Cell Metabolism
Minireview
Focus on Brown Adipose Tissue

Transcriptional Control of Brown Fat Development

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Deconvoluting the natural pathway of BAT development has defined key molecular events, which enables researchers to manipulate the amount or activity of brown fat. We review recent advances on the transcriptional regulation of BAT development and discuss the emerging questions.

Introduction

In contrast to white adipose tissue (WAT), which is specialized for the storage of excess energy, brown adipose tissue (BAT) dissipates chemical energy to produce heat as a defense against cold. Interest in the development and regulation of BAT has exploded in the last few years because of a confluence of discoveries in the biology and physiology of the brown adipocyte. Clearly, much of the interest in this cell type is due to its role in the defense against hypothermia and obesity. Observations originally made in the oncology clinic with ^{18}fluoro-labeled 2-deoxyglucose positron emission tomography (^{18}FDG-PET) scanning have led to an appreciation that most, if not all, adult humans have distinct brown fat deposits, and the activity of BAT varies depending on age, adiposity, temperature, and gender (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Furthermore, a major transcriptional regulator of brown fat cell identity, PRDM16, was recently discovered. This is the first cell-autonomous transcriptional component that is both necessary and sufficient to stimulate the development of brown fat cells. This offers a new opportunity to investigate the developmental origins of brown fat and provides a new pathway for the manipulation of BAT in vivo. In this article, we review the current understanding of the transcriptional regulation and cellular origin of brown fat development.

Transcriptional Regulators of Brown Fat Cell Development and Differentiation

Despite the differences in the developmental origins and physiological functions of brown and white adipocytes, both cell types share a very similar transcriptional cascade that controls the process of fat differentiation. Detailed studies of white fat differentiation had previously identified PPARγ (peroxisome proliferator-activated receptor-γ) and the C/EBPs (CCAAT/enhancer-binding proteins) as key transcription factors driving fat cell differentiation (reviewed in Farmer, 2006). Indeed, PPARγ is absolutely necessary for both white fat and brown fat cell development. C/EBPs function cooperatively with PPARγ and promote a transcriptional cascade that promotes and maintains the stable differentiated state of adipocytes. While C/EBPα is essential for the normal insulin sensitivity of mature fat cells, it is required only for the formation of white fat, not brown fat, suggesting a possible role for other C/EBP family members in brown fat development. C/EBPβ and δ, as well as other transcription factors, also participate in the transcriptional cascade of adipogenesis by regulating PPARγ gene expression. Brown fat cell differentiation requires PPARγ but, importantly, this factor alone is not sufficient to drive mesenchymal cells into a brown fat program. This led us and other investigators to hunt for transcriptional components that would specifically promote a brown fat genetic program, including the expression of UCP1. As described below, several transcriptional factors and cofactors that affect the expression of UCP1 and that of other key brown fat-selective genes have been identified.

Forkhead Box C2

Forkhead box C2 (FOXC2) is a member of the forkhead/winged helix transcription factor family whose gene expression is enriched in the adipose tissues of human and mouse. Enerback and colleagues reported that transgenic expression of FOXC2 in the adipose tissue induced the emergence of brown fat-like cells in WAT, with increased mitochondria and elevated expression of thermogenic genes, including UCP1 and PPARγ-coactivator-1α (PGC-1α) (Cederberg et al., 2001). Notably, transgenic expression of FOXC2 counteracts many obesity-associated pathologies, including insulin resistance and hypertriglyceridemia (Cederberg et al., 2001). This "browning" effect stimulated by FOXC2 seems mainly a result of inducing the expression of the RIα subunit of cAMP-dependent protein kinase (PKA). This sensitizes cells to cAMP signaling through the β-adrenergic pathway.

PGC-1α and Its Transcriptional Regulators

PGC-1α was originally identified from brown fat cells as a cold-inducible coactivator of PPARγ (Puigserver et al., 1998). Accumulating evidence indicates that PGC-1α is a master regulator of mitochondrial biogenesis and oxidative metabolism in most cell types, including brown fat and skeletal muscle. Indeed, genetic ablation of PGC-1α results in reduced capacity for cold-induced thermogenesis in vivo and in a blunted response to cAMP signaling in cultured brown fat cells. Similarly, ectopic expression of PGC-1α in white fat cells induces a number of mitochondrial genes and thermogenic genes, such as *Ucp1* (Puigserver et al., 1998).

Several transcriptional regulators have been shown to control brown fat development and function, at least in part, through regulating the transcriptional activity or gene expression of PGC-1α. RIP140, a corepressor of many nuclear receptors,

has been shown to suppress the transcriptional activity of PGC-1α through a physical interaction (Hallberg et al., 2008). Genetic ablation of RIP140 causes the emergence of brown fat-like cells in WAT (Leonardsson et al., 2004). Similarly, SRC2/TIF2/GRIP1, a member of the steroid receptor coactivator (SRC) family, represses PGC-1α transcriptional activity. Loss of SRC2 function leads to an increase in adaptive thermogenesis and energy expenditure in vivo (Picard et al., 2002). Rb (retinoblastoma) protein and p107, another member of the Rb pocket protein family, also negatively regulate PGC-1α gene expression. Adipocytes derived from pRb-deficient fibroblasts or embryonic stem cells exhibit a brown fat phenotype with high mitochondrial content and elevated expression of UCP1, PGC-1α, and mitochondrial genes (Hansen et al., 2004). p107-deficient mice display a striking accumulation of brown fat-like cells in WAT, with multilocular lipid droplets, abundant mitochondria, and high levels of UCP1 and PGC-1α expression (Scimè et al., 2005). The biological effect of p107 on brown fat development appears to be mediated through the repression of pRb, because pRb levels are significantly reduced in Sca-1$^+$ CD31$^-$ Lin$^-$ adipogenic precursors isolated from p107-deficient mice. Lastly, TWIST1, a helix-loop-helix-containing transcriptional regulator, has recently been reported as a negative regulator of PGC-1α function in brown fat. Genetic ablation or depletion of TWIST1 induces the expression of brown fat-selective genes, while overexpression of TWIST1 represses them in a PGC-1α-dependent fashion (Pan et al., 2009).

Taken together, these data suggest a dominant role for PGC-1α in brown fat development and its thermogenic function. However, the mass of BAT and the expression of many brown fat-selective genes are not affected by the depletion of PGC-1α. Thus, while PGC-1α is a crucial regulator of adaptive thermogenesis and mitochondrial biogenesis, it does not determine the cellular specification of brown fat.

PRD1-BF-1-RIZ1 Homologous Domain Containing Protein-16

PRD1-BF-1-RIZ1 homologous domain containing protein-16 (PRDM16) is a 140 kDa zinc finger protein that was originally identified at a chromosomal breakpoint of t(1;3)(p36;q21)-positive human acute myeloid leukemia cells (Mochizuki et al., 2000). Our previous study showed that PRDM16 expression was highly enriched in BAT compared with WAT (Seale et al., 2007). When ectopically expressed in white fat preadipocytes or myoblasts, PRDM16 induces a nearly complete brown fat genetic program. This includes mitochondrial biogenesis, increased cellular respiration, and expression of brown fat-selective genes, both the cAMP-inducible thermogenic genes (*Ucp1*, *Pgc-1α*, and *Deiodinase-d2*) and those BAT-selective genes that are not sensitive to cAMP (such as *Cidea* and *Elovl3*). Furthermore, transgenic expression of PRDM16 in adipose tissue increases the formation of pockets of multilocular brown-like adipocytes in WAT depots under stimulation with a β-adrenergic agonist.

PRDM16 had previously been shown to bind directly to a specific DNA sequence via two sets of zinc fingers (ZF1 and ZF2 domains) in vitro. Interestingly, abrogation of DNA binding using a point mutation did not substantially alter the ability of PRDM16 to induce the brown fat phenotype when compared to the wild-type protein. This suggested that PRDM16 was not working as a classical DNA-binding transcription factor. Further study demonstrated that, in addition to inducing the gene expression of PGC-1α, PRDM16 directly binds to both PGC-1α and PGC-1β to increase their transcriptional activities (Seale et al., 2007). These results directed us to search for other binding partners that further specified the actions of PRDM16. In fact, PRDM16 interacts with a variety of canonical DNA-binding transcription factors, such as PPARα, PPARγ, p53, and several members of the C/EBP family (Kajimura et al., 2009; Seale et al., 2008). In each case, binding is via one or more of PRDM16's zinc finger domains, resulting in powerful coactivation of their transcriptional activities (Figure 1A). On the other hand, PRDM16 expression in white fat precursors or in myoblasts robustly represses gene expression of selective markers for white fat cells or skeletal muscle, respectively. The repressive effect of PRDM16 on white fat cell-selective genes is mediated through its regulated association with C-terminal binding proteins (CtBP1 and 2), well-known corepressor proteins (Kajimura et al., 2008). These results indicate that PRDM16 is a coregulatory protein that can function as a bidirectional switch in brown fat development through multiple protein-protein interactions.

Depletion of PRDM16 in primary brown fat cell precursors not only causes a near-total loss of brown fat characteristics but also, unexpectedly, causes the emergence of distinct morphological and genetic characteristics of skeletal myotubes in culture. These include the formation of syncytia and ectopic activation of skeletal muscle-specific genes. Consistent with this, BAT from PRDM16-deficient mice exhibits an abnormal morphology, with reduced expression of brown fat-selective marker genes and elevated expression of skeletal muscle-specific genes (Seale et al., 2008). Conversely, expression of PRDM16 in myogenic precursors drives a robust and functional program of brown adipogenesis. These results suggest that PRDM16 is a critical determinant of the brown fat lineage from myoblast progenitors during the embryonic development.

How does PRDM16 control the conversion of myoblastic precursors to brown fat? Recent studies from our group demonstrated that PRDM16 forms a transcriptional complex with the active form of C/EBPβ (LAP) that is abundantly expressed both in brown fat and myoblasts (Kajimura et al., 2009). It has also been reported that C/EBPβ is a dominant transcription factor that controls cAMP-induced gene expression in brown fat cells (Karamitri et al., 2009). Indeed, depletion of C/EBPβ significantly blunts the ability of PRDM16 to induce the brown fat differentiation and specific fat gene program in myoblasts. Consistent with this finding, BAT from C/EBPβ-deficient mice displays a similar molecular signature to BAT from PRDM16-deficient mice, with reduced expression of BAT-selective genes and elevated expression of skeletal muscle-selective genes. These studies indicate that a PRDM16-C/EBPβ complex controls the initiating events of the conversion from myoblastic precursors to brown fat cells (Figure 1B).

Surprisingly, the combination of these two factors (PRDM16 and C/EBPβ) is sufficient to induce a fully functional brown fat program in nonadipogenic cells such as embryonic fibroblasts and skin fibroblasts from mouse and man. Cells expressing these two factors differentiate into mature adipocytes with extremely high oxygen consumption and high expression of brown fat-selective genes, including *Ucp*1 and *Pgc-1α*. Furthermore, when transplanted into immunocompromised mice,

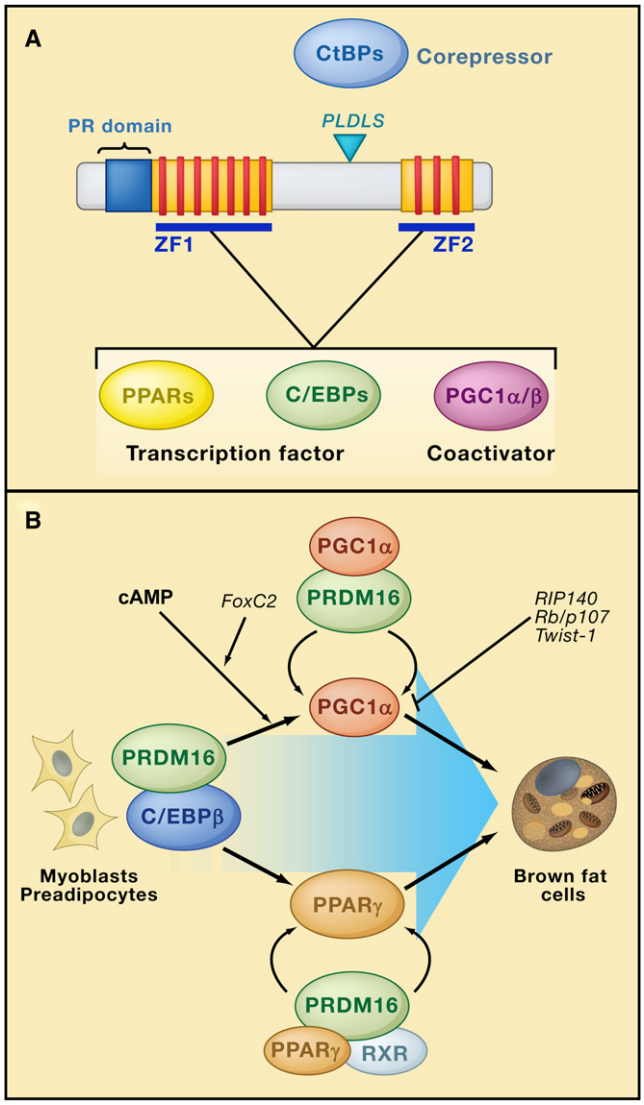

Figure 1. Transcriptional Control of Brown Fat Development through PRDM16

(A) Structure of PRDM16 and key domains of its function. PRDM16 directly interacts with canonical transcription factors such as PPARα, PPARγ, and C/EBP family members and transcriptional coactivators PGC-1α and PGC-1β through the two sets of zinc finger domains (ZF1 and ZF2). PRDM16 is also associated with the corepressors CtBP1 and 2 through its PLDLS motif. This interaction mediates the repressive action of PRDM16 on the expression of white fat cell-specific genes.

(B) PRDM16-C/EBPβ transcriptional complex acts in *Myf5*-positive myoblastic precursors or preadipocytes to induce the expression of PPARγ and PGC-1α. PRDM16 coactivates PPARγ and PGC-1α, which then drives a brown fat differentiation program. The cAMP-dependent thermogenic gene program is potentiated by FOXC2 and PRDM16. RIP140, Rb/p107, and TWIST1 antagonize the expression or transcriptional activity of PGC-1α and repress brown fat genetic program.

fibroblasts expressing these two factors give rise to an ectopic fat pad that displays a brown fat-like phenotype: multilocular adipocytes expressing UCP1. Importantly, similar to endogenous BAT, this engineered brown fat tissue can function as a sink for active glucose disposal, as determined by FDG-PET scanning (Kajimura et al., 2009).

Developmental Origin of Brown Fat

Because WAT and BAT share many common features, including a conserved PPARγ-driven transcriptional program of adipogenesis, these tissues have been assumed to share a direct common progenitor. However, recent studies indicate that brown adipocytes are developmentally closer to skeletal muscle than to white adipose cells (Figure 2). In particular, genetic fate-mapping experiments indicate that BAT in the interscapular region and skeletal muscle but not white adipose cells arise from cells that express *Myf5*, a gene previously assumed to be present almost exclusively in committed skeletal muscle precursors (Seale et al., 2008). Similarly, *Engrailed-1* (*En1*)-expressing cells in the central dermomyotome form BAT, skeletal muscle, and dermis (Atit et al., 2006). Whether *En1*-expressing cells give rise to any white adipose lineages was not examined. In addition, global gene expression analyses by the Cannon and Nedergaard group show that brown but not white adipocyte precursors express a gene profile related to that of skeletal muscle cells (Timmons et al., 2007). Most recently, the mitochondrial proteomic signature of BAT was shown to be highly related to that of skeletal muscle but not to that of WAT (Forner et al., 2009).

As described above, PRDM16 appears to function as a major determinant of a brown adipose/skeletal muscle fate switch from a common cellular precursor compartment. Interestingly, genetic ablation of PRDM16 in mouse caused a significant but relatively modest reduction in the molecular and morphological characteristics of brown fat (Seale et al., 2008). This suggests a parallel or independent pathway or pathways that direct a subset of myoblasts into brown adipocyte lineage in vivo. There are 17 members of PRDM16 family in mammals, and it is possible that one of more of these may compensate for the chronic loss of PRDM16 during embryogenesis. Notably, myogenin-deficient mice that completely lack differentiated skeletal muscle have an expanded BAT depot in the interscapular region. Together, these findings are consistent with the hypothesis that BAT shares a direct common upstream precursor with skeletal muscle cells. It will now be important to examine whether *Myf5*-expressing cells can clonally give rise to both cell lineages.

The signaling molecules that control the timing and specificity of PRDM16 expression and commitment to the brown adipose lineage are unknown. Bone morphogenic proteins (BMPs), members of the TGF-β superfamily of secreted factors, have been suggested to facilitate adipogenic differentiation. Of particular interest is BMP7, which has been described as a selective and potent inducer of brown but not white adipogenesis in preadipocyte and multipotent fibroblast cultures (Tseng et al., 2008). BMP7 treatment of fibroblast cultures is associated with induction of key brown adipogenic regulators such as PRDM16 and PGC-1α, although the mechanisms that mediate this effect are not known. Importantly, *BMP7*-deficient embryos possess significantly reduced amounts of BAT that lack UCP1 expression. The cellular specificity and timing of BMP7 signaling in BAT is an important open question for future experiments. Interestingly, certain BMPs also negatively regulate skeletal myogenesis, suggesting that BMP7 or a related family member may act on early somitic precursor cells to direct brown adipocyte versus skeletal muscle cell determination. Another member of the TGF-β superfamily, GDF-3, has been shown to negatively regulate a

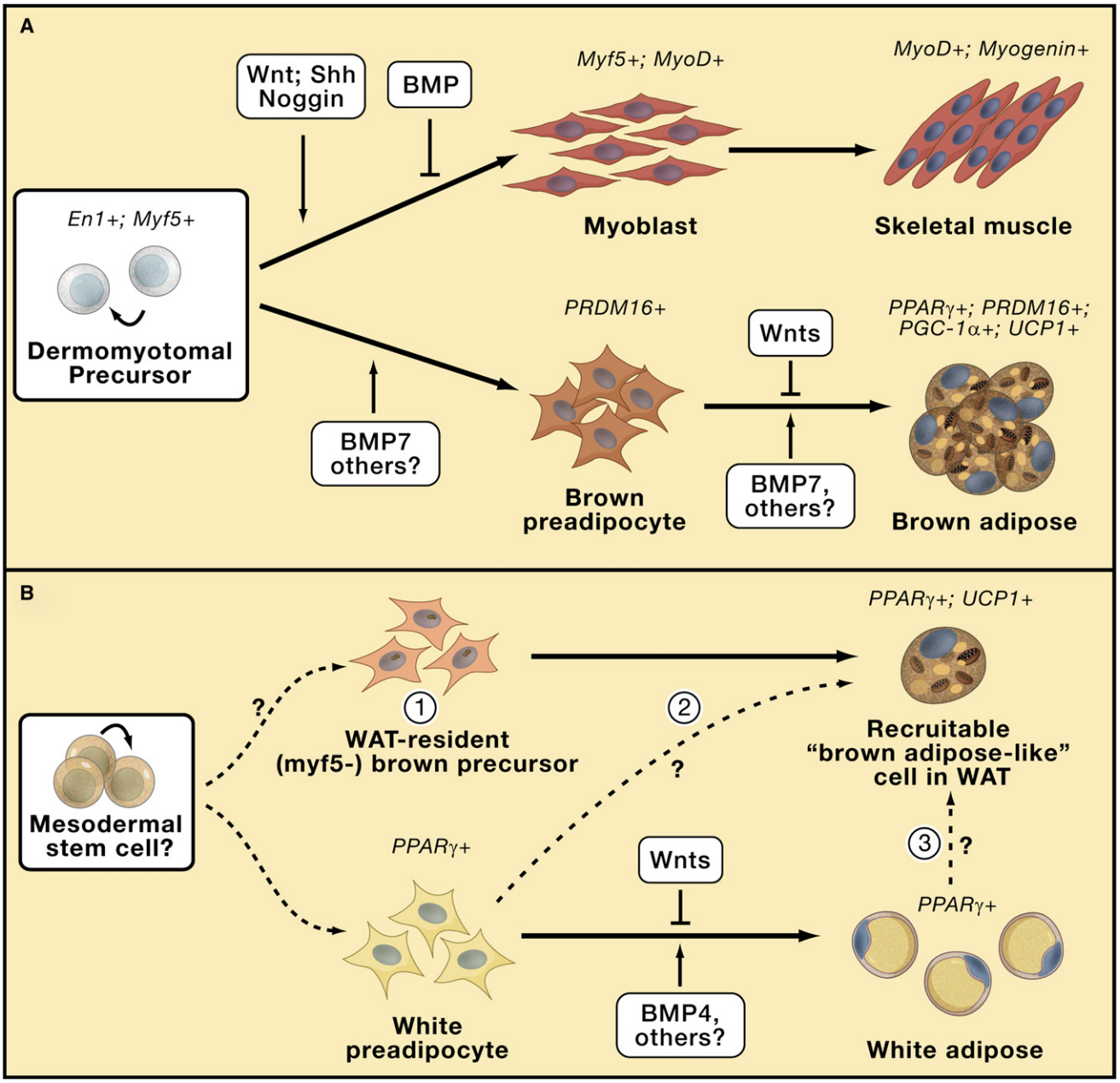

Figure 2. Hierarchical Developmental Relationships in Adipose

(A) BAT (A) and WAT (B) have separate developmental origins in the embryo. BAT and skeletal muscle originate from precursors in the dermomyotome that express *Engrailed-1* (*En1*) and *Myf5*. Brown adipose fate in the somite may be controlled by members of the transforming growth factor (TGF)-β superfamily of secreted factors, such as BMP7. Canonical Wnt signaling represses the differentiation of brown preadipocytes into mature brown adipose cells. PRDM16, PPARγ, PGC-1α, and UCP1 are functional markers of brown adipose cells in the developmental, homogenous deposits of BAT. MyoD expression and skeletal muscle commitment are positively regulated by Wnt, Sonic Hedgehog (Shh), and Noggin. BMPs suppress myogenic commitment.

(B) The embryonic stem cells of the white adipose lineage remain to be well defined. White preadipocytes that express PPARγ differentiate into mature white adipose cells in a process that also appears to be stimulated by certain BMPs. The adaptive *Ucp1*-expressing brown-like adipose cells that develop in WAT in response to cold or β-adrenergic stimulation are not descendent from *Myf5*-expressing cells. These cells may be derived from (1) a specialized compartment of *Myf5*-negative but committed brown precursors, (2) directed differentiation from white preadipocytes, and/or (3) transdifferentiation from mature white adipocytes (as indicated). The broken arrows depict hypothetical precursor-product relationships.

thermogenic gene program in white fat depots; hence, this factor could also play a role in BAT development (Shen et al., 2009).

Activation of the canonical Wingless (Wnt) signaling pathway is also associated with opposing effects on adipose and skeletal muscle lineages. Specifically, Wnt activation represses both brown and white adipogenesis by suppressing the induction of PPARγ and C/EBPα in precursor cells (Ross et al., 2000). By contrast, Wnt ligands are crucial inductive cues for embryonic

muscle formation through direct activation of muscle determination factors. Fibroblast growth factors (FGFs) −16, −19 (FGF15 in mouse), and −21 have also been implicated in the regulation of brown adipogenesis and thermogenic activity of brown fat (Konishi et al., 2000).

In addition to the dedicated depots of brown adipocytes that are formed before birth, "brown adipocyte-like" cells are also found interspersed in WATs of adult animals that have been acclimated to cold or chronically treated with selective β3-adrenergic agonists. These induced "brown adipocyte-like" cells express UCP1 and have a multilocular morphology similar to their counterparts in preformed depots. However, these cells have a distinct developmental origin, since they are not descendent from a *Myf5*-expressing progenitor (Seale et al., 2008). It thus remains to be determined whether mature white adipose cells, committed populations of preadipocytes, or undetermined stem cells are the source of these brown fat-like cells. Strain-dependent variability in the expression of UCP1 in brown adipose cells from retroperitoneal WAT but not in those from interscapular BAT depots suggests that these cell types are genetically dissimilar (Xue et al., 2007). The developmental origins and molecular characteristics of these cells will be very important to understand. While the supraclavicular depots of brown fat in humans form distinct pads in a predictable location and are thus likely analogous to the BAT arising from muscle in mice, brown fat cells interspersed in white depots are also found in humans.

Emerging Questions and Therapeutic Opportunities

Recent advances in brown fat biology and physiology further suggest its pivotal role in controlling energy homeostasis and its promise as a therapeutic approach to combat obesity in mice and humans. For example, a number of studies in animal models (e.g., fat-selective *Ucp1* transgenic mouse) have demonstrated that increases in amount or activity of brown fat can counteract obesity through increasing whole-body energy expenditure. The new findings on the regulation of brown fat development have identified key cellular and molecular events, as reviewed above, which now enable researchers to ask a variety of fundamental questions in the field. For example, how much brown fat is needed to alter whole-body energy expenditure and counteract obesity? This can be determined either by transplantation of brown fat cells or with transgenic animal models. Since skin fibroblasts or the stromal vascular fraction from adipose tissues are relatively easy to obtain, even from humans, autologous transplantation of engineered brown fat cells generated by expressing PRDM16 and C/EBPβ would be a feasible way to explore this question. It is also extremely important to clarify, via key transcriptional regulators such as FOXC2, pRb, RIP140, PRDM16, and PGC-1α, if there is a single, unified pathway/mechanism(s) acting to induce brown fat development. Better understanding of the molecular circuits that regulate brown fat specification and development will lead to identification of novel and specific pharmacological targets for antiobesity drugs.

One of the major unsolved questions is the molecular identity and cellular origins of the cAMP-inducible "brown fat-like" cells residing in the WAT. As described above, it does seem clear that a cAMP-induced transformation of WAT to BAT, presumably from a *Myf5*-independent cell lineage, is associated with a protection against obesity and metabolic diseases (Cederberg et al., 2001; Leonardsson et al., 2004). Identifying specific cell surface markers and establishing methods to isolate this cell population will advance our understanding of this issue. In addition, the role of BAT developmental regulators such as PRDM16 and PGC-1α in the formation of cAMP-induced "brown fat-like" cells and metabolic benefit must now be assessed.

Since gene transfer or cellular transplantation may not be an optimal method for human therapeutics in the metabolic syndrome, we also need to explore alternative ways to induce brown fat activity and development. Screening chemical compounds or drugs to induce dominant brown fat regulators such as PRDM16, FOXC2, or PGC-1α is certainly plausible. Alternatively, characterizing the upstream inductive components, such as endogenous hormones/polypeptides that stimulate the formation of brown fat cells during development, will be valuable. Likewise, new drugs or polypeptides that increase differentiation or activity of BAT that exists in adult humans may offer new treatments for obesity and diabetes.

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