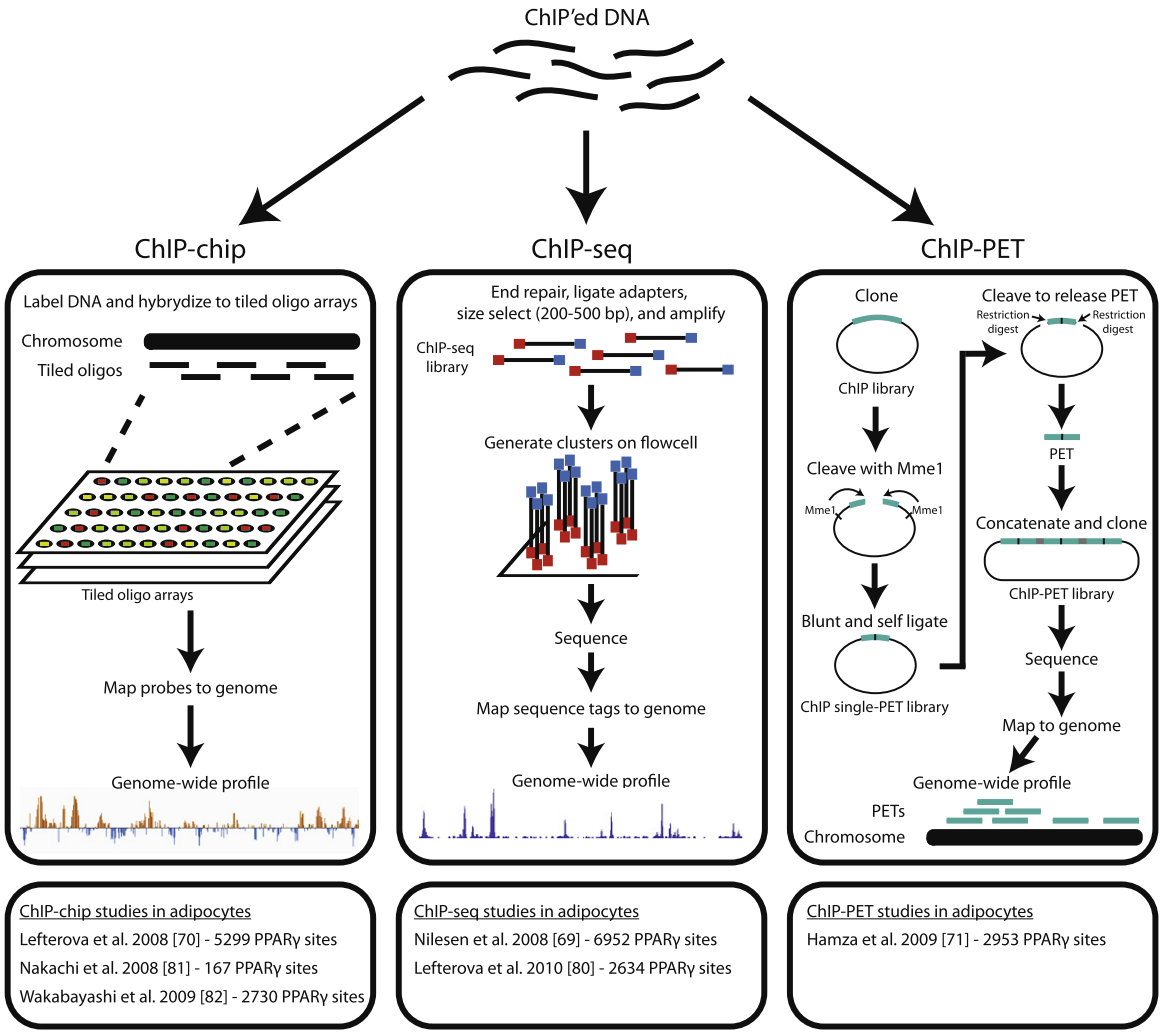

Fig. 1. Methods used to study genome-wide PPARγ binding in adipocytes. Overview of the major steps in ChIP-chip, ChIP-seq, and ChIP-PET (Paired end diTags) technologies used for genome-wide profiling of PPARγ in adipocytes. In ChIP-chip (left), input and ChIP'ed DNA is labeled with fluorescent dyes and hybridized to tiled oligo arrays spanning the entire genome or defined genomic regions (e.g. gene promoters). Probes are mapped to the genome and transcription factor binding sites are identified as regions with a significant fluorescent signal in the ChIP'ed DNA sample compared to the input. ChIP-seq (middle) is fundamentally different from ChIP-chip, as the ChIP'ed DNA is analyzed by sequencing instead of hybridization to arrays. In this method, adapters are ligated to the ChIP'ed DNA and short fragments (200–500 bp) are selected and amplified by PCR to generate a ChIP-seq library. These fragments are hybridized to a flow cell and each fragment is amplified by bridge amplification on the flow cell to generate clusters that each originate from a single DNA fragment. The clusters are then sequenced simultaneously and sequence tags are subsequently mapped to the genome. Peaks of sequence tags represent transcription factor binding sites. Finally, ChIP'ed DNA can be analyzed by ChIP-PET (right). In this method, ChIP'ed DNA is cloned to yield a ChIP library. This is subsequently digested with a restriction enzyme (e.g. MmeI), blunted, and self-ligated to give a ChIP single-PET library. The paired end diTag is then released by restriction enzyme digestion, concatenated, and cloned to give a ChIP-PET library. The paired end diTags are then sequenced and mapped to the genome. All of the methods described above give an unbiased genome-wide profile of transcription factor binding, and they have all been used to study PPARγ binding in 3T3-L1 adipocytes (bottom).

platforms has paved the way for unbiased experimental identification of transcription factor binding sites. The advantage of such approaches over in silico predictions, which only take the DNA sequence and not the chromatin structure or other associated proteins into account, are obvious [65,66]. ChIP-chip was the first genome-wide ChIP technology to be widely employed; however, this technique is relatively expensive to use for complete genome-wide studies, and cross-hybridization to tiled oligo arrays may increase the number of false positives. ChIP-seq is currently the method of choice in transcriptional genomics as this technique is cost efficient, very sensitive, and has a resolution that is much higher (up to 1 bp) than ChIP-chip. Two additional platforms for analyzing DNA from ChIP experiments termed ChIP-cloning and ChIP-PET (Paired end diTags) (Fig. 1) have also been used to study global transcription factor binding [67,68] although to a lesser extent than the two platforms described above. Both techniques offer a global unbiased view of transcription factor binding; however, they employ extensive cloning of ChIP'ed DNA followed by sequencing and are consequently more laborious than ChIP-chip and ChIP-seq.

Given the importance of PPARγ in adipogenesis, we and others have recently determined the genome-wide binding profile of PPARγ and its heterodimerization partner RXR using ChIP-seq [69], ChIP-chip [70], and ChIP-PET [71] (Fig. 1). All studies reported a very high degree of overlap between PPARγ and RXR binding clearly showing that PPARγ associates with endogenous PPARγ target sites as a heterodimer with RXR on a global scale (as exemplified by the stearoyl-CoA desaturase 1 (*Scd1*) locus in Fig. 2A). This is in line with previous findings showing that PPARγ dimerization with RXR is obligate for direct DNA binding [72,73]. Another interesting finding from these unbiased genome-wide studies is that although PPARγ:RXR binding sites are enriched at promoters, the

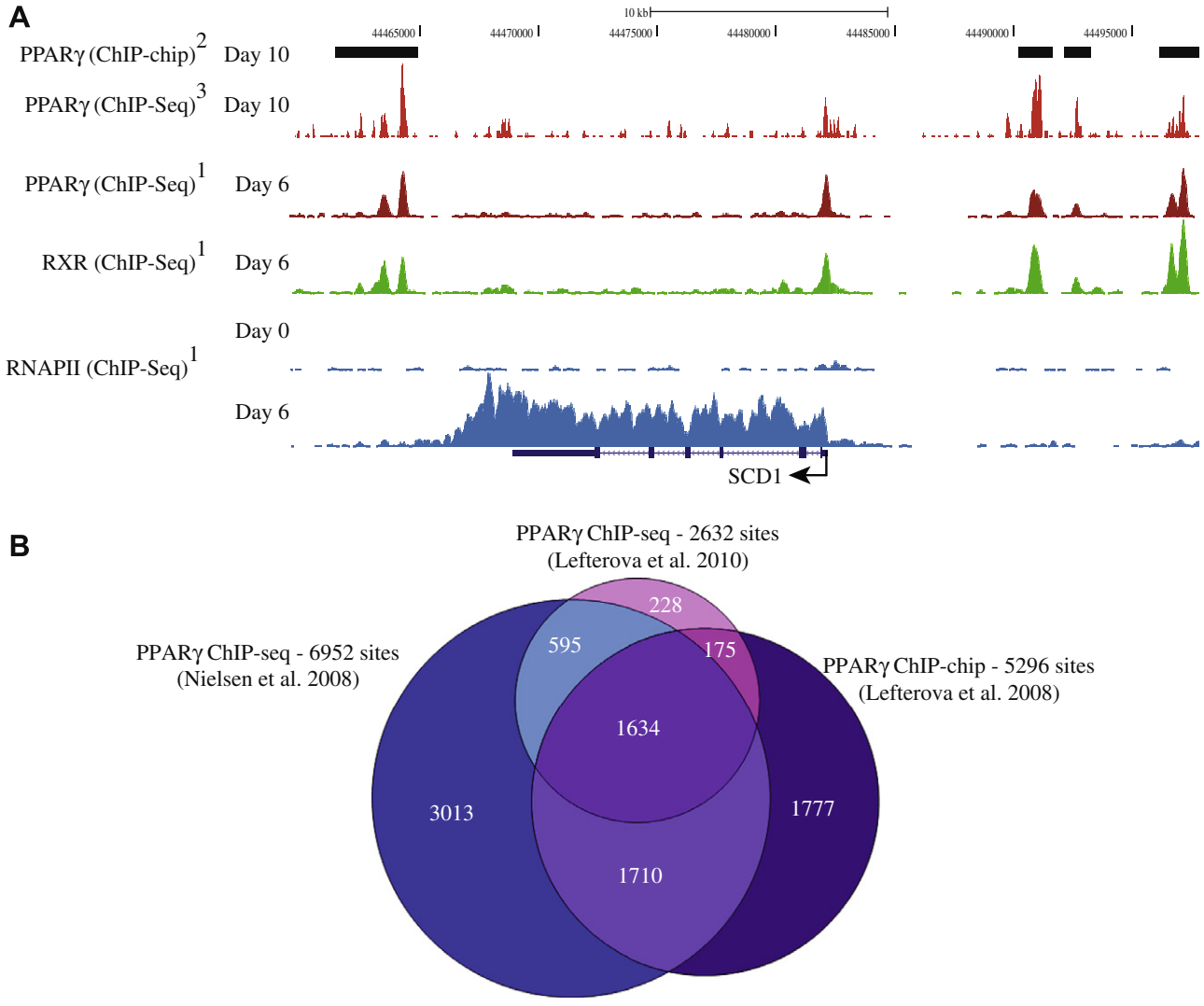

Fig. 2. Genome-wide profiling of PPARγ binding sites in adipocyte differentiation. (A) ChIP-seq and ChIP-chip data from 3T3-L1 adipocytes viewed in the UCSC genome browser showing PPARγ- and RXR–binding sites and RNAPII occupancy for the stearoyl-CoA desaturase 1 (*Scd1*) locus ([1]Nielsen et al. 2008 ChIP-Seq [69], [2]Lefterova et al. 2008 ChIP-chip [70], and [3]Lefterova et al. 2010 ChIP-Seq [80]). The height of the peaks indicates the binding intensity of the factors to DNA at the respective genomic position. Black bars for PPARγ ChIP-chip data indicate peak detected target sites at day 10. (B) Venn diagram representing overlap between the three adipocyte genome-wide PPARγ binding studies shown in A (Nielsen et al. 2008, FDR < 0.1%; Lefterova et al. 2008, FDR < 1%; Lefterova et al. 2010, FDR < 0.5%). The total numbers of PPARγ binding sites in the Venn diagram from Lefterova et al. 2008 and 2010 deviate slightly from the numbers previously published [70,80] because the data was transformed from the mm8 to the mm9 Mouse Genome Assembly.

majority of PPARγ:RXR binding sites are located in regions far away from transcription start sites (TSS). Similar findings have been reported for the estrogen receptor (ER) [65,74], glucocorticoid receptor (GR) [75], androgen receptor (AR) [76], and vitamin D receptor (VDR) [77], suggesting that this pattern of binding is a general feature of several nuclear receptors and many other transcription factors. Importantly, global profiling of H3K9 acetylation revealed that PPARγ binding sites are associated with high levels of this activating histone mark, thereby indicating that the identified binding sites are in fact functional regulatory sites [70]. These findings show that PPARγ as well as other nuclear receptors act at a distance from target gene promoters and suggest that looping between enhancer elements and promoters is a common mechanism of PPARγ transactivation of target genes, as has been demonstrated for ER [78]. In keeping with this, we have recently shown that an enhancer in intron 1 of the uncoupling protein (UCP) 3 not only regulates expression of UCP3 but also loops down to specifically interact with and most likely activate the adjacent UCP2 gene promoter [79].

The overlap between PPARγ binding from Nielsen et al. 2008 [69], Lefterova et al. 2008 [70], and Lefterova et al. 2010 [80] is shown for the *Scd1* locus in Fig. 2A and by a genome-wide Venn diagram in Fig. 2B. These studies reveal that 1634 PPARγ target sites are found in mature 3T3-L1 adipocytes irrespective of the laboratory, ChIP detection method, and peak detection program. This pool of PPARγ binding sites thus represents high-confidence binding sites occupied by PPARγ in adipocytes. Interestingly, it also shows that in total 9132 PPARγ sites have been detected by the two laboratories, showing that the number of sites that can be associated with PPARγ in adipocytes is much higher than the number of high-confidence sites. This indicates that other factors (e.g. transcription factors, cofactors, epigenetic factors) might influence the PPARγ cistrome and thereby fine tune PPARγ action in adipocytes.

Two other recent studies used ChIP in combination with tiled oligo promoter arrays to identify PPARγ binding sites during adipogenesis (Fig. 1). Nakachi et al. [81] used this approach together with microarray gene expression analysis to identify 20 genes that

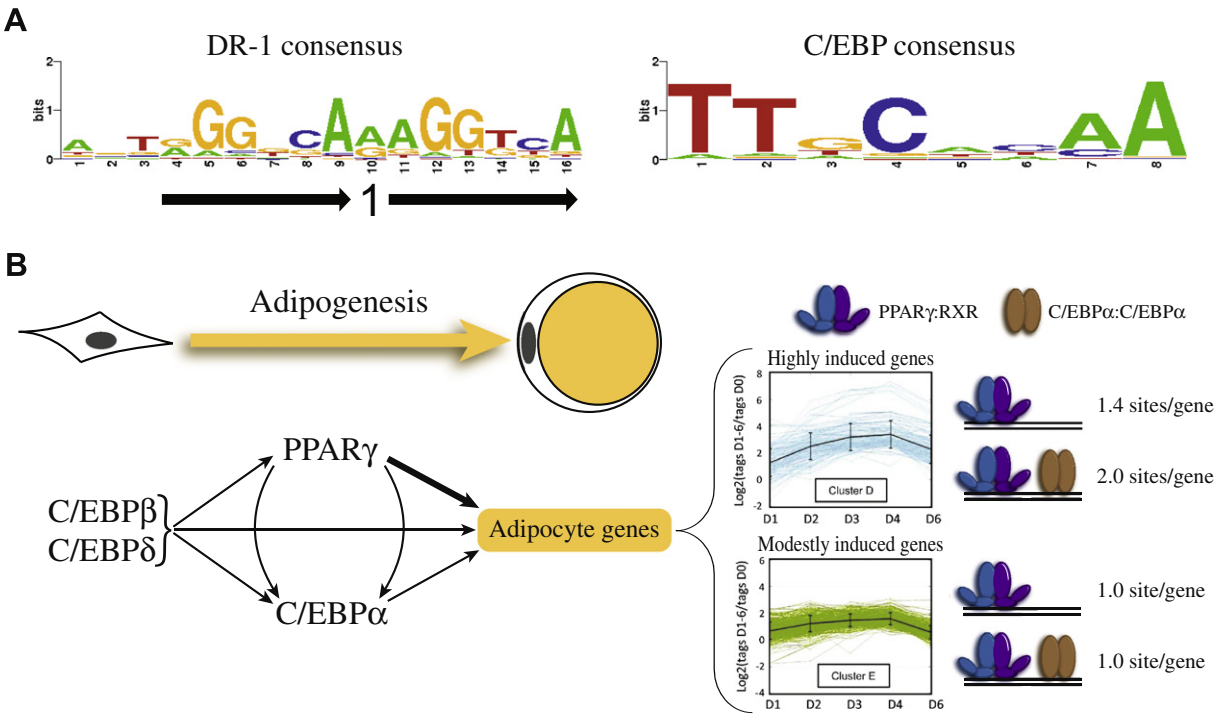

Fig. 3. C/EBP and PPARγ crosstalk during adipogenesis. (A) Web logo of DR-1 type PPRE and C/EBP consensus motif Position Weight Matrix (PWM) generated based on de novo motif search of PPARγ:RXR–binding sites (Nielsen et al. 2008 [69]). (B) Schematic overview of the transcriptional network controlling adipogenesis (left) and prevalence of common PPARγ:RXR–C/EBP sites within 100 kb of the TSS of genes in RNAPII cluster D (highly upregulated genes) and E (modestly upregulated genes) (Nielsen et al. 2008 [69]) (right).

have a PPARγ binding sites in the promoter and were upregulated during 3T3-L1 adipocyte differentiation. Sixteen of these genes were novel PPARγ target genes. Wakabayashi et al. [82] identified 2730 PPARγ target sites in mature 3T3-L1 adipocytes using a promoter array tiling from −6 kb to +2.5 kb relative to the TSS of 28 000 mouse proximal promoters. Interestingly, they combined binding information from the PPARγ ChIP-chip with gene expression data and identified a cluster of eight genes encoding protein methyltransferases containing SET domains as direct PPARγ target genes during adipogenesis. One of these termed Setd8 was shown to be important for PPARγ target gene activation and adipogenesis by increasing H4K20 mono-methylation at PPARγ target genes. These findings indicated a positive feedback loop between PPARγ and SET domain methyltransferases that may be important for adipocyte differentiation.

4. PPARγ activates most genes linked to adipogenesis and metabolism

RNAPII occupancy at ER target gene promoters as determined by ChIP in combination with tiled oligo promoter arrays has previously been used to study gene transcription in response to estrogen treatment [83]. We have recently used ChIP-seq to determine the genome-wide occupancy of RNAPII during adipogenesis and used RNAPII occupancy within genes as a measure of transcriptional activity [69]. Briefly, sequence tags from +250 to the end of Ensembl genes were counted on the different days of differentiation for all genes >1 kb in length, and the number of sequence tags within each gene was expressed relative to the number at day 0 of differentiation and was used a measure of relative transcription level. In these analyses, promoter regions were omitted to avoid including tags from RNAPII stalled at the promoter. RNAPII occupancy within genes was shown to correlate well with primary transcript levels as measured by qPCR using intron-exon primers (Median Spearman correlation factor 0.83). Cluster analysis of the regulated genes identified five distinct clusters based on their temporal profile of RNAPII occupancy (i.e. transcription) during differentiation. Genes in clusters A and B were linked to cell cycle and cell proliferation and downregulated during adipogenesis. Genes in cluster C were linked to cell cycle and the ribosome and were transiently upregulated and then downregulated later in the differentiation process. Finally, genes in cluster D and E were linked to glucose and lipid metabolism and upregulated during differentiation. Assigning peaks to the nearest gene revealed that PPARγ:RXR sites in mature adipocytes are associated with 74% and 47% of the upregulated genes in cluster D and E, respectively, thereby suggesting that PPARγ is involved in the induction of a hitherto unrecognized high number of genes activated during adipogenesis. Notably, almost all genes involved in lipid and glucose metabolism had PPARγ:RXR target sites assigned. These findings were supported by the Lazar group who showed that 63% of the genes that are upregulated more than threefold during adipogenesis, as determined by gene expression microarray, have at least one PPARγ binding site within 50 kb of their TSS [70]. Both studies also showed that a subset of these binding sites can drive gene expression in a heterologous promoter reporter assay. Furthermore, knockdown of PPARγ expression using siRNA approaches or activation of PPARγ by its high-affinity ligand rosiglitazone was demonstrated to decrease and increase expression of these genes, respectively [69,70]. These findings indicate that PPARγ in addition to being a central coordinator of the adipogenic program also acts as a direct activator of most adipocyte specific genes.

5. PPARγ and C/EBPα bind adjacent on a genomic scale in adipocytes

Motif analyses on the DNA sequences from PPARγ:RXR binding sites in mature adipocytes revealed that the DR-1 type PPRE

including the reported 5′ extension consensus sequence identified by in vitro studies [72,73], is highly enriched at PPARγ:RXR binding sites [69,70] (Fig. 3A). PPARγ has also been shown to associate with DR-2 elements in vitro [84]; however, DR-2 elements were not found to be enriched at PPARγ:RXR target sites, thereby indicating that the DR-1 element is the main motif directing PPARγ action in adipocytes.

Interestingly, both Lefterova et al. [70] and Nielsen et al. [69] reported the enrichment of a C/EBP binding motif at PPARγ target sites in addition to the DR-1 element, suggesting that these adipogenic transcription factors work together at common regulatory sites to regulate target genes (Fig. 3A). C/EBPα ChIP at a few selected loci confirmed that C/EBPα and PPARγ do occupy some of the same sites in mature adipocytes [69]. These findings were extended by Lefterova et al. [70], who showed that 63% of the PPARγ target sites identified by ChIP-chip in mature adipocytes were also bound by C/EBPα, thereby identifying a novel direct crosstalk between these two key adipogenic transcription factors at common regulatory sites on a global scale. Interestingly, C/EBPβ, which is important early in adipogenesis has a binding profile similar to C/EBPα in mature adipocytes [70]. Importantly, knock down of any of these two C/EBP family members or PPARγ in mature adipocytes, reduced the expression of genes associated with common PPARγ:RXR–C/EBP binding sites. This suggests that crosstalk between PPARγ:RXR and C/EBPs at common regulatory sites is important for regulating the expression of adipocyte genes (Fig. 3B). Interestingly, genes contained in RNAPII cluster D described above, which are highly induced during adipogenesis, have on average two common PPARγ:RXR–C/EBPα sites within 100 kb of their TSS, whereas genes in cluster E only have one common site on average in the proximity of their TSS (Fig. 3B). This may at least partly explain why genes in cluster D are more highly induced than genes in cluster E, and it suggests that the crosstalk between C/EBPs and PPARγ is especially important for the induction of the highly induced adipocyte genes.

The finding that PPARγ and C/EBPs occupy the same regulatory sites and directly coordinate the expression of most adipocyte genes has important implications for our understanding of the transcriptional network controlling adipogenesis. Although it is evident that PPARγ is a key regulator of adipogenesis, the data from the genome-wide analyses described above indicate that members of the C/EBP family may directly promote or modulate PPARγ activity at the majority of its binding sites. Interestingly, the co-occupancy of C/EBPs with PPARγ in adipocytes is not restricted to C/EBPα, since C/EBPβ is also bound to many target sites even in the mature adipocytes [69,70]. This indicates that C/EBPβ, in addition to being an early inducer of PPARγ expression, may also cooperate with PPARγ in mature adipocytes (Fig. 2B). Furthermore, C/EBPβ occupies many PPARγ target sites prior to binding of PPARγ [69], indicating that this subtype may also function as a priming factor preparing the chromatin template for PPARγ. The partial redundancy between the C/EBP subtypes may have made it difficult to observe such functions of C/EBPs in previous genetic studies. Further investigations are necessary to delineate the functional and molecular relationship between members of the C/EBP family and PPARγ on common regulatory target sites of adipocyte genes.

6. Concluding remarks

The recent genome-wide studies discussed in this review have greatly increased our understanding of the mechanisms underlying PPARγ action during adipogenesis. Most interestingly, these studies have revealed a novel crosstalk between PPARγ and C/EBPs, which has revised our view on the transcriptional network controlling adipogenesis and adipocyte function. Concurrently, these studies have inspired new interesting questions. First, how do PPARγ and C/EBPs work together on the chromatin template? In particular, it will be interesting to investigate if they occupy common sites at the same time as a part of the same complex or if they work in a sequential manner. In this regard, it is interesting to speculate that PPARγ and C/EBPs directly interact as has been demonstrated for C/EBPs and several other nuclear receptors including GR and PPARα [85,86]. Second, how do epigenetic marks and the chromatin structure change at common PPARγ:RXR–C/EBP sites during adipogenesis? Given the early binding of C/EBPβ to common PPARγ:RXR–C/EBP sites, it will be especially interesting to investigate how the chromatin landscape changes early in adipogenesis and how this affects PPARγ target gene activation. Finally, are other transcription factors directly involved in the PPARγ:RXR–C/EBP crosstalk? In this regard, the Krüppel-like factor (Klf) family of transcription factors is of particular interest, as several members of this family have been shown to be involved in adipocyte differentiation [52–54]. It is likely that a more extensive crosstalk involving several adipogenic transcription factors will be revealed as more genome-wide profiles emerge in the near future.

Acknowledgements

The authors would like to thank Dr. Mitchell A. Lazar for sharing PPARγ ChIP-seq data used in this review. This work was supported by the Danish Council for Independent Research – Natural Sciences, the Novo Nordisk Foundation and by grants to the EU FP6 STREP project, X-TRA-NET.

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