Adipogenic hotspots: where the action is

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Transcription factors (TFs) orchestrate cell-fate decisions by programming the expression of many genes, including those of other TFs that further drive lineage specification. C/EBP proteins and glucocorticoid receptor (GR) activate transcription of the master regulator of adipocyte differentiation, PPAR γ . A study in this issue of *The EMBO Journal* sheds light on how C/EBPs, GR, STAT5, and RXR cooperate during early adipogenesis to globally remodel the epigenome.

Adipocytes, the major lipid-containing component of adipose tissue, benefit metabolism by coordinating lipid and glucose homeostasis. They are post-mitotic, terminally differentiated cells derived from mesenchymal precursors.

In cell culture models, temporary exposure of preadipocytes to a mix of insulin, glucocorticoid, and an inducer of cAMP signaling triggers adipogenesis, changing the expression of hundreds of structural genes, and a variety of TFs (Farmer, 2006). Upon their induction, C/EBPs and PPAR γ occupy sites near most of the genes that are upregulated during adipogenesis, including their own and each other's genes, forming a transcription network that helps to explain how they establish and maintain the adipocyte phenotype (Lefterova *et al*, 2008; Nielsen *et al*, 2008).

Comparative analysis of chromatin state maps at different stages of adipogenesis has recently provided further insight into an adipogenic transcription network (Mikkelsen *et al*,

Figure 1 Chromatin opening during adipogenesis. Temporary exposure of preadipocytes to adipogenic stimuli induces DNase I hypersensitive 'hotspots' with characteristics of enhancers including binding of multiple TFs, coactivator recruitment (e.g. p300), histone acetylation (stars), and increased chromatin accessibility. (A) Transient DNase I hypersensitive sites (DHSs). These represent a transition state not observed in either the preadipocyte or the adipocyte. They are present within the first day and disappear later in adipogenesis. One such hotspot functions as an enhancer of the master adipogenic TF, PPAR γ . (B) Stable DHSs. Chromatin hotspots that open early and remain open through differentiation, with early TFs replaced by PPAR γ and C/EBP α about 2 days after the start of adipogenesis. (C) *De novo* DHSs. Many DHSs are adipocyte specific and bound by PPAR γ , which may be sufficient to open chromatin at these regions.

2010; Steger *et al*, 2010). Thousands of putative preadipocyte- and adipocyte-specific *cis*-regulatory regions were identified through examination of several histone modifications associated with transcriptionally active or repressed chromatin. Bioinformatics identified enriched TF motifs, and PLZF, SRF (Mikkelsen *et al*, 2010) and GR (Steger *et al*, 2010) were validated as sequence-specific regulators of adipogenesis.

Mandrup and colleagues now present a genome-wide analysis of chromatin remodeling events occurring during adipocyte differentiation (Siersbaek *et al*, 2011). By monitoring differential sensitivity to DNase I, they detected dynamic changes to chromatin, identified enriched TF motifs at DNase I hypersensitive sites (DHSs), and validated binding of C/EBP proteins, GR, RXR, and STAT5 to the regions remodeled at early time points. Remarkably, these TFs co-localize at over 900 regions of open chromatin, which they termed 'hotspots'. Most of the hotspots are transiently open only during the early stages of adipogenesis, and many of these correspond to enhancer regions marked by transient histone acetylation, TF binding, and coactivator recruitment that define an epigenomic transition state during adipogenesis (Figure 1A) (Steger *et al*, 2010).

Interestingly, chromatin sites that open early and remain open through adipogenesis are frequently occupied by PPAR γ in adipocytes (Figure 1B), suggesting that early adipogenic TFs may facilitate its binding. It is important to note, however, that most PPAR γ -binding sites lie outside of early DHSs, leading to the possibility that PPAR γ does not require pioneer factors to open up chromatin before gaining access to these targets (Figure 1C). Indeed, forced expression of PPAR γ in preadipocytes caused binding to regions normally occupied only in adipocytes, which was accompanied by increased DNA accessibility and histone acetylation (Lefterova *et al*, 2010).

It is intriguing to compare adipogenic hotspots with another recently identified DNA element termed highly occupied target (HOT) regions (Gerstein *et al*, 2010; modENCODE Consortium *et al*, 2010). HOT regions bind many different TFs (at least 15 in *Caenorhabditis elegans*) but are not enriched in the known motifs to which they bind, suggesting that the interactions are indirect. Adipogenic hotspots appear to be functionally distinct from HOT regions, as they display enrichment for motifs and tissue-specific expression of targets. Yet, the existence of hotspots and HOT regions indicates that genomic binding of many TFs may not be distributed evenly across sites containing cognate binding motifs, but rather, concentrated at a smaller number of regions of open chromatin. This may in part explain the specificity of TF binding that goes far beyond simple recognition of high-affinity motifs in the genome (Carroll *et al*, 2005).

Adipogenic hotspots likely regulate gene expression, but it is unknown whether they function solely in this regard. Could TF occupancy accompanied by increased chromatin accessibility and enriched histone modification define all active enhancers? An answer awaits development of high-throughput methods that determine the function of putative *cis*-regulatory elements. Indeed, although many DHSs co-localize with active histone modifications, thousands are unmarked, and it is possible that various combinations of chromatin features may signal distinct three-dimensional structures that positively or negatively regulate mRNA and/or non-coding RNA synthesis as well as DNA replication and repair. Future studies will need to determine all the action going on at the hotspots.

Conflict of interest

The authors declare that they have no conflict of interest.

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