Unraveling the complexity of thermogenic remodeling of white fat reveals potential antiobesity therapies

Nabil Rabhi and Stephen R. Farmer

Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118, USA

Adipose tissue is a complex organ consisting of a mixture of mature adipocytes and stromal vascular cells. It displays a remarkable ability to adapt to environmental and dietary cues by changing its morphology and metabolic capacity. This plasticity is demonstrated by the emergence of interspersed thermogenic beige adipocytes within white depots in response to catecholamines secretion. Coordinated cellular interaction between different cell types within the tissue and a fine-tuned transcriptional program synergistically take place to promote beige remodeling. However, both cell–cell interactions and molecular mechanisms governing beige adipocyte appearance and maintenance are poorly understood. In this and the previous issue of *Genes & Development*, Shao and colleagues (pp. 1461–1474) and Shan and colleagues (pp. 1333–1338) advance our understanding of these issues and, in doing so, highlight potential therapeutic strategies to combat obesity-associated diseases.

At the transcriptional level, white adipocyte identity is maintained by concomitant action of Pparγ promoting white adipocyte gene programs and Zfp423 suppressing thermogenic gene programs by antagonizing Ebf2, a brown fat determination factor (Shao et al. 2016; Shapira et al. 2017). Beige adipocyte emergence in response to adrenergic stimulation is blunted in mice lacking Ebf2. In contrast, overexpression of Ebf2 or inducible deletion of Zfp423 in adipocytes strongly promotes beige adipocyte formation (Stine et al. 2015). Direct interaction between Zfp423 and Ebf2 is an important determinant for the activation of the thermogenic program by Ebf2 in adipocytes (Shapira et al. 2017). However, the molecular mechanisms and physiological relevance of this interaction in white adipocytes have been unclear.

Zfp423–Ebf2 interaction is mediated through the C-terminal C2H2 zinc fingers (ZFs) of Zfp423 (Shao et al. 2016). After in vitro screening of several histidine-to-asparagine substitutions in ZFs, Shao et al. (2021) show that only the H1285N mutant failed to suppress thermogenic genes while maintaining its ability to drive Ppary. This discovery facilitated their investigation of the functional role of the Zfp423–Ebf2 interaction in vivo by combining CRISPR–Cas9 and proximity labeling technologies to generate an inducible adipocyte-specific mouse model that expresses either WT Zfp423 or the H1285N mutant. The investigators found that $Zfp423^{H1285N}$ mutant mice recapitulate the phenotype of the adipocyte Zfp423 knockout model with regard to maintaining the inguinal white adipocyte phenotype when housed at room temperature. To further demonstrate that the Zfp423–Ebf2 interaction is key for suppression of the thermogenic program, Shao et al. (2021) used chromatin affinity purification (ChAP) of the endogenous Avi-tagged $Zfp423^{WT\text{-}Avi}$ and $Zfp423^{H1285N\text{-}Avi}$ proteins. The investigators show that Zfp423 occupancy of Ebf2 target genes is dependent on its interaction with Ebf2. Importantly, the investigators found that $Zfp423^{H1285N}$ retained its ability to occupy Ebf2-independent targets, including genes implicated in extracellular matrix expression. In fact, loss of the Zfp423–Ebf2 interaction does not impact Ebf2 chromatin binding activity but rather promotes recruitment of the BRG1-containing BAF chromatin remodeling complex to thermogenic genes, suggesting that Zfp423 blocks Ebf2 coactivator recruitment. They further demonstrated that all core components of nucleosome remodeling deacetylase (NuRD) copartner with Zfp423 to promote suppression of thermogenic gene expression. To support these results, the investigators used ChIP-seq to show that disturbance of the Zfp423–Ebf2 interaction modifies the composition of complexes at the chromatin level and thus leads to the recruitment of Ppary to thermogenic genes, which ultimately leads to loss of the white adipocyte phenotype. Having established that Ppary occupies thermogenic genes in the absence of Zfp423, the investigators thought to delete Zfp423 in mature adipocytes in an obese

[*Keywords*: EBF2; PPARγ; ZFP423; beige adipocytes; rosiglitazone; white adipocytes]
Corresponding author: sfarmer@bu.edu
Article is online at http://www.genesdev.org/cgi/doi/10.1101/gad.349053.121.

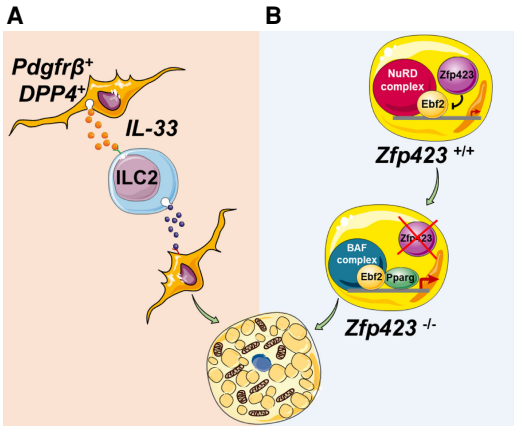

Figure 1. Roads to beige adipocytes. (*A*) Adipocyte precursors cells (APCs) expressing DPP4 and PDGFRβ from subcutaneous adipose tissue (ScAT) release IL-33 in response to cold exposure. IL-33 will increase the recruitment of the innate lymphoid type 2 cells (ILC2), promoting beige adipogenesis. (*B*) The white adipocyte gene program is regulated by Zfp423 by suppressing PPARγ occupancy of thermogenic genes. Interaction between ZFP423–EBF2 facilitates the recruitment of the NuRD repressor complex to thermogenic genes. Upon the loss of ZFP423, free EBF2 recruits the activator complex BAF and facilitates the PPARγ occupancy of thermogenic gene enhancers.

context in order to pursue potential therapeutic strategies. Although Zfp423 deletion alone did not restore the metabolic health of the mice on the high-fat diet, it did amplify the beneficial metabolic effects of antidiabetic rosiglitazone while blocking the weight-gaining effects of the drug. Consequently, a potential strategy would be a combination of thiazolidinediones (Ppary agonists) with the means to selectively inactivate Zfp423 in white adipocytes.

Overall, Shao et al. (2021) uncovered the molecular mechanism by which Zfp423 maintains the white adipocyte gene program, shedding light yet again on the high plasticity of adipocytes at the cellular level (Fig. 1). An important question arising from these findings is how many white adipocytes will be responsive to Zfp423 inhibition in an obese context. Is the Zfp423–Ebf2 interaction necessary to maintain the white adipocyte phenotype only in white adipocytes that arise from a beige lineage, as is the case in the subcutaneous adipose depot, or could it control "true" white adipocytes present in visceral and other depots?

We previously showed that cold exposure induces a shift in the immune cell composition within the local adipose tissue microenvironment (Rabhi et al. 2020). In an the previous issue of *Genes & Development*, Shan et al. (2021) added a new layer, providing insight into how immune cellular regulation promotes cold adaptation. The investigators identified a subpopulation of Dpp4+ Pdgfrβ + adipocyte precursors cells (APC) as highly responsive to adrenergic stimulation. Transcriptomic analysis revealed that interleukin 33 (IL-33), a cytokine that has been implicated previously in WAT thermogenesis, was among the top up-regulated genes responding to cold. IL-33 is the primary survival cytokine that regulates ILC2s in WAT (Brestoff et al. 2015; Lee et al. 2015). Many multipotent stromal cells, including mesenchymal stem cells, have been shown to act as a reservoir for IL-33 (Mahlakõiv, et al. 2019; Spallanzani et al. 2019). Using IL-33-driven EGFP reporter mice, Shan et al. (2021) demonstrated that DPP4+ PDGFRβ+ cells are the predominant source of IL-33. Next, these investigators determined that IL-33 expression peaked after 1 d of cold exposure and then began to decline. This peak correlated with an increase in ILC2 frequency within the adipose tissue and the tissue level of IL-33. Interestingly, the investigators found that tissue levels of IL-33 in DPP4+ cells were selectively reduced when mice were moved to thermoneutrality, while no changes were observed in DPP4– cells. The investigators concluded that DPP4+ APCs and IL-33 expression are thermosensitive. Using CRISPR–Cas9 to knock out CREB, the investigators demonstrated that IL-33 expression is directly regulated by the classical β-adrenergic receptor–CREB signaling pathway. The investigators found that conditionally deleting *Il33* from Pdgfrβ-expressing cells impacted inguinal WAT remodeling by decreasing ILC2 cells and multilocular adipocytes while enhancing white adipocyte transcripts. Injection of methionine-enkephalin (MetENK) partially restored the beige phenotype, supporting a role for this ILC2-derived peptide in IL33-mediated beige adipocyte formation (Brestoff et al. 2015). This work by Shan et al. (2021) highlights the complexity of endocrine signaling that facilitates adipose tissue plasticity (Fig. 1). More studies on mechanisms and stromal-derived factors that may promote adipose tissue remolding are needed. A better understanding of those mechanisms may uncover new therapeutic strategies for metabolic disease treatment.

Acknowledgments

S.R.F. was supported by National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases grants DK117161 and DK117163. N.R was supported by a Société Francophone du Diabète (SFD) and American Heart Association (AHA) fellowship (17POST33660875).

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