Enhancing brown fat with NFIA

Suzanne N. Shapira and Patrick Seale

Brown adipose tissue is a key metabolic organ that oxidizes fatty acids and glucose to generate heat. Through epigenomic analyses of multiple adipose depots, the transcription factor nuclear factor I-A (NFIA) is now shown to drive the brown fat genetic program through binding to lineage-specific *cis*-regulatory elements.

Brown fat is a mammalian-specific tissue that can burn chemical energy to generate heat. Unlike white adipose tissue, which is highly adapted for energy storage and has relatively low mitochondrial abundance, brown fat is packed with mitochondria that contain uncoupling protein-1 (UCP1). When activated by long-chain fatty acids, UCP1 uncouples the mitochondrial proton gradient from energy (ATP) synthesis; this drives accelerated rates of substrate oxidation and culminates in the production of heat[1]. Brown fat can therefore act as a sink for lipids, glucose, and possibly other metabolites that can be harmful to other tissues. Indeed, genetic manipulations in mice that increase the amount of brown fat lead to improved glucose and triglyceride clearance[2], higher energy expenditure, and resistance to weight gain in response to a high-fat diet. Promisingly, recent studies also show that cold exposure or β3-agonist treatment in humans activates thermogenic fat cells, leading to increased energy expenditure and decreased body fat mass[3,4]. A more complete understanding of the mechanisms that control brown fat activity may reveal new therapeutic approaches to reduce metabolic disease.

The coordinated recruitment of transcription factors to tissue-specific regulatory elements directs lineage specification during cellular differentiation. Thus, unravelling the transcriptional hierarchies that direct adipogenesis will advance our understanding of how adipocyte identity is established and maintained. In this issue, Hiraike *et al.* utilize genome-wide analyses of accessible (that is, 'open') chromatin to identify NFIA as a positive transcriptional regulator of brown adipogenesis, thus deepening our understanding of the transcriptional dynamics that activate the brown fat gene program[5].

Adipocyte development begins when multipotent precursors undergo determination to become committed preadipocytes. Extracellular signals then initiate a transcriptional cascade that drives terminal differentiation — the process by which preadipocytes acquire the molecular and functional attributes of mature adipocytes. Brown adipocytes arise from a population of multipotent precursors in the dermomyotome that also give rise to skeletal muscle and dermis[6]. White adipocytes have a variety of developmental origins; however, most white adipose depots in mice possess an intrinsic ability to activate a brown-fat-like program (termed beiging) under conditions of β-adrenergic stimulation[7]. Despite having divergent developmental origins and metabolic functions, brown and white adipocytes share transcriptional regulators that orchestrate the general adipogenic differentiation process. In particular, general adipogenesis is dependent on the nuclear receptor PPARγ, the 'master regulator' of fat cell development. PPARγ directly binds and regulates numerous genes involved in adipogenesis. PPARγ also interacts with many other transcription factors and co-regulators that determine white or brown lineage-specific gene expression[8]. While many factors that influence adipocyte fate have been described, our understanding of chromatin dynamics, particularly during the early stages of brown adipogenesis, requires further illumination.

Hiraike *et al.* performed formaldehyde-assisted isolation of regulatory elements (FAIRE) followed by deep-sequencing analysis of brown and white adipose depots. This unbiased technique exploits the different crosslinking efficiencies of exposed versus protein-associated DNA, thus allowing for specific detection of open chromatin that is characteristic of active *cis*-regulatory regions[9]. DNA motif analysis of the brown-fat-selective open chromatin regions revealed enrichment for the NFI motif. The NFI family of transcription factors are expressed in distinctive patterns and play roles in both gene activation and repression[10]. The NFIA isoform was selectively enriched in brown and activated beige adipose tissue compared with white adipose tissue. Ectopic expression of NFIA in skeletal myoblast precursors induced adipocyte differentiation with concomitant repression of the muscle program. Further, NFIA-expressing adipocytes responded to cyclic AMP (cAMP) by increasing oxygen consumption, a hallmark of brown fat cell function. Conversely, short hairpin RNA (shRNA) studies in brown fat cells demonstrated that NFIA is required for both induction and maintenance of brown-fat-specific gene expression in adipocytes.

To investigate the mechanism of NFIA action, the authors performed chromatin immunoprecipitation sequencing (ChIP-seq) experiments to localize the genomic binding sites of NFIA in brown adipocytes. NFIA co-localized with brown-fat-selective active enhancer marks along with other transcriptional regulators previously found to bind at brown fat genes, including PPARγ, C/EBPβ, and EBF2. In particular, NFIA and PPARγ

Suzanne N. Shapira and Patrick Seale are at the Institute for Diabetes, Obesity, and Metabolism, Department of Cell and Developmental Biology, Smilow Center for Translational Research, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.
e-mail: sealep@mail.med.upenn.edu

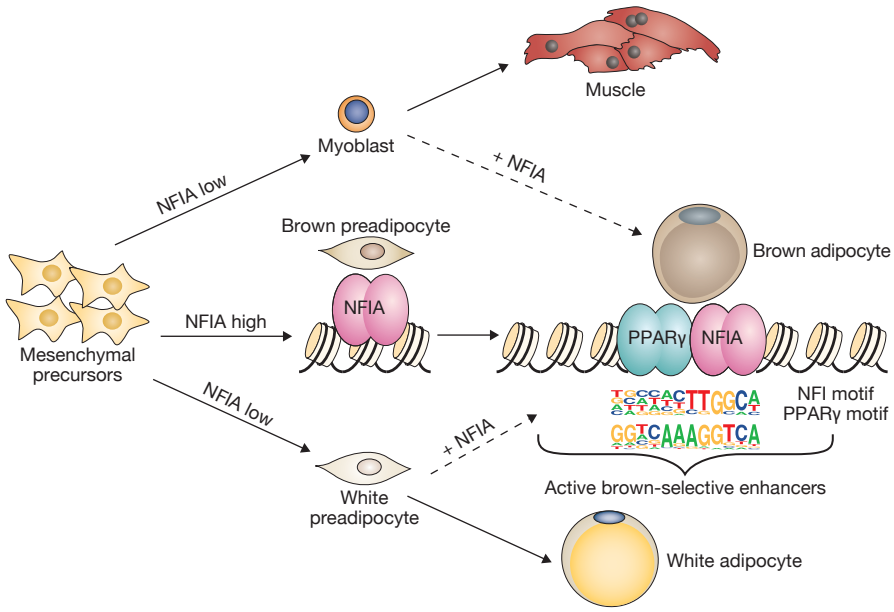

Figure 1 Regulation of brown adipocyte commitment by NFIA. Multipotent mesenchymal precursors are directed along specific lineages by master transcriptional regulators. Brown preadipocytes are characterized by early NFIA occupancy at *cis*-regulatory elements. During terminal differentiation, NFIA cooperates with the lineage-determining factor PPARγ to activate brown-fat-specific genes. Following ectopic overexpression of NFIA (dashed arrows), brown fat enhancers are activated in myoblasts and white preadipocytes with concomitant recruitment of PPARγ and downstream gene activation.

binding were strongly correlated at brown-fat-selective genes. Notably, NFIA was uniquely present at a number of brown fat enhancers prior to inducing differentiation and before PPARγ recruitment to these sites. This suggests that NFIA plays an early role in controlling the chromatin structure at lineage-specific *cis*-regulatory elements. In support of this concept, ectopic expression of NFIA was sufficient to direct exogenously expressed PPARγ to its brown-fat-selective binding sites leading to downstream gene activation (Fig. 1).

To determine the requirement for NFIA *in vivo*, the authors analysed brown adipose tissue (BAT) from whole-body knockout (KO) animals. BAT from NFIA-KO animals was morphologically similar to wild-type animals, but had reduced expression of both brown-fat-specific and mitochondrial genes, with a reciprocal increase in muscle-related genes. These findings suggest that *Nfia* is required for proper activation of the brown fat program and suppression of muscle process genes *in vivo*. Interestingly, *Nfia* levels were also decreased in the BAT of obese *db/db* mice, correlating with a reduced thermogenic profile in the tissue. Further, NFIA was expressed in human brown adipose tissue and perirenal adipose tissue from patients with the catecholamine-secreting tumour pheochromocytoma, suggesting that NFIA may have a conserved role in regulating browning of human adipose tissue.

Identifying the factors that initially drive the brown adipogenic genetic program will be key for future studies aimed at reprogramming-based therapies. The expression and binding activity at early time points in the differentiation process suggest that NFIA may function to open chromatin at brown fat gene enhancers. These early NFIA-bound sites also display high levels of H3K27ac and chromatin accessibility so it is unclear if these sites are already open prior to NFIA binding. It is also possible that NFIA itself acts as a 'pioneering' protein. This special class of transcription factors has the ability to directly control chromatin unwinding through directly binding DNA and displacing nucleosomes[11]. Of note, NFIA is known to interact with transcription factors that have pioneering activity such as FOXA1 (ref. 12) and SOX9 (ref. 13) in other cellular contexts. A pioneering mechanism has also been proposed for the transcription factor EBF2, which binds early in the differentiation program and can facilitate the recruitment of PPARγ to brown-fat-specific enhancers[14]. Thus, it is tempting to speculate that NFIA and EBF2 cooperate to direct chromatin accessibility at lineage-specific regions. Identifying NFIA binding partners in preadipocytes may help to address these questions.

Hiraike *et al.* found that NFIA was able to induce the brown fat program in myoblasts in a PRDM16-independent manner, suggesting that these factors act in separate pathways. The authors also observed that *Prdm16* expression levels were unchanged in the BAT of NFIA-KO animals. Future studies will be needed to determine the epistatic relationship between PRDM16 and NFIA in *bona fide* brown adipocytes. It will also be important to establish tissue-specific mouse models of NFIA deletion in preadipocytes and adipocytes to determine if the phenotypes observed in the whole-body KO mouse model are due to adipose cell-autonomous functions of NFIA.

A major effort in the field is to identify strategies that can reprogram precursor cells to mature and functional brown adipocytes *ex vivo* for downstream transplantation therapy[15]. Given that NFIA can reprogram white adipocytes and muscle to brown adipocytes, it would be interesting to study how NFIA itself is regulated during the development of brown adipose tissue. Importantly, NFIA is able to both activate adipogenesis and the brown fat gene program. Thus, identifying the upstream signals that activate NFIA would potentially provide an avenue to differentiate mesenchymal precursor or embryonic stem cells into brown adipocytes.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Harms, M. & Seale, P. *Nat. Med.* **19,** 1252–1263 (2013).
2. Bartelt, A. *et al. Nat. Med.* **17,** 200–205 (2011).
3. Yoneshiro, T. *et al. J. Clin. Invest.* **123,** 3404–3408 (2013).
4. Cypess, A. M. *et al. Cell Metab.* **21,** 33–38 (2015).
5. Hiraike, Y. *et al. Nat. Cell Biol.* **19,** 1081–1092 (2017).
6. Wang, W. & Seale, P. *Nat. Rev. Mol. Cell Biol.* **17,** 691–702 (2016).
7. Sharp, L. Z. *et al. PLoS ONE* **7,** e49452 (2012).
8. Siersbaek, M. S. *et al. Mol. Cell Biol.* **32,** 3452–3463 (2012).
9. Simon, J. M. *et al. Nat. Protoc.* **7,** 256–267 (2012).
10. Gronostajski, R. M. *Gene* **249,** 31–45 (2000).
11. Adam, R. C. *et al. Nature* **521,** 366–370 (2015).
12. Grabowska, M. M. *et al. Mol. Endocrinol.* **28,** 949–964 (2014).
13. Kang, P. *et al. Neuron* **74,** 79–94 (2012).
14. Shapira, S. N. *et al. Genes Dev.* **31,** 660–673 (2017).
15. Boss, O. & Farmer, S. R. *Front. Endocrinol. (Lausanne)* **3,** 14 (2012).