Feature Review

CellPress

PPARγ and the global map of adipogenesis and beyond

Martina I. Lefterova[1,2*], Anders K. Haakonsson[3*], Mitchell A. Lazar[2], and Susanne Mandrup[3]

[1] Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA
[2] Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, and Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA
[3] Department of Biochemistry and Molecular Biology, University of Southern Denmark, 5230 Odense, Denmark

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear receptor (NR) superfamily of ligand-dependent transcription factors (TFs) and function as a master regulator of adipocyte differentiation and metabolism. We review recent breakthroughs in the understanding of PPARγ gene regulation and function in the chromatin context. It is now clear that multiple TFs team up to induce PPARγ during adipogenesis, and that other TFs cooperate with PPARγ to ensure adipocyte-specific genomic binding and function. We discuss how this differs in other PPARγ-expressing cells such as macrophages and how these genome-wide mechanisms are preserved across species despite modest conservation of specific binding sites. These emerging considerations inform our understanding of PPARγ function as well as of adipocyte development and physiology.

PPARγ as a master regulator of adipocyte biology

PPARγ is a member of the NR superfamily of ligand-activated TFs that regulate essential aspects of biology from development to metabolism [1–3]. PPARγ is required for adipocyte differentiation, regulation of insulin sensitivity, lipogenesis, and adipocyte survival and function [1,4,5] (Box 1). The structure of PPARγ and its mechanism of binding to DNA are similar to those of several other NRs (Box 2). Synthetic PPARγ agonists have emerged as important pharmacologic agents in diabetes management; however, their use has been limited due to serious side effects caused by off-target PPARγ activation in non-adipose tissues [6] (Box 3). Novel strategies involve selective targeting of PPARγ in adipose tissue, for instance with compounds that modulate PPARγ activity by targeting post-translational modifications of the receptor. Thus, elucidating the gene- and tissue-selectivity of its actions could lead to the development of novel PPARγ compounds that maintain efficacy while reducing side effects. Accomplishing this would involve defining the PPARγ transcriptional network and putative regulatory elements both in their specific chromatin contexts and in different cell types. Integrating these data with gene expression profiling under conditions that affect PPARγ levels or activity may reveal cell type-specific PPARγ transcription networks that potentially could be targeted in tissue-selective ways.

We discuss here how the PPARγ transcriptional network is established during adipogenesis. We describe the molecular mechanisms underlying cell type-specific PPARγ actions, and offer insights into promises and pitfalls of translating discoveries made in murine systems to PPARγ biology in humans.

Establishment of the PPARγ transcriptional network during adipogenesis

Adipogenesis has been studied extensively *in vitro*, in particular using the murine 3T3-L1 preadipocyte cell line (Box 1) [7–10]. Based on these *in vitro* studies it appears that adipogenesis proceeds through the activation of at least two waves of TFs (Figure 1). The first is induced directly by the adipogenic cocktail, and includes TFs such as the CCAAT-enhancer-binding proteins C/EBPβ and -δ as well as the glucocorticoid receptor (GR), signal transducer and activator of transcription 5A (STAT5A), and the cAMP-response element-binding protein (CREB). These factors in turn activate TFs of the second wave, which initiate the adipocyte gene program [8,11]. PPARγ and C/EBPα appear to play the most prominent roles in this second wave – as demonstrated by loss-of-function studies [4](Box 1).

PPARγ binding during adipogenesis

Over the past decade new techniques, such as chromatin immunoprecipitation (ChIP) combined with whole genome

Glossary

ChIP-seq: chromatin immunoprecipitation (ChIP) coupled with deep sequencing to investigate protein–DNA interactions on a genome-wide scale.
DHS-seq (also called DNase-seq): DNase I hypersensitive (DHS) site analysis coupled with deep sequencing to investigate chromatin accessibility on a genome-wide scale.
FAIRE-seq: formaldehyde-assisted isolation of regulatory elements (FAIRE) coupled with deep sequencing.
Hotspot: genomic region occupied by multiple transcription factors in a given cell type.
Position weight matrix: probabilistic representation of DNA sequence motifs generated based on alignment of multiple transcription factor binding sites.

Corresponding authors: Lazar, M.A. (lazar@mail.med.upenn.edu); Mandrup, S. (s.mandrup@bmb.sdu.dk).
Keywords: PPARγ; adipogenesis; chromatin; genome-wide analyses; transcriptional network.
*These authors contributed equally to this work.

1043-2760/
Published by Elsevier Ltd. http://dx.doi.org/10.1016/j.tem.2014.04.001

Box 1. Importance of PPARγ in adipogenesis

Many studies on PPARγ have utilized adipocyte cell lines, such as 3T3-L1 cells, which are derived from mouse embryonic fibroblasts and can be induced to undergo adipocytic differentiation using a cocktail of dexamethasone, a cAMP-elevating agent (3-isobuthyl-1-methylxantine), and insulin [90,91]. Early studies demonstrated that PPARγ is induced during adipogenesis [92,93], and that PPARγ is necessary and sufficient for adipocyte differentiation [94], thereby establishing PPARγ as a master regulator of adipogenesis [1]. *In vivo*, whole-body PPARγ knockout in mice is embryonic lethal as a result of placental defects, whereas mice with chimeric PPARγ expression have shown that embryonic stem cells lacking PPARγ cannot contribute to fat formation [95,96]. Targeted fat-specific PPARγ deletion results in various abnormalities, including reduced white and brown fat, decreased adipocyte gene expression, and fatty liver [1,97], and recently a more efficient fat-specific knockout revealed a pivotal role for adipocyte PPARγ in all adipose depots including mammary gland, bone marrow, and skin [98]. PPARγ is also required for the survival of adult adipocytes as evidenced by a conditional knockout model [99].

microarrays (ChIP-chip) and deep sequencing (ChIP-seq; see Glossary), have enabled genome-wide mapping of TF binding and patterns of histone modifications. These techniques have greatly changed our view on transcriptional regulation. Genome-wide profiling of PPARγ and retinoid X receptor (RXR) binding in 3T3-L1 adipocytes has demonstrated that PPARγ:RXR bind to thousands of sites in mature adipocytes [12–16]. Bioinformatic analysis of the DNA sequence of the binding regions from these genome-wide studies has confirmed a degenerate direct repeat 1 (DR1) element with a conserved 5′ flanking sequence conforming to the depicted position weight matrix (Box 2, Figure I) as the primary binding sequence recognized by PPARγ:RXR [12,13]. This consensus sequence is close to that initially determined based on the alignment of a limited number of binding sites and is consistent with reports demonstrating that the C-terminal extension of the PPARγ DNA-binding domain (DBD) directly interacts with the 5′ flanking sequence [17,18] and facilitates binding of PPAR:RXR heterodimers to DR1 elements that are imperfect matches to the consensus [19,20].

Consistent with the finding that PPARγ can also bind to chromatin in the absence of agonists (Box 2), the genome-wide binding pattern of PPARγ in adipocytes does not change dramatically in response to synthetic agonists. However, binding of PPARγ to many preexisting binding sites in 3T3-L1 is enhanced in response to acute treatment with the PPARγ agonist rosiglitazone. This enhanced binding of PPARγ correlates with increased recruitment of mediator subunit 1 and expression of nearby genes, indicating that enhanced PPARγ recruitment plays a role in the activation of PPARγ targets in response to rosiglitazone in adipocytes [21].

The majority of PPARγ binding sites are also occupied by RXR, consistent with early findings that RXR is an obligate heterodimerization partner of PPARγ. The time-course of PPARγ binding during adipogenesis follows the induction of PPARγ protein levels, whereas RXR is already bound to many sites in the undifferentiated state, probably as a heterodimer with PPARδ or other NRs [13]. PPARγ binding sites are strongly enriched in the vicinity of genes that are induced during differentiation [12–16,22] such as

Box 2. Structure and co-factors of PPARγ

The PPARγ gene (*PPARG*/*Pparg*) is transcribed from alternative promoters giving rise to two protein isoforms, PPARγ1 and the longer PPARγ2 which is almost exclusively expressed in adipocytes [3]. PPARγ contains an N-terminal domain involved in ligand-independent activation function (AF1); a DNA binding domain (DBD), and a C-terminal ligand-binding domain (LBD) containing the ligand-dependent activation function 2 (AF2) [1] (Figure I).

PPARγ binds as an obligate heterodimer with members of the retinoid X receptor (RXR) family at consensus binding sites consisting of imperfect direct repeats of the sequence AGGTCA separated by a single base pair (DR1 elements) (Figure I) [4]. The crystal structure of the DNA-bound PPARγ–RXR heterodimer in the presence of ligand as well as coactivator peptides provides structural support for the functional findings that initially characterized the PPARγ domains [18].

PPARγ binds to its cognate binding site even in the absence of ligand. The unliganded state of PPARγ favors interactions with NR corepressor (NCoR) and silencing mediator for retinoid and thyroid receptors (SMRT), which recruit chromatin-modifying enzymes such as histone deacetylases to repress transcription. Conversely, in the presence of ligand a conformational change in the PPARγ LBD favors interactions with coactivators such as steroid receptor coactivators (SRCs), histone acetyltransferases (HATs) such as CBP and P300, and the Mediator complex, which ultimately promote gene transcription [1]. Finally, the N-terminal AF1 domain is important for agonist-independent recruitment of HATs and Mediator [100,101] (Figure I).

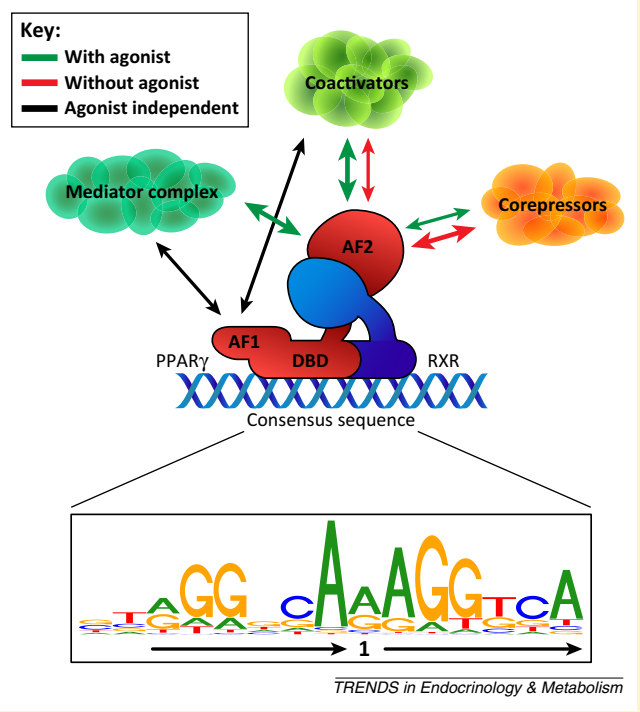

Figure I. PPARγ domains, cofactors, and mechanism of binding to DNA. The main structural domains of the PPARγ protein are shown, including the N-terminal activation function 1 (AF1) domain, the DNA-binding domain (DBD), and the C-terminal AF2 domain. PPARγ binds as a heterodimer with RXR to DNA sequences that conform to a consensus motif containing two imperfect direct repeats of the sequence AGGTCA, separated by a single nucleotide (DR1). The shown graphical representation of the PPARγ:RXR binding motif is based on the position weight matrix in the JASPAR database (http://jaspar.binf.ku.dk/) generated on PPARγ ChIP-seq data from 3T3-L1 cells [13]. Also shown is the conserved portion of the 5′ extension of the consensus motif. In the presence of agonist, the PPARγ AF2 domain facilitates agonist-dependent recruitment of coactivators and Mediator in exchange for corepressors, leading to increased expression of target genes. In the absence of agonist, the AF2 associates more strongly with corepressors. The AF2 domain is also responsible for agonist binding and heterodimerization with RXR. The AF1 domain is mediates agonist-independent recruitment of coactivators and the Mediator complex.