Double SET point: G9a makes its mark in adipogenesis

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Sequence-specific DNA-binding transcription factors (TFs) bind throughout the genome to orchestrate the process of adipocyte differentiation in an exquisitely timed and precise manner. TF binding recruits histone-modifying enzymes, resulting in dynamic epigenomic modifications that correlate with extensive gene expression changes. In this issue of *The EMBO Journal*, Wang *et al* (2013) demonstrate a critical role for the SET-containing histone methyltransferase G9a in generating repressive chromatin marks that are lost in the process of adipogenesis.

Adipocytes are central to energy homeostasis and their dysfunction is associated with metabolic diseases such as obesity. Understanding the mechanisms driving adipocyte differentiation, also known as adipogenesis, has become increasingly important as these diseases reach epidemic levels. Extensive work has revealed that this process is directed during both white and brown adipocyte differentiation by a highly regulated transcriptional cascade, culminating in the robust expression of the master adipocyte regulator PPAR γ during the terminal differentiation stage of adipogenesis (Cristancho and Lazar, 2011). PPAR γ combines with C/EBP α and C/EBP β to localize to enhancer regions neighbouring genes induced during differentiation (Lefterova *et al*, 2008). These enhancer regions are characterized by increased DNase I hypersensitivity or are enriched for histone modifications associated with increased transcription, such as Histone 3 Lysine 9 acetylation (H3K9ac) or H3K27ac (Mikkelsen *et al*, 2010; Steger *et al*, 2010; Siersbæk *et al*, 2011).

The current study from Ge and colleagues provides evidence of transcriptional repression of PPAR γ in the preadipocyte (Wang *et al*, 2013). In contrast to activating histone modifications that co-localize to common enhancer regions, they show that in precursor cells the repressive histone modifications H3K9me2 and H3K27me3 do not overlap and H3K9me2 is specifically located throughout the entire PPAR γ gene body and its enhancer regions. H3K9me2 levels decrease globally during differentiation and are undetectable at the PPAR γ locus of mature adipocytes, suggesting an epigenomic mechanism for silencing the PPAR γ locus in preadipocytes that is alleviated as transcriptional activators work in concert to promote PPAR γ expression during adipogenesis (Figure 1).

This novel layer of regulation is significant because, while histones throughout the genome are extensively modified

during adipocyte differentiation, perhaps no other locus is more extensively regulated than PPAR γ (Mikkelsen *et al*, 2010; Steger *et al*, 2010; Siersbæk *et al*, 2011). Indeed, Wang *et al* report that H3K9me2 is mostly absent from loci of other positive regulators of adipogenesis. Previous studies have revealed that an epigenomic transition state is initiated within hours of adding an adipogenic cocktail to preadipocytes. During this period early adipogenic transcription factors (TFs), such as glucocorticoid receptor (GR), C/EBP β , C/EBP δ and STAT5a, bind multiple functional enhancers upstream of the PPAR γ transcription start site, opening chromatin, recruiting co-activators, and generating activating histone modifications (Mikkelsen *et al*, 2010; Steger *et al*, 2010; Siersbæk *et al*, 2011). The time course of H3K9me2 disappearance at the PPAR γ locus in the context of the previously described epigenomic transition state could provide insight into signals governing the switch between gene repression and activation.

Key to understanding the role of H3K9me2 in preadipocytes is determining how this mark is deposited in a loci-specific manner. Ge and colleagues demonstrate that G9a is likely the histone methyltransferase that catalyses the formation of H3K9me2 in adipogenic cells. It is present at the PPAR γ locus in preadipocytes, maintains low PPAR γ levels in both white and brown precursor cells and decreases during differentiation. Furthermore, knockdown, deletion or chemical inhibition of this enzyme promotes adipogenesis. G9a, however, does not itself bind DNA in a sequence-specific manner, and thus it will be critical to determine the sequence-specific TFs that recruit it to the PPAR γ locus in preadipocytes (Shinkai and Tachibana, 2011). Candidates would include multiple TFs that have recently been identified as necessary for maintaining adipogenic competency in precursor cells (Seale *et al*, 2007; Gupta *et al*, 2010; Cristancho *et al*, 2011).

Intriguingly, Wang *et al* (2013) also demonstrate that an intact SET domain is not required for G9a to promote expression of Wnt10a, a canonical Wnt ligand that has been shown to inhibit differentiation. Many chromatin-modifying enzymes have been implicated in adipogenesis, but in some cases conflicting studies have mired our understanding of their role (Cristancho and Lazar, 2011). Potentially, one explanation for the contradictory roles of these enzymes is that histone modifiers have both

Figure 1 G9a represses PPAR γ levels in preadipocytes. In preadipocytes, the repressive H3K27me3 mark surrounds the PPAR γ locus, but Wang *et al* have shown that H3K9me2 is a repressive mark found throughout this region. G9a, an SET domain containing histone methyltransferase, is required for the deposition of H3K9me2 at this locus. However, the sequence-specific TF(s) that localize it to the genome are unknown. During early differentiation, as G9a levels begin to decrease and H3K9me2 levels also diminish at the PPAR γ locus, transcriptional activators of adipogenesis (C/EBP β , C/EBP δ , GR and STAT5a) are recruited to enhancers at hotspots of open chromatin and epigenomic change, driving the increase in PPAR γ levels. The exact temporal relation of changes in H3K9me2 levels to the epigenomic transition state remains to be determined. In mature adipocytes, the major sequence-specific positive regulators of adipogenesis (PPAR γ , C/EBP α and C/EBP β) bind enhancers characterized as regions with DNase I hypersensitivity and histone modifications of active transcription, like H3K9ac and H3K27ac, H3K9me2 is no longer present and robust PPAR γ expression is sustained.

enzyme-dependent and -independent roles. Future studies that tease out these distinct mechanisms could mark a new set of targeted therapeutics for metabolic disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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