

## Review

# A systems perspective on brown adipogenesis and metabolic activation

R.N. Pradhan<sup>1,2†</sup> M. Zachara<sup>1,2†</sup> and B. Deplancke<sup>1,2</sup>

<sup>1</sup>Institute of Bioengineering, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, and <sup>2</sup>Swiss Institute of Bioinformatics, Lausanne, Switzerland

Received 9 December 2016; accepted 12 December 2016

Address for correspondence: Professor B Deplancke, Institute of Bioengineering, École Polytechnique Fédérale de Lausanne (EPFL), AI 1147, EPFL SV-IBI UPDEPLA, AAB 145, Station 19, CH-1015 Lausanne, Switzerland. E-mail: [bart.deplancke@epfl.ch](mailto:bart.deplancke@epfl.ch)

<sup>†</sup>shared first author.

## Summary

Brown adipocytes regulate energy expenditure via mitochondrial uncoupling. This makes these fat cells attractive therapeutic targets to tackle the burgeoning issue of obesity, which itself is coupled to insulin resistance, type 2 diabetes, cardiovascular and fatty liver disease. Recent research has revealed a complex network underlying brown fat cell differentiation and thermogenic activation, involving secreted factors, signal transduction, metabolic pathways and gene regulatory components. Given that brown fat is now reported to be present in adult humans, it is desirable to harness the knowledge from each network module to design effective therapeutic strategies. In this review, we will present a systems perspective on brown adipogenesis and the subsequent metabolic activation of brown adipocytes by integrating signaling, metabolic and gene regulatory modules with a specific focus on known ‘druggable’ targets within each module.

**Keywords:** Brown adipogenesis, gene regulatory network, metabolism, signal transduction.

## Introduction

With the increase in the global incidence of obesity and its associated pathologies, collectively termed the metabolic syndrome, there is renewed interest in exploring pathways that modulate energy balance in mammalian systems (1). Disproportional energy intake over expenditure leads to energy imbalance, excessive fat storage in the white adipose tissue (WAT), and disturbance of whole body homeostasis, igniting intense investigation into pathways that would enhance energy homeostasis (2,3). The latter is in part mediated by fat depots via secretion of cytokines and interaction with various organs of the body. WAT, known as an endocrine-type storage organ, is capable of secreting adipokines such as leptin and adiponectin (4), and communicating with different organs such as the brain, muscle and liver to regulate energy homeostasis as well as the immune response. Excessive energy intake leads to WAT hyperplasia (increase in the number of mature adipocytes) and/or hypertrophy (adipose tissue expansion). Given its status as an energy reservoir, WAT has therefore been the primary target to

combat obesity. However, results have been discouraging due to lipid divergence, leading to the accumulation of lipids in other organs such as skeletal muscle, kidney and liver, ultimately causing the development of metabolic disorders. For instance, in response to the adipocyte-specific deletion of peroxisome proliferator-activated receptor gamma (*Pparg*), severe lipoatrophy is observed in mice, but the mass of *Pparg* null mice and their wild type littermates is similar, with the *Pparg* null mice demonstrating lipid divergence and afflictions such as fatty liver, diabetes and severe insulin resistance (5). This underscores the importance of designing effective therapies that lack adverse systemic side effects. While dietary restriction and exercise have a positive impact on adipose mass leading to diminished fat storage (6), novel strategies to improve whole body homeostasis and restore metabolic balance are being explored to aid metabolically challenged patients. With the recent positron emission tomography – computed tomography-based (PET-CT) discovery of metabolically active brown fat and recruitable brown-in-white fat depots in adult humans (7), current work in the field has turned to

explore ways to maximize energy expenditure via the brown adipose tissue (BAT) to combat excessive fat accumulation and restore energy homeostasis.

The presence of a second type of adipose tissue, BAT, has been originally described both in rodent models and in human infants, (8), as the thermogenic activity of BAT is essential for the endurance of small mammals in cold environments. A brown adipocyte is characterized by densely packed, iron-rich mitochondria, rendering it brown in color, and is capable of converting free fatty acids into heat via the action of the uncoupling protein 1 (UCP1) in response to various stimuli, most notably cold and diet (8). Apart from classical brown fat, WAT also harbors pockets of UCP1-expressing cells with thermogenic capacities that arise in response to various stimuli (9). These adipocytes are known as beige or brite (brown-in-white) or recruitable BAT (10) and display similar features to BAT, such as high mitochondrial content, multilocular lipid droplets and expression of a key set of brown fat marker genes (*Ucp1*, *Cidea*, *Pgc1a*) despite originating from different cellular lineages (11). Classical brown fat cells develop from pluripotent precursor cells in the somites that express transcription factors (TFs) such as PAX7, Homeobox protein engrailed-1 (EN1) and Myogenic factor 5 (MYF5). These TFs later determine the development of these precursor cells either into brown fat cells, skeletal myocytes, dorsal dermis or a subset of white adipocytes (12). On the other hand, lineage-tracing analysis showed that beige fat cells, together with another subset of white adipocytes, originate from *Myf5*-negative populations of progenitors (13) and express characteristic genes such as those coding for cluster of differentiation-137 (*Cd137*), T-box 1 (*Tbx1*) and transmembrane protein 26 (*Tmem26*) (14). Interestingly, a significant portion of the brown fat from adult humans that is present in the neck and supraclavicular regions has the molecular characteristics of beige (or recruitable) BAT rather than classical BAT (14,15). This is unlike the interscapular depots of human infants that have been shown to resemble classical BAT found in rodents using high resolution imaging, as well as histological and biochemical analyses (16). To design effective therapies promoting BAT activity in metabolically challenged human subjects, it is important to have a thorough understanding of the molecular mechanisms underlying brown fat cell development and activity. This review is an attempt to integrate the signaling, metabolic and transcriptional modules governing brown fat differentiation and metabolic activation with an additional focus on the white to brown axis. We envision that this network-based perspective will contribute to the identification of unexplored pathways or incomplete mechanistic details regarding key regulators and signaling molecules, aiding in the development of targeted therapeutic approaches.

## Part 1: Metabolic activity of brown adipose tissue

### *Uncoupling protein 1, the core player in brown adipose tissue activation*

The process of 'uncoupling' substrate oxidation from adenosine triphosphate (ATP) synthesis, i.e. thermogenesis, occurs in brown adipocytes and is catalyzed by the mitochondrial protein UCP1 (8). UCP1 is a multi-pass mitochondrion inner membrane protein and member of the mitochondrial carrier protein family (SLC25) (17–20). *Ucp1* null mice show little resistance to cold and are not capable of sustaining their body temperature during prolonged cold exposure, suggesting a crucial role for UCP1 in cold-induced thermogenesis (21). Thermogenesis at the molecular level begins when free long chain fatty acids (LCFAs) liberated from the abundant multilocular fat droplets within the brown adipocyte are activated by acyl-CoA synthase to acyl CoAs and transferred into the mitochondria. This results in beta-oxidation of LCFAs (now acyl-CoAs), which, in combination with the Krebs cycle, produces the reduced electron carriers, Flavin adenine dinucleotide (FADH<sub>2</sub>) and Nicotinamide adenine dinucleotide (NADH). The latter are subsequently oxidized within the respiratory chain, which causes the protons to be pumped out of the mitochondria, ultimately resulting in the formation of a proton-motive force. UCP1 then mediates the influx of protons back into the mitochondrial matrix without ATP being synthesized. Rather, the energy stored in the proton-motive force is liberated as heat (8,22). More specifically, the mechanism by which UCP1 transports H<sup>+</sup> ions has recently been elucidated using the patch clamp technique. The latter allowed direct measurement of UCP1 currents in the native inner membrane of BAT mitochondria, demonstrating that UCP1 is a symporter of LCFAs/H<sup>+</sup>. Thus, LCFAs serve as primary substrates that associate with UCP1 via hydrophobic interactions and enable UCP1 to transport H<sup>+</sup> ions, thereby mediating thermogenesis (23).

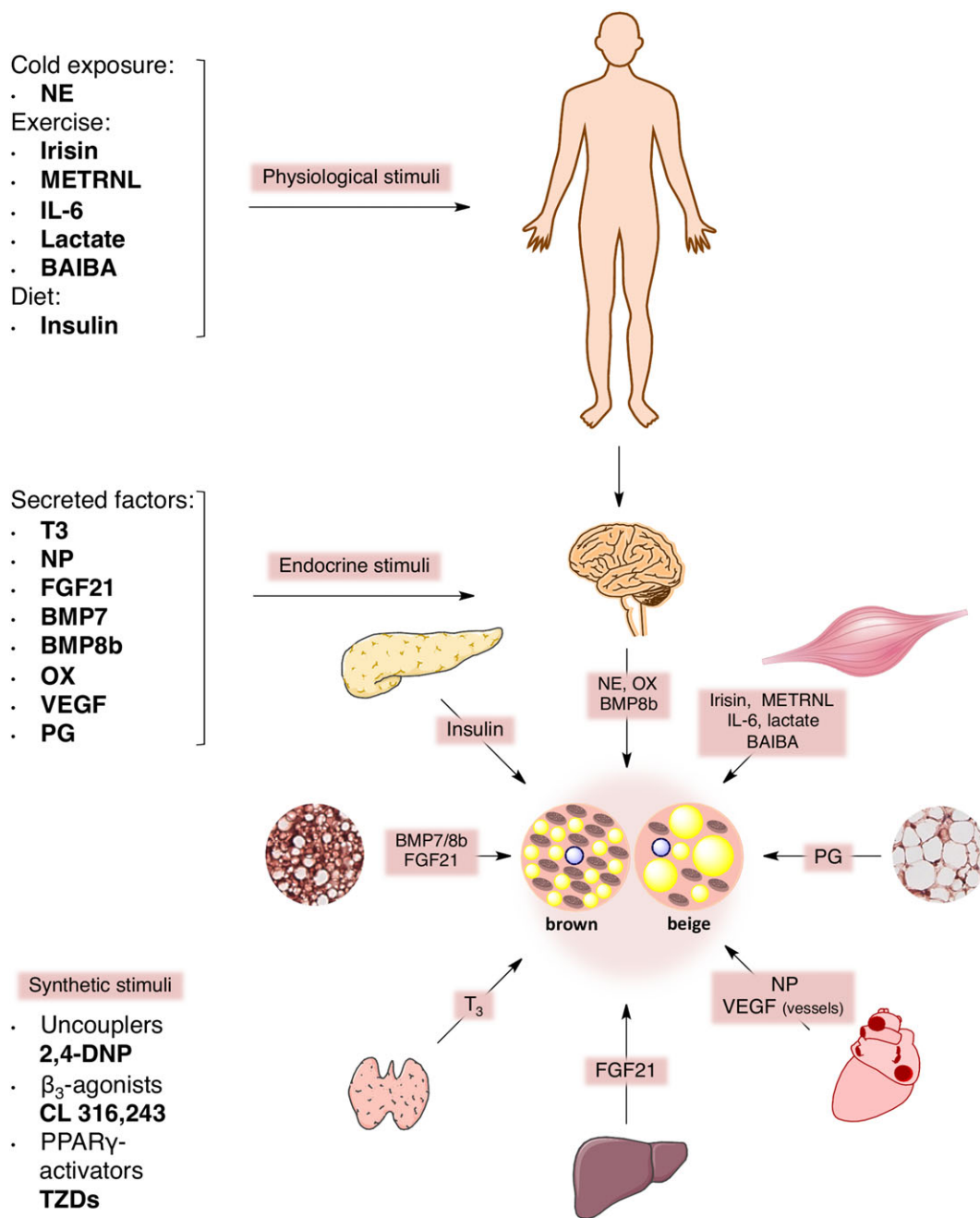
## Part 2: Known therapeutic interventions in humans

### *Non-drug-based approach*

Methods that are employed to induce BAT development and activation, aiming to improve whole body energy homeostasis, include the following:

### *Cold exposure*

Cold exposure, to our knowledge, is the most efficient approach to induce BAT development and activation (24,25), and it does so via the sympathetic nervous system (SNS) as well as the norepinephrine (NE)-dependent signal transduction pathway (Fig. 1). Numerous studies showed that the effects of chronic cold exposure are compelling and significantly improve systemic metabolic homeostasis, including insulin sensitivity as well as glucose and lipid profiles (24–27).



**Figure 1** Schematic representation of factors that promote development and activity of brown and beige adipocytes. Shown are factors with therapeutic potential as BAT potentiators. Physiological stimuli originating at the systemic level can be divided into 1) cold exposure-derived factors namely norepinephrine (NE) that is secreted by the SNS; 2) exercise-induced myokines including irisin, Meteorin-like (METRNL), interleukin 6 (IL-6), lactate and  $\beta$ -aminoisobutyric acid (BAIBA); and 3) insulin as a post-prandially secreted factor. In addition, endocrine stimuli are also visualized including thyroid hormone T<sub>3</sub>, natriuretic peptides (NP), fibroblast growth factor 21 (FGF21), bone morphogenetic protein 7 (BMP7), bone morphogenetic protein 8b (BMP8b), orexin (OX), vascular endothelial growth factor (VEGF) and prostaglandins (PG). Finally, several synthetic molecules are also shown that can act as BAT inducers but that are no longer used given their adverse secondary effects: mitochondrial uncoupler: 2,4-dinitrophenol,  $\beta_3$ -adrenoreceptor agonist: CL 316,243 and PPAR $\gamma$  activators: thiazolidinediones (TZDs). The presented list of factors activating BAT is not exhaustive. A few organ schemes originate from Servier Medical Art. Figure legend: Organs from top (clockwise): Brain, Skeletal muscle, WAT, Heart, Liver, Thyroid, BAT and Pancreas.

Nevertheless, results of adult human-based studies revealed important discrepancies, perhaps due to varying study conditions and experimental designs (10). This illuminates the need for

standardized experimental protocols to better assess and compare the actual impact of cold exposure on human BAT activity across individuals and studies.

### Exercise

The role of exercise in enhancing and maintaining overall fitness, health and well-being is incontestable. It is an established physiological method for improving muscle strength and cardiovascular system condition, decreasing body weight and allowing its maintenance, protecting against type 2 diabetes, as well as awarding enjoyment through endorphin production (28,29). In light of recent interest in BAT physiology, the cocktail of metabolic factors that is secreted during exercise was analysed in various studies in the context of BAT development and activation (12). This led to the identification of a number of molecules such as Irisin, Meteorin-like, IL-6, lactate, beta-aminoisobutyric acid amongst others (30–35) (Fig. 1). However, as with chronic cold exposure, it remains to be established how much of the beneficial effect of exercise occurs via BAT activation.

### Bioactive food ingredients

Another non-drug-based factor discussed in the context of BAT activity enhancement is diet, which along with exercise has a powerful beneficial impact on overall health and fitness. Alternative dietary options, including substances such as green tea, ginger, capsaicin (36) amongst others, have been reported to induce BAT activity. Green tea, for instance, contains caffeine and remarkable quantities of catechin-polyphenols (notably epigallocatechin gallate). These molecules interact to induce 24 h energy expenditure and fat oxidation, thereby implicating them as effective activators of sympathetically mediated thermogenesis (37). Similarly, ginger, with its bioactive constituents gingerols and shogaols (38), was shown to enhance the thermic effect of food (39). Although it was reported that ginger-supplemented high-fat diet fed rats presented lower insulin, glucose and lipids versus the control rats (38), no such effect was observed in an equivalent study conducted in humans (39).

### Drug-based approach

The abundance of brown fat and its activation by cold vary according to the extent of obesity of human subjects (7,24,26,40). Studies that directly measure oxidative metabolism in BAT by  $^{15}\text{O}$  PET blood flow demonstrated that the level of cold-inducible thermogenesis in most individuals is less than 10 kcal/day (41)–(42). In contrast to cold adapted rats where the blood flow to BAT can reach up to 1,000 ml/min/100 g (43,44), in acute cold-stressed, but chronically warm-adapted humans, the rate is 15–20 ml/min/100 g (41,42,45). To achieve a similar effect, for instance, via exercise, the thermogenic activation of BAT in humans needs to be increased 40–50-fold (46). Hence, drug-based therapies have been proposed to enhance the activity of BAT in metabolically challenged individuals where diet and exercise regimen alone is not

sufficient to promote the metabolic activity of BAT. These include chemical uncouplers such as dinitrophenol, which was used extensively but is associated with complications such as hyperthermia and has since been banned. Alternatively, several groups demonstrated the effect of UCP1-specific activators and other futile cycles that may provide metabolic benefit within a tolerable safety profile. This is evidenced by the directed release of mitochondrial protonophores that can improve insulin resistance, diabetes, hypertriglyceridaemia and hepatic steatosis by safely uncoupling in the liver of rats (47). Other drugs include beta-3 selective adrenergic agonist CL-316,243 that can potently activate brown and beige fat in rodents and is known to improve insulin action in human clinical trials (48). Currently, acute dosage of Mirabegron, a drug that has been used to treat an overactive urinary bladder, has been shown to activate brown fat. Mirabegron is therefore a candidate in consideration for a putative drug under the condition that its effect is constant upon chronic dosing and without adverse cardiovascular side effects (49). Thiazolidinediones (TZDs), well-known PPAR $\gamma$  agonists, are known to cause browning of white fat by stabilizing PR domain containing 16 (PRDM16), a transcriptional co-regulator of the early brown fat differentiation program. However, they also have a rather negative impact such as weight gain, fluid retention and cardiovascular events, reducing interest in their use. Additional molecules of interest include bone morphogenetic proteins such as BMP7 and BMP8b, cyclooxygenase-2 (COX2) and natriuretic peptides, and fibroblast growth factor 21 (FGF-21). However, these molecules have pleiotropic effects, underscoring the importance of delineating signaling and transcriptional pathways both upstream and downstream of the targeted factor. The following sections will therefore focus on key signaling and gene regulatory modules and mechanisms as well as highlight putative 'druggable' targets.

### Part 3: Secreted factors for brown adipose tissue recruitment

Numerous factors were demonstrated to positively recruit BAT (or beige adipose tissue) activity via endocrine, paracrine or autocrine mechanisms (10,50). These include (i) natriuretic peptides that bind to natriuretic receptors on brown or beige adipocytes, and that trigger lipolysis and thermogenesis by activating cyclic GMP-dependent protein kinase (PKG) (10,51); (ii) irisin, a newly identified hormone that is induced in mouse and human muscle by exercise and that stimulates UCP1 expression (10,30); and (iii) orexin, a neuropeptide that triggers the brown fat cell differentiation program by impacting the sympathetic outflow via p38 MAPK as well as the bone morphogenetic protein receptor 1-A-dependent Smad1/5 signaling (10,52) and FGF family members including



FGF-21 and FGF-15/19 that mediate browning and glucose homeostasis (53).

#### Part 4: Signal transduction pathways

Although lipid-storing cells evolved late during the course of evolution, it has been observed that evolutionarily ancient factors and pathways are highly active during adipogenesis and have either an activatory or inhibitory effect on adipocyte development. Some pathways are also known to exhibit dichotomic effects and are poorly characterized in the context of adipogenesis, particularly regarding how they converge on key transcriptional programs (54). As these signaling pathways control tightly regulated transcriptional cascades, further investigation into the transmission of signals is valuable. The core adipogenic circuit comprises the master regulator PPAR $\gamma$  along with CCAAT/enhancer binding protein alpha (C/EBP $\alpha$ ), and many signaling events are focused on the activation of this regulatory circuit. On the other hand, initiation of brown fat cell differentiation and activation of the thermogenic circuit require additional transcriptional regulators and co-regulators such as PRDM16, peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1 $\alpha$ ) that are also stimulated by specific signal transduction pathways. In this part, we highlight and summarize important signaling events that impact brown adipogenesis and identify key 'drug-able' targets where possible. A comprehensive overview of key signaling pathways and gene regulatory networks is presented in Fig. 2, highlighting the complexity of the network.

##### *Beta-adrenergic signaling*

Beta-adrenergic receptor signaling functions via three types of adrenergic receptors:  $\beta$ ,  $\alpha_2$  and  $\alpha_1$  that respond to the catecholamine, NE which is released as a consequence of cold-induced or leptin-mediated activation of the SNS. The activation of beta-adrenergic receptor signaling further promotes the activation of different signaling pathways in brown adipocytes. Of the three subtypes of beta-adrenergic receptors, beta 3 adrenergic receptor (ADRB3) is predominantly found in mature brown adipocytes, while beta 1 adrenergic receptor (ADRB1) is found in brown pre-adipocytes. Beta-adrenergic receptors are known to couple to G proteins of the G $\beta$  subtype and execute the thermogenic signaling cascade via adenylyl cyclase activation. It has been demonstrated that ADRB-1 and -3 mediated thermogenic activation and upregulation of oxidative metabolism are highly effective in elevating whole-body thermogenesis in both rodents (44,55) and humans (56).

##### *Thyroid signaling*

Thyroid hormones bind to thyroid hormone receptors (TR)  $\alpha_1$ - $\alpha_2$  and  $\beta_1$ - $\beta_2$  to execute pleiotropic effects such as growth

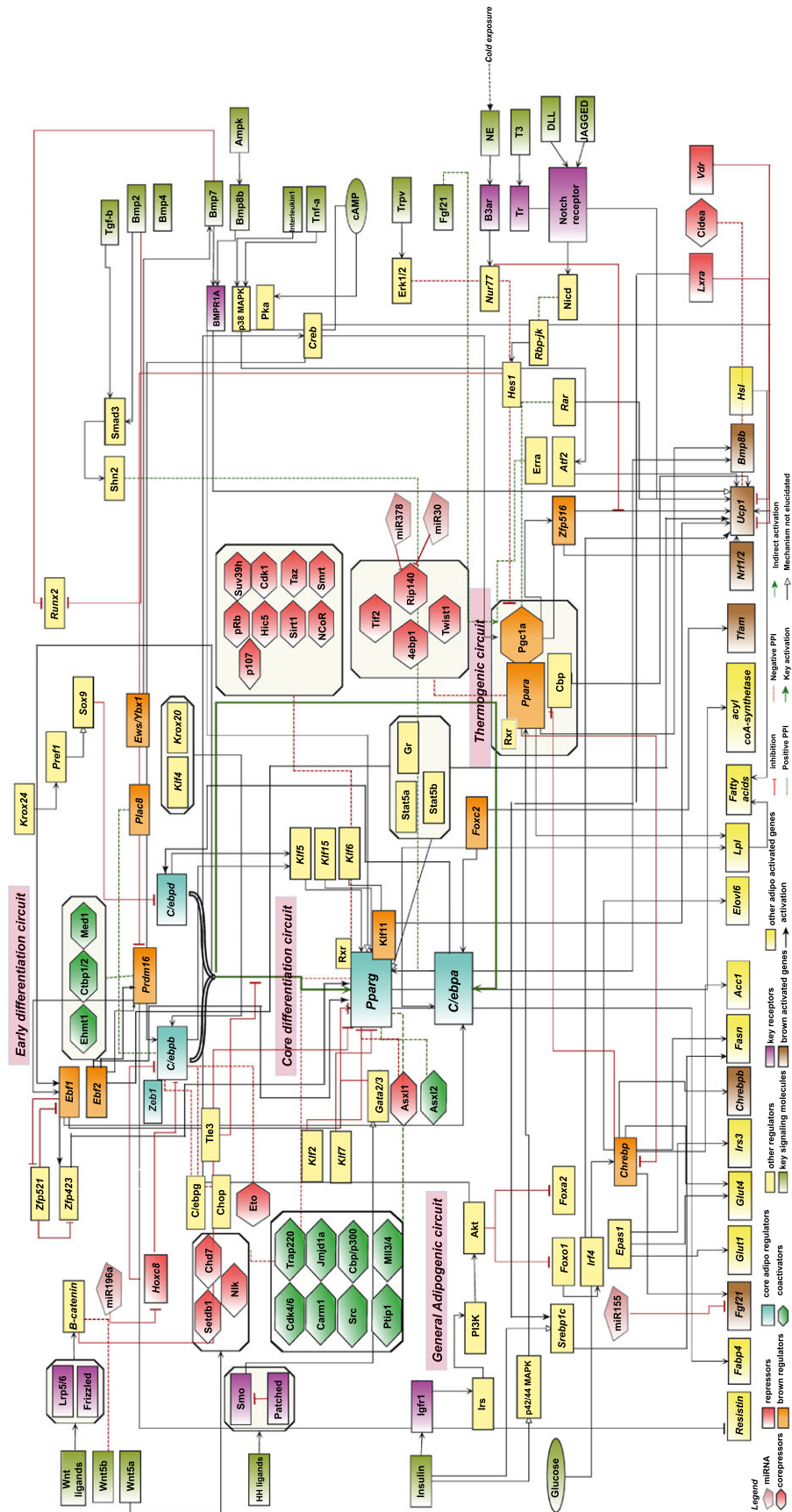
and metabolism in several target tissues throughout the body, such as liver, bone, fat and heart. Particularly in brown fat, Thyroxine or T $_4$  is converted to its metabolically active form, Triiodothyronine or T $_3$ , by type 2 deiodinase (DIO2) following sympathetic stimulation (57). T $_3$  further acts in concert with norepinephrine to stimulate *Ucp1* gene expression in the BAT in rats (58,59). To determine the TR isoforms regulated by T $_3$ , pharmacological and genetic screening approaches have been used, leading to the identification of TR $\beta$ -1 agonist Gc-1, capable of stimulating *Ucp1* expression but not affecting the regulation of whole body temperature. Further, genetic mouse models showed that TR $\alpha$  activation is important for thermogenesis while TR $\beta$  is necessary for cholesterol metabolism (60). Thus, drug designs targeting thyroid receptors must take this into account.

##### *Gq signaling*

G protein-coupled receptors (GPCRs) are a family of seven transmembrane proteins that regulate biological processes in different tissues such as the adipose tissue, via the action of heterotrimeric G proteins composed of three subunits: alpha ( $\alpha$ ), beta ( $\beta$ ) and gamma ( $\gamma$ ). Upon activation of GPCRs, G $\alpha$  dissociates from the G $\beta\gamma$  dimer causing the activation of downstream signaling events determined by G protein coupling. Of the four classes of G $\alpha$  proteins, Gq protein is known to activate multiple intracellular signaling pathways such as ERK, IP3/DAG and RHO/ROCK (61,62). A GPCR profiling-based approach revealed Gq-coupled GPCRs to be one of the highly expressed groups of signaling molecules in murine brown adipocytes that inhibit adipocyte differentiation through the RHO/ROCK signaling cascade. This phenotypic effect was manifested via the Endothelin-1/Endothelin receptor type A (ET-1/EDNRA) signaling axis. Further, overexpression of Gq *in vivo* induced a phenotype similar to the effect of 'whitening' in BAT, preventing 'browning' of WAT in cold-exposed mice (63-65). Given that approximately 25% of the drugs available in the market target GPCRs, it seems feasible to target receptors coupled to Gq to enhance browning in white fat depots (66).

##### *Wnt signaling*

Cell fate and development processes are influenced by the para- and autocrine activity of the Wnt family of secreted glycoproteins. The binding of Wnt proteins to frizzled receptors triggers beta-catenin-dependent, canonical signaling pathways. Canonical Wnt signaling ligands such as Wnt10b promote osteogenesis in mesenchymal stem cells (MSCs) and suppress both white and brown adipogenesis. It has previously been demonstrated that Wnt10b blocks brown adipose differentiation and affects the expression of *Ucp1* via repression of *Pgc1 $\alpha$*  in mature brown adipocytes leading to a switch to white adipocytes. Wnt signaling



**Figure 2 (Continued)** Network visualization of key signalling and gene regulatory modules that are implicated in brown adipogenesis. Key signal transduction pathways converging on gene regulatory modules as well as additional transcriptional regulators mediating brown adipogenesis are represented in the network. Nodes in bold italics represent genes, while the ones in bold represent proteins that interact with regulators to control downstream brown-specific and general adipocyte gene expression. The gene regulatory network comprises the following circuits (in chronological order of execution): 1. Early differentiation and 3. General adipogenic and thermogenic circuit. The network derives from a comprehensive mining exercise of current literature on both general adipogenic and brown signalling and regulatory cascades. Full protein and gene names of the nodes in this network are as follows: *Igf1r*: Insulin-like growth factor 1 receptor, *Irs*: Insulin receptor substrate, *Pik3k*: Phosphoinositide 3-kinase, *Akt*: Protein kinase B (PKB), *Lrp5/6*: Low density lipoprotein receptor-related protein, *Hh Ligands*: Hedgehog ligands, *Smo*: Smoothened, *Tgfb*: Transforming growth factor beta, *Bmp2/4/7/8B*: Bone morphogenetic proteins 2/4/7/8B, *Bmpr1A*: Bone morphogenetic protein receptor, type IA, *Smad3*: SMAD, Mothers against DPP homolog 3, *Ampk*: AMP-activated protein kinase, *Sirt6*: Sirtuin 6, *TnfA*: Tumour necrosis factor-alpha, *Camp*: Cyclic adenosine monophosphate, *Trpv*: Transient receptor potential (TRP) ion channel, *Fgfr2*: Fibroblast growth factor receptor 2, *B3A*: Beta adrenergic receptor 3, *Ne*: Norepinephrine, *Dli*: Delta like, *Nicd*: Notch intracellular domain, *T3*: Triiodothyronine, *Tr*: Thyroid receptor, *P42/44 Mapk*: Mitogen-activated protein kinase 1, *Erfk1/2*: Mitogen-activated protein kinase, *P38 Mapk*: Mitogen-activated protein kinase, *Prdm16*: PR domain containing 16, *C/EBpb*: CCAAT/enhancer binding protein beta, *C/EBpd*: CCAAT/enhancer binding protein delta, *Ebf1*: Early B-cell factor 1, *Ebf2*: Early B-cell factor 2, *Zfp521*: Zinc finger protein 521, *Zfp423*: Zinc finger protein 423, *Zeb1*: Zinc finger E-box binding homeobox 1, *Krox24*: Early growth response protein 1 (Egr1), *Pref1*: Preadipocyte factor 1 (aka Dlk1), *Sox9*: SOX9 (sex determining region Y)-box 9, *Plac8*: Placenta-specific 8, *Ews/Ybx1*: EWS RNA-binding protein 1/Y box binding protein 1, *Runx2*: runt-related transcription factor 2, *Hoxc8*: Homeobox C8, *PPARg*: Peroxisome proliferator-activated receptor gamma, *C/EBPa*: CCAAT/enhancer binding protein alpha, *KLF11*: Kruppel like factor 11, *KLF2*: Kruppel like factor 2, *KLF7*: Kruppel like factor 7, *KLF5*: Kruppel like factor 5, *KLF15*: Kruppel like factor 15, *KLF6*: Kruppel like factor 6, *Rxr*: Retinoid X receptor, *Gata2/3*: Globin transcription factor 2/3, *Epas1*: Endothelial PAS domain protein 1, *Chrebp*: Carbohydrate response element binding protein, *Srebp1c*: Sterol regulatory element binding protein 1, *Foxo*: Forkhead box O1, *Foxa2*: Forkhead box A2, *Ifi4*: Interferon regulatory factor 4, *Erra*: Estrogen-related receptor alpha, *Pparz*: Peroxisome proliferator-activated receptor alpha, *Cbp*: CREB binding protein, *Zfp516*: Zinc finger protein 516, *Foxc2*: Forkhead box C2, *Tle3*: Transducin-like enhancer of split 3, *Vdr*: Vitamin D receptor, *Lxra*: Liver X receptor alpha, *Rbp-Jk*: Recombination signal binding protein for immunoglobulin kappa J, *Hes1*: Hes family BHLH transcription factor 1, *Nurr77*: Nuclear hormone receptor NUR77, *Atf2*: Activating transcription factor 2, *Rar*: Retinoic acid receptor, *C/ebpb*: CCAAT/enhancer binding protein gamma, *Chop*: C/EBP-homologous protein 10, *Ehmt1*: Euchromatic histone N-methyltransferase 1, *Ctbp1/2*: C-terminal binding protein 1/2, *Medf1*: Mediator complex subunit 1, *Eio*: Runt-related transcription factor 1, translocated to, 1 (cyclin D related), *Setdb1*: SET domain, bifurcated 1, *Chrd7*: Chromodomain helicase DNA binding protein 7, *Nfk*: Nemo like kinase, *Asx1/2*: Additional sex comb like 1, *Asx2*: Additional sex comb like 2, *p107*: Retinoblastoma-like 1, *pRb*: Retinoblastoma protein, *Suv39*: Suppressor of variegation 3-9 Homolog 1, *Hic5*: Hydrogen peroxide-inducible clone 5 protein, *Cdk1*: Cyclin-dependent kinase 1, *Sirt1*: Sirtuin 1, *Taz*: Transcriptional coactivator with PDZ-binding motif, *NCoR*: Nuclear receptor corepressor 1, *Smrt*: Silencing mediator for retinoid and thyroid hormone receptors, *Tif2*: Transcriptional intermediary factor 2, *4ebp1*: Eukaryotic translation initiation factor 4E-binding protein 1, *Rip1/140*: Nuclear receptor interacting protein 1, *Twist1*: Twist family BHLH transcription factor 1, *Pgc1a*: Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha, *Cidea*: Cell death-inducing DFFA-like effector A, *Stat5a*: Signal transducer and activator of transcription 5a, *Stat5b*: Signal transducer and activator of transcription 5b, *Gr*: Glucocorticoid receptor, *Tfam*: Transcription factor A, mitochondrial, *Nrf1/2*: Nuclear respiratory factor 1/2, *Ucp1*: Uncoupling protein 1, *Hsl*: Hormone-sensitive lipase, *Fabp4*: Fatty acid binding protein 4, *Fgfr2*: Fibroblast growth factor 2, *Glut1*: Glucose transporter type 1, *Glut4*: Glucose transporter type 4, *Irs3*: Insulin receptor substrate 3, *Chrebp*: Carbohydrate response element, *Fasn*: Fatty acid synthase, *Acc1*: Acetyl-CoA carboxylase Alpha, *Elovl6*: ELOVL fatty acid elongase 6.

may suppress *Pgc1a* either directly via a beta-catenin LEF1 complex, or interference with transcriptional activity of *Pgc1a* regulators such as ATF-2, CREB, FOXO1 or via the inhibition of upstream signaling molecules such as protein kinase A or p38 MAPK (67). Other Wnts such as Wnt6, Wnt10a are also known to suppress adipogenesis and promote osteoblastogenesis (68). In contrast, Wnt5b positively regulates adipogenesis by countering the nuclear translocation of beta-catenin, thus inhibiting the canonical Wnt signaling pathway (69).

### Hedgehog signaling

Hedgehog (HH) ligands bind to the Patched-1 (PTCH1) receptor and liberate Smoothened (SMO), a downstream protein in the pathway, from the inhibitory effect of PTCH1 causing SMO to regulate gene transcription of GLI proteins that negatively regulate adipogenesis (54). Also, it is likely that SMO indirectly activates the GATA factors that are known to be anti-adipogenic (70). Extrapolation of results from a systems level genetic dissection of adipose regulation in *Drosophila melanogaster* to the murine system implicated HH signaling pathway as being inhibitory for white but not for brown fat formation (71). In contrast, a more recent study demonstrated that HH signaling operates in a cell-autonomous manner to block brown adipocyte differentiation, inhibit *in vivo* BAT formation and replace neck BAT in rodents into poorly differentiated skeletal muscle, partially via the upregulation of chicken ovalbumin upstream promoter transcription factor II (Coup-TFII) (72). The differences between these two studies could be attributed to the *in vivo* system used to assess the phenotypic effect of HH signaling on adipose depots thereby highlighting the importance of interpreting phenotypic outcomes obtained from *in vivo* data (72).

### Janus kinase–signal transducers and activators of transcription signaling

Janus kinases (JAKs) are associated with various cell surface receptors, whose activity is triggered via ligands such as interferon, interleukin and growth factors. Activated JAKs recruit SH2 domain containing proteins (signal transducers and activators of transcription (STATs)) to the receptors via phosphorylation and are further tyrosine phosphorylated by JAKs. These activated STATs then translocate to the nucleus, form hetero- or homo-dimers and regulate transcription of target genes. Recently, Janus kinase inhibitors were identified as molecules that possess ‘browning’ potential, using a screening approach to discover small molecules capable of promoting a switch from white to brown in human adipocytes. It was demonstrated that two compounds, tofacitinib and R406, strongly induce *UCP1* while remodeling lipids via the JAK–STAT1/3 pathway, thereby implicating the IFN–JAK–STAT–SHH axis in human adipocyte biology. Of the two potential drugs identified, the drug

tofacitinib is sanctioned in the United States for rheumatoid arthritis (73). Additionally, because the JAK–STAT pathway is involved in immune system regulation, local administration of the drug or *ex vivo* transplantation of cells post-treatment is recommended to limit deregulated immune activation.

#### Transforming growth factor-beta signaling

Transforming growth factor beta (TGF- $\beta$ ), myostatin and bone morphogenetic proteins regulate the differentiation of many cell types including adipocytes. While subsequent signaling events have common properties, specific TGF- $\beta$  family members influence distinct cell fate decisions by binding to serine/threonine kinase receptors and functioning through SMAD-dependent and -independent mechanisms. Transcriptional regulation of target genes is executed by receptor-regulated SMAD1 and SMAD3 TFs, which, upon phosphorylation, engage in dimer formation with SMAD4, and subsequently translocate to the nucleus. In adipogenesis, the canonical TGF- $\beta$  signaling pathway has an unclear molecular function. While TGF- $\beta$  expression correlates positively with obesity in humans, it inhibits *in vitro* adipogenesis of 3T3-F442A cells by signaling through SMAD3. It has been observed that supplementing wild-type mice with exogenous TGF- $\beta$ 1 reduced thermogenic gene expression in fat (74), implicating TGF- $\beta$  signaling in the negative regulation of browning. This has been exploited for therapeutic purposes as evidenced in a study where a dominant negative form of activin receptor type II-b fusion protein was used to promote thermogenesis in mice by binding to TGF- $\beta$ , thereby preventing downstream signaling (75).

#### Bone morphogenetic protein signaling

Bone morphogenetic proteins (BMPs) are categorized as a family within the TGF- $\beta$  superfamily with the ability to stimulate white and brown fat cell differentiation programs. While BMP2 and BMP4 are known to induce commitment to the white adipocyte fate when supplemented by a hormonal induction cocktail, BMP7 is known to singularly support brown fat cell differentiation in both MSC progenitors and committed pre-adipocytes even in the absence of a differentiation cocktail via the p38 MAPK pathway (76). p38 MAPK further executes the thermogenesis program by phosphorylating and activating distinct nuclear receptors (NRs), ATF2 and PGC1 $\alpha$ , which in turn promote *Ucp1* expression by binding to a cAMP and PPAR-response element that resides in a key enhancer of the *Ucp1* gene (77). This phenotypic effect was also observed *in vivo*, because *Bmp7* null mice show a reduction in brown fat mass that was rescued upon adenovirus-mediated overexpression of *Bmp7*, thereby protecting the mice from diet-induced obesity while triggering energy expenditure (76). BMP7 is also known to function synergistically with rosiglitazone, a TZD, to give rise to inducible brown

adipocytes from SCA1+ adipocyte progenitor cells that arise from different fat depots as well as skeletal muscle in mice (78). Additionally, *in vivo* studies showed that BMP7 enhances *Ucp1* expression in WAT in combination with the beta3-adrenergic agonist CL-316,243 to levels higher than those achieved by CL-316,243 treatment alone (78). This study underscores the importance of the BMP7 signaling pathway in inducing browning and as a viable therapeutic option.

BMP8b is yet another BMP signaling molecule capable of regulating whole body energy metabolism both centrally, through AMPK activation in hypothalamic nuclei, and peripherally, by activating the p38 MAPK signaling pathway in differentiating and mature brown adipocytes. Using *Bmp8b* knockout mice, it was demonstrated that BMP8b increases the peripheral response of BAT to adrenergic stimulation and acts centrally to increase sympathetic output to BAT. BMP8b signaling specifically acts on the thermogenic pathway, as *Bmp8b* knockout mice do not show gross abnormality in BAT morphology. Additionally, it has been proposed that AMPK has a counter-regulatory role, acting in opposition to BMP8b to regulate energy expenditure, supported by the observation of greater thermogenic activation upon localized expression of the dominant negative AMPK alpha isoform. As AMPK is a known druggable target in peripheral tissues to treat insulin resistance (79), its relationship with BMP8b offers new opportunities in targeted drug design. It is worth noting that the ability of BMP8b to execute central actions to reduce whole body weight without compensatory food intake offers an exciting premise for new therapeutic options, demonstrating the importance of understanding thermogenesis at a central regulatory level.

#### NOTCH signaling

The Delta-like (DLL) and Serrate/Jagged (JAG) family of membrane bound ligands bind to transmembrane NOTCH receptors causing gamma-secretase-mediated proteolytic cleavage of NOTCH, leading to the nuclear translocation of the NOTCH intracellular domain (NICD). Once in the nucleus, the NICD activates the RBP-jk transcriptional complex and subsequently its downstream targets such as the *Hes* and *Hey* family genes (80). Constitutive activation of NOTCH signaling inhibits *Pgc1a* and *Prdm16* transcription in white adipocytes via HES1-mediated transcriptional repression and causes ‘whitening’ of BAT. In contrast, inhibition of NOTCH signaling is associated with ‘browning’ of WAT, improvement of insulin sensitivity and whole-body energy homeostasis (81). This differential response is intriguing and highlights the involvement of different gene regulatory programs that are cell-context dependent. As NOTCH signaling is an evolutionarily conserved pathway, these findings can be extended to human studies; however, this merits further investigation into identifying molecular



mechanisms operating in human white to brown conversion.

## Part 5: Gene regulatory networks

Once adipogenic precursors commit to the adipogenesis program, a transcriptional circuit is switched on, leading to the expression of genes associated with fat cells. While the white and brown differentiation programs share a set of regulators and adipogenic stimuli, they both have unique requirements that determine their phenotypic differences. It is therefore important to delineate both, i.e. the core adipogenic differentiation and the thermogenic programs, to get a full picture of the gene regulatory events that give rise to fully functional brown and beige fat. In this section, we present an overview of the individual circuits such as the early and core differentiation, thermogenic and adipogenic modules within the overall gene regulatory network. A schematized overview is presented in Fig. 2.

### Early differentiation circuit

#### Early B-cell factors

The early B-cell factors (EBF), especially EBF1 and EBF2, play an important regulatory function in the early differentiation circuit along with Zinc Finger Protein (ZFP) 423 and its close paralog ZFP521, both implicated as early regulators of adipose commitment and differentiation (82,83). ZFP521 functions in a repressive feedback loop with EBF1, while also repressing ZFP423, thereby influencing early regulatory events in the lineage switch between bone and fat (83). ZFP423, on the other hand, functions by promoting *Pparg* expression through amplification of the BMP signaling pathway via SMAD binding (82). Further, *Ebf1* is induced by both CCAAT/enhancer binding protein delta (C/EBPd) and CCAAT/enhancer binding protein beta (C/EBPb) and then directly activates both *Pparg* (via binding to the *Pparg1* promoter) and *Cebpa*, which positively feeds back to *Clebpd* (84). A previous study demonstrated EBF2 to regulate PPARg binding to determine brown versus white fat fate, leading to its classification as a brown-specific transcriptional regulator (85). It was further suggested that BAT-specific target genes of PPARg were categorically bound by EBF2 that then served as a mark for the later recruitment of PPARg at sites regulating the expression of brown fat selective genes such as *Prdm16* and *Ucp1* (85).

#### PR domain containing 16

The 140 kDa PRD1-BF-1-RIZ1 homologous domain containing protein-16, PRDM16, is a core transcriptional co-regulator in both brown and beige fat, showing selective expression in brown over white fat (13). *In vitro* cell culture studies in classical brown fat cells demonstrated the

requirement of PRDM16 to develop and maintain a thermogenic gene program. However, *in vivo* deletion of *Prdm16* did not affect the function of classical brown fat but had a significant impact on the development of beige fat cells. Additionally, deletion of *Prdm16* along with its closest homolog *Prdm3* led to an early and extreme loss of brown fat thermogenic gene expression (86). Most importantly, PRDM16 has been demonstrated to function as a molecular switch between white and brown programs by coupling with diverse protein interaction partners simultaneously, mediating both activating and repressive gene expression programs. These dichotomous activities are executed through the use of chromatin remodellers, C-terminal binding proteins (CtBPs) that function via the recruitment of histone deacetylases (87). PRDM16 interacts with either PGC1a or CtBPs to, respectively, activate the brown or suppress the white gene expression program. During brown fat activation, recruitment of PGC1a displaces CtBPs from the complex to activate brown fat-specific genes. Additionally, it has been suggested that PRDM16 binds to specific TFs linked to the promoters/enhancers of target genes, further guiding the recruitment of PGC1a or CtBPs to initiate the formation of a co-activator or a co-repressor complex (87). The TFs that guide PRDM16 to its respective target genes and their molecular underpinnings have not been mapped yet, and it is quite likely that numerous factors are involved in this regulatory process.

#### Placenta-specific 8

Placenta-specific 8 (PLAC8), a key upstream molecule in the brown adipogenic regulatory network, functions by associating with *Clepbp* and binding to its promoter to induce transcription (88). *In vivo* genetic deletion of *Plac8* results in the loss of expression of several brown fat-specific components such as *Prdm16* and *Pgc1a*. However, general adipocyte differentiation factors such as PPARg and AP2 are not downregulated, indicating the importance of PLAC8 in recruiting BAT during thermogenic challenges. Consequently, *in vivo* genetic inactivation of *Plac8* leads to errors in BAT differentiation and thermogenic programs, causing cold intolerance and late onset obesity (88). However, the upstream signaling activators of *Plac8* induction and those that promote PLAC8 binding to C/EBPb are as yet uncharacterized and merit further investigation.

#### CCAAT/enhancer binding protein beta and CCAAT/enhancer binding protein delta

PPARg and C/EBPs, the key molecular players of adipogenesis, vary in their molecular function in brown versus white fat programs. In response to pro-adipogenic stimuli, C/EBPb and C/EBPd transition from a repressive complex, involving the nuclear co-repressors NCoR1 and SMRT (89), into an active one. This in turn allows these TFs to induce expression of PPARg and C/EBPa in cooperation

with the epithelial–mesenchymal transition TF ZEB1 (90), which drives terminal fat cell differentiation. C/EBP $\beta$  is important for adipogenic differentiation in immortalized adipocytes with slightly inconspicuous effect in mouse embryonic fibroblasts (MEFs). While it has been demonstrated that C/EBP $\beta$ -deficient mice have reduced adiposity, this might be attributed to impairment in lipogenic and not adipogenic programs (54,91). It is quite likely that C/EBP $\delta$  may take over for C/EBP $\beta$  in a knock out model, as a double knockout mouse model for *C/ebp $\beta$*  and *C/ebp $\delta$*  shows a further decline in adipose tissue mass (91). Moreover, C/EBP $\beta$  has been shown to work in collaboration with PPAR $\gamma$  to induce the expression of *C/ebp $\alpha$* , as PPAR $\gamma$  is required to release the inhibitory chromatin remodeler HDAC1 from the *C/ebp $\alpha$*  promoter (92). Thus, C/EBP - $\beta$  and - $\delta$  work synergistically with other members of the core differentiation circuit to execute downstream gene regulatory programs.

### Core differentiation circuit

#### *Peroxisome proliferator-activated receptor gamma*

The ligand-dependent nuclear receptor PPAR $\gamma$  expressed primarily in adipocytes is induced during adipogenesis and is central to the adipogenic regulatory network. It has two different isoforms, PPAR $\gamma$ 1 and PPAR $\gamma$ 2, of which PPAR $\gamma$ 2 is exclusively present in the adipose tissue in contrast to PPAR $\gamma$ 1, which is ubiquitously expressed. PPAR $\gamma$  functions as a regulator in adipogenic programs common to both white and brown fat differentiation, and no other factor has so far been found that can promote adipogenesis in the absence of PPAR $\gamma$ . All pro-adipogenic transcriptional regulators such as C/EBPs and some KLFs such as KLF5, KLF15 converge on one of the two *Pparg* promoters while anti-adipogenic transcriptional regulators such as the GATA factors function by repressing *Pparg* expression (93–95). Additionally, key adipogenic signaling pathways also converge on *Pparg* expression and activity. Upon activation by ligands, PPAR $\gamma$  forms a heterodimer with RXR and binds to a Ppar response element (PPRE) in cooperative fashion (96,97), enabling it to control the expression of a specific subset of PPRE-containing genes such as *Ap2*, *Fabp4* and *Lpl* in white adipocytes (98). Studies showed that stimulation of white adipocytes *in vitro* or *in vivo* by strong PPAR $\gamma$  agonists such as rosiglitazone leads to ‘browning’ of the white cells (99). The latter triggers an upregulation of mitochondrial genes such as *Ucp1*, *Cox7a1* and *Cox8b*, subsequently causing an increase in mitochondrial mass, enhanced oxygen consumption and lipid oxidation (100,101). This browning effect is characterized by a suppression of adipokine production such as Resistin, Alpha1-acidoglycoprotein and Haptoglobin (102–104). The phenotypic outcome of supplementing white adipocytes already known to express *Pparg* and its endogenous ligands

with additional PPAR $\gamma$  agonists suggests that brown adipocytes produce a distinct repertoire of PPAR $\gamma$  ligands that differ in activity from those produced in white adipocytes (105). It is likely that this regulatory mechanism is then executed via the actions of PRDM16/PGC1 $\alpha$  and PRDM16/CtBP complexes as they are known to mediate brown versus white programs.

#### *CCAAT/enhancer binding protein alpha*

C/EBP $\alpha$  plays a crucial role in differentiated adipocytes and works in concert with PPAR $\gamma$  to constitute the core differentiation circuit. The molecular function of C/EBP $\alpha$  is central to the acquisition of insulin sensitivity as opposed to accumulation of lipids or expression of adipocyte genes (106).

#### *Nuclear Factor IA*

The Nuclear Factor I (NFI) family of TFs including NFIA, NFIB, NFIC and NFIX are widely expressed, with each TF regulating a distinct set of target genes as evidenced by different phenotypes yielded by mouse knockout models (107). Previously, both NFIA and NFIB have been identified as positive regulators of white adipocyte differentiation via *in vitro* cell culture studies in the 3T3L1 cell line (108). Using an integrative genomics approach, we discovered that NFIA is also involved in the brown adipogenesis gene regulatory network (109). Notably, we observed that *Nfia* is differentially expressed during brown fat cell differentiation and has a strong positive correlation with *Ucp1* expression levels in the mouse genetic reference panel (BXD) (110) and human clonal BAT cell lines (111). Further, genome-wide localization analyses based on ChIP-seq revealed that, while NFIA does not directly bind to *Ucp1*, it does, however, bind to general adipogenic marker genes such as *Zfp423*, core adipogenic regulators such as *Pparg* and *Rxrg*, early brown specific TFs such as *Ebf2* and finally drivers of the thermogenic module such as *Ppara* and *Pgc1a*. A follow-up on the mechanistic details of NFIA action in brown versus white is necessary, especially to delineate its mode of regulatory action in these tissue types.

Apart from the core members of this differentiation circuit, additional adipogenic regulators include positive regulators such as ZEB1 (90), ASLX2 (112), Kruppel like factors (KLF)-5 (93), -15 (113) and 6 (114), STAT5 $\alpha$  (115,116), while negative regulators include GATA transcription factors (94,95), ASLX1 (112), KLF-2 (117). These transcriptional details have been reviewed extensively elsewhere (54,105).

### Thermogenic circuit

#### *Peroxisome proliferator-activated receptor alpha*

The peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ), a protein from the nuclear receptor family comprising PPAR $\gamma$ /d/b, is a fatty acid-activated TF that executes transcriptional regulation of genes involved in

cellular metabolism (118). It is particularly detected in tissues such as brown fat, liver or heart, known to have high rates of fatty acid oxidation and peroxisomal metabolism. High expression of *Ppara* distinguishes brown from white fat and is related to the lipid oxidation capacity of brown fat. It was shown that PPAR $\alpha$  activators stimulate *Ucp1* gene expression both in brown adipocytes and mature BAT, acting through a PPRE element situated in the upstream enhancer of the *Ucp1* gene that is also responsible for PPAR $\gamma$ -dependent regulation (119). PPAR $\alpha$  interacts with CBP and PGC1 $\alpha$ , forming a tight regulatory complex to activate *Ucp1* gene expression. An interesting regulatory mechanism exists between the two PPARs, PPAR $\alpha$  and PPAR $\gamma$ , during brown adipogenesis. While it was demonstrated that PPAR $\alpha$  and PPAR $\gamma$  bind and activate *Ucp1* gene transcription through the same *Ucp1*-PPRE element, the predominant molecular function of any particular subtype depends on the relative amount of each subtype. For example, crosstalk with other signaling pathways such as regulation of PPAR transcriptional activity by MAPK-dependent phosphorylation enhances PPAR $\alpha$  (120) but decreases PPAR $\gamma$  activity (121), as well as ligand availability and interaction with co-regulators such as PGC1 $\alpha$ . The interaction of PGC1 $\alpha$  with PPAR $\alpha$  is ligand dependent, whereas the one with PPAR $\gamma$  is not (122). Therefore, these and possibly other uncharacterized events may determine which PPAR subtype activates *Ucp1* transcription in response to brown adipocyte-inducing conditions. In other words, it could be PPAR $\gamma$  in association with differentiation-dependent events, or PPAR $\alpha$  in conjunction with increased lipid catabolism in active BAT, highlighting the importance of understanding this regulatory mechanism. Further, it was observed that acute administration of the PPAR $\alpha$  ligand Wy 14,643 upregulates *Ucp1* mRNA expression in those physiological situations in which endogenous PPAR $\alpha$  ligands are expected to be low. This is in agreement with previous findings demonstrating that PPAR $\alpha$  sensitivity *in vivo* depends upon the status of lipid metabolism (123).

#### *Carbohydrate response element binding protein*

Yet another interesting mechanism is the differential regulation of PPAR $\alpha$  and carbohydrate response element binding protein (ChREBP), a TF that is responsive to glucose signaling and is more highly expressed in brown compared to white fat tissue, influencing lipid storage and lipolysis in brown adipocytes. It was demonstrated that ChREBP and PPAR $\alpha$  regulate each other via an inhibitory feedback mechanism that is activated during conditions of high and low glucose, respectively (124). As such, ChREBP and PPAR $\alpha$  coordinately establish a feedback loop between lipogenesis and lipolysis in brown adipocytes. The molecular mechanism underlying this crosstalk remains largely unexplored and may serve as an avenue for further research to develop novel therapeutics.

#### *Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha*

Given that PPAR $\gamma$  alone is insufficient to induce browning, extensive investigation into other putative regulators led to the identification of PGC1 $\alpha$ . The involvement of PGC1 $\alpha$  was first demonstrated in the thermogenic circuit when a dramatic increase in *Pgc1a* expression was observed in mice exposed to cold in both skeletal and brown fat (122). PGC1 $\alpha$  is also triggered in brown fat cell lines upon treatment with the beta-adrenergic receptor agonist isoproterenol (122). It interacts with multiple nuclear hormone receptors that bind to the *Ucp1* gene enhancer in either a ligand-dependent or -independent manner as in the case of Retinoic Acid Receptor (RAR) and Thyroid Receptor (TR) or PPAR $\gamma$ , respectively. PGC1 $\alpha$  not only boosts the efficiency of transcription but also plays a key role in specifying the genes that are targeted by its partner TFs. This is illustrated by overexpression of PGC1 $\alpha$  in white fat cells leading to the induction of *Ucp1* through the PPAR $\gamma$  binding sites but a failure to express the PPAR $\gamma$  target gene *Ap2*. As PGC1 $\alpha$  is a co-activator and is dependent on the binding of particular TFs to implement different gene expression programs, repression of binding events could affect different biological responses. Thus, targeting singular binding events between PGC1 $\alpha$  and particular TFs might offer new therapeutic opportunities.

However, while PGC1 $\alpha$  is a critical regulator of adaptive thermogenesis, it is not the master regulator of brown differentiation. This is evidenced by the fact that reduction in PGC1 $\alpha$  levels during brown fat cell differentiation has no significant impact on brown adipogenesis. However, it has a strong impact on the induction of thermogenic genes by cAMP (105). Apart from its molecular involvement in regulating thermogenesis, it also aids in inducing angiogenesis via the co-activation of the orphan nuclear receptor ERRA on the *Vegf* promoter. PGC1 $\alpha$  was stimulated by an inadequacy in nutrients as well as oxygen leading to increased *Vegf* expression and subsequently angiogenesis in BAT and skeletal muscle *in vivo* (125). Thus, the transcriptional functions of PGC1 $\alpha$  are key to the development and thermogenic activity of brown adipocytes.

#### *Forkhead box C2*

Forkhead box protein C2 (FOXC2), a member of the Forkhead box family of TFs, regulates different aspects of adipocyte metabolism by activating PPAR $\gamma$  and C/EBP $\alpha$  as well as by enhancing cAMP-mediated PKA signaling (126). Most importantly, FOXC2 responds strongly to high caloric load via strong induction of cAMP signaling as demonstrated by high fat feeding experiments in which FOXC2-overexpressing mice showed reduced weight gain in response to the amount of food consumed. This categorizes FOXC2 as a putative therapeutic target based on its molecular function as a metabolic regulator and a factor that can induce adaptive thermogenesis (126). Additionally,

enhanced expression of *Foxc2* has also been implicated in mitochondrial biogenesis via trans-activation of the nuclear encoded mitochondrial transcription factor A as well as induction of mitochondrial fusion via activation of Mitofusin 1 and 2, and Optic atrophy 1 homolog (127). This correlation was observed in humans, making FOXC2 an attractive target for therapy. *Foxc2* expression was also shown to increase in response to high caloric feeding, whilst repressed by cold (127). This suggests that FOXC2 has a distinct role in regulating mitochondrial function from that observed in cold-adapted thermogenesis, known as metabolo-regulatory thermogenesis. This is different from thermo-regulatory thermogenesis, which is categorically induced by cold and is dependent on expression of *Pgc1a* and *Ucp1* (8).

In addition to the key factors described above, new factors have recently been implicated in early brown adipogenesis. These include the Kruppel like factor 11 that binds to PPAR $\gamma$  and activates brown fat genes in rosiglitazone-treated human adipocytes (128) as well as the EWS/YBX1/BMP7 axis, in which the RNA-binding protein EWS along with its partner YBX1 transcriptionally activates *Bmp7* to induce browning (129). Additionally, new factors implicated in the thermogenic program are Zinc-finger protein 516 (ZFP516) that is involved in the activation of *Ucp1* gene expression by interacting with PRDM16 (130) and interferon regulatory factor-4 (IRF4), an important TF partner of PGC1a that both induces and interacts with PGC1a to activate thermogenic genes (131). A new regulatory layer comprising small non-coding RNAs, miRNAs such as miR-193b-365, miR-133, miR-196a, miR-155, miR-27, miR-378, miR-34a along with BAT-specific long-noncoding RNAs such as *lnc-BATE1* and *Blnc1* is also known to promote brown and/or beige adipocyte differentiation and physiological function (132).

### Anti-adipogenic regulators affecting the PGC1a–UCP1 axis

Given the central function of PGC1a and UCP1 in the thermogenic program, their expression and activity are tightly regulated by a multitude of factors that serve as negative regulators. This mode of negative regulation most likely functions to restrain thermogenesis, whose prolonged activation may lead to adverse conditions such as hyperthermia, which in turn has the potential to cause organ failure and death (133).

#### *Nuclear receptor interacting protein 140*

Nuclear receptor (NR) interaction protein 140 (Rip140) is a ligand-dependent transcriptional repressor of nuclear receptors including estrogen receptor and PPARs and also functions as a transcriptional co-repressor that shares a number of downstream targets with PGC1a and inhibits PGC1a's transcriptional activity by binding to it directly

(134). Along with suppressing PGC1a target genes, Rip140 also represses *Ucp1* expression by recruiting inhibitory histone modifying and DNA methylation enzymes to the promoter of *Ucp1* (135). Lack of Rip140 increases *Ucp1* expression by de-repression of PPAR $\alpha$ , PPAR $\gamma$  and PGC1a. Studies suggest that, given Rip140's high expression in WAT compared to BAT, it functions to suppress the brown phenotype. This is evidenced by significant enhancement of brown-related genes upon knockdown of *Rip140* in white fat cells (136). Further, observations from *in vivo* studies demonstrated that *Rip140* null mice exhibit expenditure rather than storage of the consumed fat (137). However, in-depth investigation into the molecular mechanisms underlying Rip140-mediated 'browning' suppression is pending.

#### *Liver X receptor alpha*

The Liver X receptor alpha (LXR $\alpha$ ) does not directly interfere with PGC1a's transcriptional activity or binding, rather, when ligand-activated, this TF interferes with the induction of *Ucp1* expression by displacing PPAR $\gamma$  from the *Ucp1* enhancer and by drafting RIP140 as a corepressor to its binding site (133). It has also been demonstrated that mice lacking LXR $\alpha$  show increased *Ucp1* expression in both BAT and WAT with no significant change in the expression of *Pgc1a*, thus manifesting a lean phenotype (133,138).

#### *Retinoblastoma protein*

pRb acts as a molecular toggle between the white and brown adipocyte differentiation programs (139) by direct binding to the *Pgc1a* promoter leading to transcriptional repression (140). Additionally, pRb functions as a PPAR $\gamma$  co-repressor, thereby suppressing its transcriptional activity (141).

#### *Twist basic helix–loop–helix TF 1*

Twist basic helix–loop–helix TF 1 (TWIST1) functions as an antagonist of brown fat-mediated thermogenesis by binding directly to PGC1a and suppressing its transcriptional activity. This is executed by Twist1-mediated recruitment of the histone deacetylase HDAC5 to the promoters of PGC1a target genes, causing transcriptional repression (142).

Other key anti-adipogenic factors suppressing the PGC1a and UCP1 axis include unliganded Vitamin D receptor that directly suppresses *Ucp1* expression via binding to a Vitamin D receptor element in the proximal region of the *Ucp1* promoter (133), steroid receptor co-activators such as SRC2 and SRC3 that repress the transcriptional activity of PGC1a, TRPV4 (a transient receptor potential cation channel 4) that represses PGC1a via the ERK1/2 protein kinases (143) and SHP (orphan nuclear receptor) that inhibits the ERR-g mediated promoter transactivation of PGC1a (133).



### Outlook

While the white adipocyte differentiation network is well characterized, the brown adipogenic signaling and gene regulatory network remains rather sparse, warranting further investigation to annotate missing nodes and interactions. The latter include additional regulators that are capable of inducing browning in concert with key molecular players, thereby providing alternative points of safe therapeutic intervention. Most notably, through a comprehensive literature-based reconstruction of the brown signaling and gene regulatory network, we illustrate the requirement to focus on delineating implicated signaling pathways and on how they converge on the transcriptional components. This will enable a more thorough understanding of key molecular events and guide better drug discovery efforts. An additional focus should be on elucidating mechanisms that govern the white to brown switch, aiding in the development of drugs that can safely and specifically mediate browning.

Recent studies have also elucidated alternative modes of thermogenesis that function without the need for UCP1 activation, wherein beige and white adipocytes can directly respond to cool temperature (27–33°C) and activate a thermogenic program in a cell-autonomous manner. This particular type of thermogenic program was determined to be independent of the canonical cAMP/protein kinase/CREB pathway downstream of beta-adrenergic signaling (144). Furthermore, in a study conducted on human subjects, it was observed that elevated thermogenesis upon cold exposure is not inhibited by the chemical blockade of beta-adrenergic signaling alone (145). This suggests the existence of alternative pathways for induction of a thermogenic program through a cell-autonomous effect of temperature on certain fat depots. Recently, a futile cycle of creatine metabolism has been identified as an alternative pathway for energy expenditure and thermal homeostasis (146). The implicated proteins Creatine Kinase Mitochondrial 1 and 2 (CKMT1 and CKMT2) were enriched in human BAT, raising the possibility of exploiting this pathway in manipulating energy consumption in patients with metabolic diseases, particularly via dietary supplementation and/or administration of novel drugs.

One of the main challenges in the field of translational research is the design of safe therapeutic interventions. Several treatment options targeting BAT are being explored, but more often than not, they suffer from potential systemic side effects. We believe that a systems perspective summarizing various input stimuli, downstream signaling and transcriptional components will provide a strong framework to aid in the development of targeted drug design. Furthermore, the field's focus should not only be on identifying specific druggable pathways but also strategies for drug delivery such as the development of controllable microenvironments, scaffolds, biomaterials for transplantation of

energy expending cells as well as focused pharmacological intervention to stimulate the activity of resident progenitor cells *in situ* by triggering endogenous brown adipogenesis.

### Acknowledgements

This work was supported by SNSF grant (31003A\_162735) and by Institutional Support by the Swiss Federal Institute of Technology in Lausanne (EPFL). The authors would like to thank Dr. Petra C. Schwalie, Dr. Wanze Chen, Dr. Daniil Alpern and Dr. Vincent Gardeux for constructive discussions.

### Conflict of interest statement

All authors declare no conflict of interest.

### References

1. Cohen P, Spiegelman BM. Brown and beige fat: molecular parts of a thermogenic machine. *Diabetes* 2015; **64**: 2346–2351.
2. Klaus S. Adipose tissue as a regulator of energy balance. *Curr. Drug Targets* 2004; **5**(3): 241–250.
3. Spiegelman BM, Flier JS. Adipogenesis and obesity: rounding out the big picture. *Cell* 1996; **87**(3): 377–389.
4. Guerre-Millo M. Adipose tissue and adipokines: for better or worse. *Diabetes Metab.* 2004; **30**(1): 13–19.
5. Wang F, Mullican SE, DiSpirito JR, Peed LC, Lazar MA. Lipatrophy and severe metabolic disturbance in mice with fat-specific deletion of PPAR $\gamma$ . *Proc. Natl. Acad. Sci. U. S. A.* 2013; **110**(46): 18656–18661.
6. Poehlman ET, Melby CL, Goran MI. The impact of exercise and diet restriction on daily energy expenditure. *Sport. Med* 1991; **11**(2): 78–101.
7. Virtanen KA, Lidell ME, Orava J *et al.* Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 2009; **360**(15): 1518–1525.
8. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004; **277**–359.
9. Vitali A, Murano I, Zingaretti MC *et al.* The adipose organ of obesity-prone C57BL/6 J mice is composed of mixed white and brown adipocytes. *J. Lipid Res.* 2012; **53**(4): 619–629.
10. Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. *Nat. Med.* 2013; **19**(10): 1252–1263.
11. Sidossis L, Kajimura S. Brown and beige fat in humans: thermogenic adipocytes that control energy and glucose homeostasis. *J. Clin. Invest.* 2015; **125**(2): 478–486.
12. Kajimura S, Spiegelman BM, Seale P. Brown and beige fat: physiological roles beyond heat generation. *Cell Metab.* 2015; **22**(4): 546–559.
13. Seale P, Bjork B, Yang W *et al.* PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 2008; **454**(7207): 961–967.
14. Wu J, Boström P, Sparks LM *et al.* Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 2012; **150**(2): 366–376.
15. Shinoda K, Luijten IHN, Hasegawa Y *et al.* Genetic and functional characterization of clonally derived adult human brown adipocytes. *Nat. Med.* 2015; **21**(4): 389–394.
16. Lidell ME, Betz MJ, Enerbäck S. Two types of brown adipose tissue in humans. *Adipocyte* 2014; **3**(1): 63–66.

17. Aquila H, Link TA, Klingenberg M. The uncoupling protein from brown fat mitochondria is related to the mitochondrial ADP/ATP carrier. Analysis of sequence homologies and of folding of the protein in the membrane. *EMBO J.* 1985; **4**(9): 2369–2376.
18. Bouillaud F, Weissenbach J, Ricquier D. Complete cDNA-derived amino acid sequence of rat brown fat uncoupling protein. *J. Biol. Chem.* 1986; **261**(4): 1487–1490.
19. Heaton GM, Wagenvoort RJ, Kemp A, Nicholls DG. Brown-adipose-tissue mitochondria: photoaffinity labelling of the regulatory site of energy dissipation. *Eur. J. Biochem.* 1978; **82**(2): 515–521.
20. Ridley RG, Patel HV, Gerber GE, Morton RC, Freeman KB. Complete nucleotide and derived amino acid sequence of cDNA encoding the mitochondrial uncoupling protein of rat brown adipose tissue: lack of a mitochondrial targeting presequence. *Nucleic Acids Res* 1986; **14**(10): 4025–4035.
21. Enerbäck S, Jacobsson A, Simpson EM *et al.* Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 1997; **387**(6628): 90–94.
22. Nicholls DG. The physiological regulation of uncoupling proteins. *Biochim. Biophys. Acta* 2006; **1757**(5–6): 459–466.
23. Fedorenko A, Lishko PV, Kirichok Y. Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. *Cell* 2012; **151**(2): 400–413.
24. Saito M, Okamatsu-ogura Y, Matsushita M *et al.* High incidence of metabolically active brown adipose effects of cold exposure and adiposity. *Diabetes* 2009; **58** (JULY): 1526–1531.
25. Yoneshiro T, Aita S, Matsushita M *et al.* Recruited brown adipose tissue as an antiobesity agent in humans. *J. Clin. Invest.* 2013; **123**(8): 3404–3408.
26. Cypess AM, Lehman S, Williams G *et al.* Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 2009; **360**(15): 1509–1517.
27. Yoneshiro T, Matsushita M, Nakae S *et al.* Brown adipose tissue is involved in the seasonal variation of cold-induced thermogenesis in humans. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* 2016; **310**: R999–R1009.
28. Stampfer MJ, Hu FB, Manson JE, Rimm EB, Willett WC. Primary prevention of coronary heart disease in women through diet and lifestyle. *N. Engl. J. Med.* 2000; **343**(1): 16–22.
29. Hu FB, Manson JE, Mj S *et al.* Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N. Engl. J. Med.* 2001; **345**(11): 790–797.
30. Boström P, Wu J, Jedrychowski MP *et al.* A PGC1- $\alpha$ -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012; **481**(7382): 463–468.
31. Jedrychowski MP, Wrann CD, Paulo JA *et al.* Detection and quantitation of circulating human irisin by tandem mass spectrometry. *Cell Metab.* 2015; **22**(4): 734–740.
32. Rao RR, Long JZ, White JP, *et al.* Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. *NIH public access.* 2015; **157**(6): 1279–1291.
33. Knudsen JG, Murholm M, Carey AL *et al.* Role of IL-6 in exercise training- and cold-induced UCP1 expression in subcutaneous white adipose tissue. *PLoS One* 2014; **9**(1).
34. Carrière A, Jeanson Y, Berger-Müller S *et al.* Browning of white adipose cells by intermediate metabolites: an adaptive mechanism to alleviate redox pressure. *Diabetes* 2014; **63**(10): 3253–3265.
35. Roberts LD, Boström P, O'Sullivan JF *et al.*  $\beta$ -Aminoisobutyric acid induces browning of white fat and hepatic  $\beta$ -oxidation and is inversely correlated with cardiometabolic risk factors. *Cell Metab.* 2014; **19**(1): 96–108.
36. Saito M, Yoneshiro T. Capsinoids and related food ingredients activating brown fat thermogenesis and reducing body fat in humans. *Curr. Opin. Lipidol.* 2013; **24**(1): 71–77.
37. Dulloo A, Seydoux J, Girardier L, Chantre P, Vandermander J. Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. *Int. J. Obes.* 2000; **24**: 252–258.
38. Nammi S, Sreemantula S, Roufogalis BD. Protective effects of ethanolic extract of *Zingiber officinale* rhizome on the development of metabolic syndrome in high-fat diet-fed rats. *Basic Clin Pharmacol Toxicol.* 2009; 366–373.
39. Mansour MS, Ni YM, Roberts AL *et al.* Ginger consumption enhances the thermic effect of food and promotes feelings of satiety without affecting metabolic and hormonal parameters in overweight men: a pilot study. *Metabolism.* 2012; **61**(10): 1347–1352.
40. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM *et al.* Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 2009; **360**(15): 1500–1508.
41. Muzik O, Mangner TJ, Granneman JG. Assessment of oxidative metabolism in brown fat using PET imaging. *Front. Endocrinol. (Lausanne)* 2012; **3**: 15.
42. Orava J, Nuutila P, Noponen T *et al.* Blunted metabolic responses to cold and insulin stimulation in brown adipose tissue of obese humans. *Obesity* 2013; **21**(11): 2279–2287.
43. Foster DO, Frydman ML. Nonshivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorogenesis induced by noradrenaline. *Can. J. Physiol. Pharmacol.* 1978; **56**(1): 110–122.
44. Foster DO, Depocas F, Frydman ML. Noradrenaline-induced calorogenesis in warm- and cold-acclimated rats: relations between concentration of noradrenaline in arterial plasma, blood flow to differently located masses of brown adipose tissue, and calorogenic response. *Can. J. Physiol. Pharmacol.* 1980; **58**(8): 915–924.
45. Muzik O, Mangner T. 15O PET measurement of blood flow and oxygen consumption in cold-activated human brown fat. *J. Nucl. Med.* 2013; **54**(4): 523–531.
46. Ramseyer VD, Granneman JG. Adrenergic regulation of cellular plasticity in brown, beige/brite and white adipose tissues. *Adipocyte* 2016; **0**(0): 1–11.
47. Perry RJ, Zhang D, Zhang X-M, Boyer JL, Shulman GI. Controlled-release mitochondrial protonophore reverses diabetes and steatohepatitis in rats. *Science* 2015; **347**(6227): 1253–1256.
48. Weyer C, Tataranni PA, Snitker S, Danforth E, Ravussin E. Increase in insulin action and fat oxidation after treatment with CL 316,243, a highly selective beta3-adrenoceptor agonist in humans. *Diabetes* 1998; **47**(10): 1555–1561.
49. Cypess AM, Weiner LS, Roberts-Toler C *et al.* Activation of human brown adipose tissue by a  $\beta$ 3-adrenergic receptor agonist. *Cell Metab.* 2015; **21**(1): 33–38.
50. Betz MJ, Enerbäck S. Human brown adipose tissue: what we have learned so far. *Diabetes* 2015; **64**(7): 2352–2360.
51. Bordicchia M, Liu D, Amri E *et al.* Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin Invest* 2012; **122**(3): 1022–1036.
52. Sellayah D, Bhara P, Sikder D. Orexin is required for brown adipose tissue development, differentiation, and function. *Cell Metab.* 2011; **14**(4): 478–490.
53. Owen BM, Mangelsdorf DJ, Kliewer SA. Tissue-specific actions of the metabolic hormones FGF15/19 and FGF21. *Trends Endocrinol. Metab.* 2015; **26**(1): 22–29.

54. Rosen ED, MacDougald O. a. Adipocyte differentiation from the inside out. *Nat. Rev. Mol. Cell Biol.* 2006; 7(12): 885–896.
55. Granneman JG, Li P, Zhu Z, Lu Y. Metabolic and cellular plasticity in white adipose tissue I: effects of beta3-adrenergic receptor activation. *Am. J. Physiol. Endocrinol. Metab.* 2005; 289(4): E608–E616.
56. Arch JRS, Wilson S. beta3-Adrenoceptors and the regulation of metabolism in adipose tissues. *Biochem Soc Trans* 1994; 24: 574–579.
57. Martinez De Mena R, Scanlan TS, Obregon MJ. The T3 receptor  $\beta 1$  isoform regulates UCP1 and D2 deiodinase in rat brown adipocytes. *Endocrinology* 2010; 151(10): 5074–5083.
58. Bianco AC, Sheng X, Silva JE. Triiodothyronine amplifies nor-epinephrine stimulation of uncoupling protein gene transcription by a mechanism not requiring protein synthesis. *J. Biol. Chem.* 1988; 263(34): 18168–18175.
59. Guerra C, Roncero C, Porras A, Fernandez M, Benito M. Triiodothyronine induces the transcription of the uncoupling protein gene and stabilizes its mRNA in fetal rat brown adipocyte primary cultures. *J. Biol. Chem.* 1996; 271(4): 2076–2081.
60. Obregon M. Metabolic Effects of Thyroid Hormones. *Thyroid* 2008; 18(2): 185–195.
61. Buhl AM, Johnson NL, Dhanasekaran N, Johnson GL. G alpha 12 and G alpha 13 stimulate Rho-dependent stress fiber formation and focal adhesion assembly. *J. Biol. Chem.* 1995; 270(42): 24631–24634.
62. Wang Y, Falting JM, Mattsson CL, Holmstrom TE, Nedergaard J. In brown adipocytes, adrenergically induced beta(1)-/beta(3)-(Gs)-, alpha(2)-(Gi)- and alpha(1)-(Gq)-signalling to Erk1/2 activation is not mediated via EGF receptor transactivation. *Exp Cell Res* 2013; 319(17): 2718–2727.
63. Sbarbati A, Morroni M, Zancanaro C, Cinti S. Rat interscapular brown adipose tissue at different ages: a morphometric study. *Int. J. Obes.* 1991; 15(9): 581–587.
64. Cinti S, Frederich RC, Zingaretti MC *et al.* Immunohistochemical localization of leptin and uncoupling protein in white and brown adipose tissue. *Endocrinology* 1997; 138(2): 797–804.
65. Himms-Hagen J. Defective brown adipose tissue thermogenesis in obese mice. *Int J Obes* 1985; 9(Suppl 2): 17–24.
66. Klepac K, Kilić A, Gnad T *et al.* The Gq signalling pathway inhibits brown and beige adipose tissue. *Nat. Commun.* 2016; 7: 10895.
67. Kang S, Bajnok L, Longo KA *et al.* Effects of Wnt signaling on brown adipocyte differentiation and metabolism mediated by PGC-1alpha. *Mol. Cell. Biol.* 2005; 25(4): 1272–1282.
68. Cawthorn WP, Bree AJ, Yao Y *et al.* Wnt6, Wnt10a and Wnt10b inhibit adipogenesis and stimulate osteoblastogenesis through a  $\beta$ -catenin-dependent mechanism. *Bone* 2012; 50(2): 477–489.
69. van Tienen FH, Laeremans H, van der Kallen CJ, Smeets HJ. Wnt5b stimulates adipogenesis by activating PPARgamma, and inhibiting the beta-catenin dependent Wnt signaling pathway together with Wnt5a. *Biochem Biophys Res Commun* 2009; 387(1): 207–211.
70. Suh JM, Gao X, McKay J *et al.* Hedgehog signaling plays a conserved role in inhibiting fat formation. *Cell Metab.* 2006; 3(1): 25–34.
71. Pospisilik JA, Schramek D, Schnidar H *et al.* Drosophila genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. *Cell* 2010; 140(1): 148–160.
72. Nosavanh L, Yu D-H, Jaehnig EJ *et al.* Cell-autonomous activation of Hedgehog signaling inhibits brown adipose tissue development. *Proc. Natl. Acad. Sci. U. S. A.* 2015; 112(16): 5069–5074.
73. Moisan A, Lee Y-K, Zhang JD *et al.* White-to-brown metabolic conversion of human adipocytes by JAK inhibition. *Nat. Cell Biol.* 2014; 17(1): 57–67.
74. Yadav H, Quijano C, Kamaraju AK *et al.* Protection from obesity and diabetes by blockade of TGF- $\beta$ /Smad3 signaling. *Cell Metab.* 2011; 14(1): 67–79.
75. Koncarevic A, Kajimura S, Cornwall-Brady M *et al.* A novel therapeutic approach to treating obesity through modulation of TGF $\beta$  signaling. *Endocrinology* 2012; 153(7): 3133–3146.
76. Tseng Y, Kokkotou E, Schulz T *et al.* New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 2008; 454(7207): 1000–1004.
77. Cao W, Daniel KW, Robidoux J *et al.* p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. *Mol Cell Biol* 2004; 24(7): 3057–3067.
78. Schulz TJ, Huang TL, Tran TT *et al.* Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc. Natl. Acad. Sci. U. S. A.* 2011; 108(1): 143–148.
79. Zhou G, Myers R, Li Y *et al.* Role of AMP-activated protein kinase in mechanism of metformin action. *J. Clin. Invest.* 2001; 108(8): 1167–1174.
80. Schroeter EH, Kisslinger JA, Kopan R. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature* 1998; 393(6683): 382–386.
81. Bi P, Shan T, Liu W *et al.* Inhibition of Notch signaling promotes browning of white adipose tissue and ameliorates obesity. *Nat. Med.* 2014; 20(8): 911–918.
82. Gupta RK, Arany Z, Seale P *et al.* Transcriptional control of preadipocyte determination by Zfp423. *Nature* 2010; 464(7288): 619–623.
83. Kang S, Akerblad P, Kiviranta R *et al.* Regulation of early adipose commitment by Zfp521. *PLoS Biol.* 2012; 10(11): e1001433.
84. Jimenez MA, Akerblad P, Sigvardsson M, Rosen ED. Critical role for Ebf1 and Ebf2 in the adipogenic transcriptional cascade. *Mol. Cell. Biol.* 2006; 27(2): 743–757.
85. Rajakumari S, Wu J, Ishibashi J *et al.* EBF2 determines and maintains brown adipocyte identity. *Cell Metab.* 2013; 17(4): 562–574.
86. Harms MJ, Ishibashi J, Wang W *et al.* Prdm16 is required for the maintenance of brown adipocyte identity and function in adult mice. *Cell Metab.* 2014; 19(4): 593–604.
87. Kajimura S, Seale P, Tomaru T *et al.* Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. *Genes Dev.* 2008; 22(10): 1397–1409.
88. Jimenez-Preitner M, Berney X, Uldry M *et al.* Plac8 is an inducer of C/EBP $\beta$  required for brown fat differentiation, thermoregulation, and control of body weight. *Cell Metab.* 2011; 14(5): 658–670.
89. Raghav SK, Waszak SM, Krier I *et al.* Integrative genomics identifies the corepressor SMRT as a gatekeeper of adipogenesis through the transcription factors C/EBP $\beta$  and KAISO. *Mol. Cell* 2012; 46(3): 335–350.
90. Gubelmann C, Schwalie PC, Raghav SK *et al.* Identification of the transcription factor ZEB1 as a central component of the adipogenic gene regulatory network. *Elife* 2014; 3: e03346.
91. Tanaka T, Yoshida N, Kishimoto T, Akira S. Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene. *EMBO J.* 1997; 16(24): 7432–7443.
92. Zuo Y, Qiang L, Farmer SR. Activation of CCAAT/enhancer-binding protein (C/EBP) alpha expression by C/EBP beta during adipogenesis requires a peroxisome proliferator-activated



- receptor-gamma-associated repression of HDAC1 at the *C/ebp alpha* gene promoter. *J. Biol. Chem.* 2006; **281**(12): 7960–7967.
93. Oishi Y, Manabe I, Tobe K *et al.* Krüppel-like transcription factor KLF5 is a key regulator of adipocyte differentiation. *Cell Metab.* 2005; **1**(1): 27–39.
94. Tong Q, Dalgin G, Xu H *et al.* Function of GATA transcription factors in preadipocyte-adipocyte transition. *Science* 2000; **290**(5489): 134–138.
95. Tong Q, Tsai J, Tan G, Dalgin G, Hotamisligil GS. Interaction between GATA and the C/EBP family of transcription factors is critical in GATA-mediated suppression of adipocyte differentiation. *Mol. Cell. Biol.* 2005; **25**(2): 706–715.
96. Isakova A, Berset Y, Hatzimanikatis V, Deplancke B. Quantification of Cooperativity in heterodimer-DNA binding improves the accuracy of binding specificity models. *J. Biol. Chem.* 2016; **291**(19): 10293–10306.
97. Willson TM, Lambert MH, Kliewer SA. Peroxisome proliferator-activated receptor gamma and metabolic disease. *Annu. Rev. Biochem.* 2001; **70**: 341–367.
98. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* 1994; **79**(7): 1147–1156.
99. Ohno H, Shinoda K, Spiegelman BM, Kajimura S. PPAR $\gamma$  agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein. *Cell Metab.* 2012; **15**(3): 395–404.
100. Wilson-fritch L, Burkart A, Bell G *et al.* Mitochondrial biogenesis and remodeling during adipogenesis and in response to the insulin sensitizer rosiglitazone mitochondrial biogenesis and remodeling during adipogenesis and in response to the insulin sensitizer rosiglitazone. *Mol. Cell. Biol.* 2003; **23**(3): 1085–1094.
101. Wilson-Fritch L, Nicoloso S, Chouinard M *et al.* Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. *J. Clin. Invest.* 2004; **114**(9): 1281–1289.
102. Stepan CM, Bailey ST, Bhat S *et al.* The hormone resistin links obesity to diabetes. *Nature* 2001; **409**: 307–312.
103. Do Nascimento CO, Hunter L, Trayhurn P. Regulation of haptoglobin gene expression in 3T3-L1 adipocytes by cytokines, catecholamines, and PPAR $\gamma$ . *Biochem. Biophys. Res. Commun.* 2004; **313**(3): 702–708.
104. Castriota G, Thompson GM, Lin Y *et al.* Peroxisome proliferator-activated receptor gamma agonists inhibit adipocyte expression of alpha1-acid glycoprotein. *Cell Biol. Int.* 2007; **31**(6): 586–591.
105. Farmer SR. Molecular determinants of brown adipocyte formation and function. *Genes Dev.* 2008; **22**(10): 1269–1275.
106. Rosen ED, Hsu C, Wang X *et al.* C/EBP $\alpha$  induces adipogenesis through PPAR $\gamma$ : a unified pathway. *Genes Dev.* 2002; **22**: 22–26.
107. Gronostajski RM. Roles of the NFI/CTF gene family in transcription and development. *Gene* 2000; **249**: 31–45.
108. Waki H, Nakamura M, Yamauchi T, Wakabayashi K, Yu J. Global mapping of cell type-specific open chromatin by FAIRE-seq reveals the regulatory role of the NFI family in adipocyte differentiation. *PLoS Genetics* 2011; **7**(10): e1002311.
109. Pradhan RN, Bues Johannes, Gardeux V, Schwalie PC, Alpern D, Chen W, Ruesseil J, Raghav SK, Deplancke B. Dissecting the brown adipogenic regulatory network using integrative genomics. *Scientific Reports* 2016.
110. Williams EG, Mouchiroud L, Frochoux M *et al.* An evolutionarily conserved role for the aryl hydrocarbon receptor in the regulation of movement. *PLoS Genetic* 2014; **10**(9): e1004673.
111. Xue R, Lynes MD, Dreyfuss JM *et al.* Clonal analyses and gene profiling identify genetic biomarkers of human brown and white thermogenic potential. *HHS Public Access* 2016; **21**(7): 760–768.
112. Park UH, Yoon SK, Park T, Kim EJ, Um SJ. Additional sex comb-like (ASXL) proteins 1 and 2 play opposite roles in adipogenesis via reciprocal regulation of peroxisome proliferator-activated receptor  $\gamma$ . *J. Biol. Chem.* 2011; **286**(2): 1354–1363.
113. Mori T, Sakaue H, Iguchi H *et al.* Role of krüppel-like factor 15 (KLF15) in transcriptional regulation of adipogenesis. *J. Biol. Chem.* 2005; **280**(13): 12867–12875.
114. Li D, Yea S, Li S *et al.* Krüppel-like factor-6 promotes preadipocyte differentiation through histone deacetylase 3-dependent repression of DLK1. *J. Biol. Chem.* 2005; **280**(29): 26941–26952.
115. Floyd ZE, Stephens JM. STAT5A promotes adipogenesis in nonprecursor cells and associates with the glucocorticoid receptor during adipocyte differentiation. *Diabetes* 2003; **52**(2): 308–314.
116. Nanbu-Wakao R, Morikawa Y, Matsumura I *et al.* Stimulation of 3 T3-L1 adipogenesis by signal transducer and activator of transcription 5. *Mol. Endocrinol.* 2002; **16**(7): 1565–1576.
117. Wu J, Srinivasan SV, Neumann JC, Lingrel JB. The KLF2 transcription factor does not affect the formation of preadipocytes but inhibits their differentiation into adipocytes. *Biochemistry* 2005; **44**(33): 11098–11105.
118. Tyagi S, Gupta P, Saini AS, Kaushal C, Sharma S. The peroxisome proliferator-activated receptor: a family of nuclear receptors role in various diseases. *J. Adv. Pharm. Technol. Res.* 2011; **2**: 236–240.
119. Barberá MJ, Schlüter A, Pedraza N *et al.* Peroxisome proliferator-activated receptor  $\alpha$  activates transcription of the brown fat uncoupling protein-1 gene. A link between regulation of the thermogenic and lipid oxidation pathways in the brown fat cell. *J. Biol. Chem.* 2001; **276**(2): 1486–1493.
120. Valmaseda A, Carmona MC, Barberá MJ *et al.* Opposite regulation of PPAR- $\alpha$  and - $\gamma$  gene expression by both their ligands and retinoic acid in brown adipocytes. *Mol. Cell. Endocrinol.* 1999; **154**(1–2): 101–109.
121. Hu E, Kim JB, Sarraf P, Spiegelman BM. Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPAR $\gamma$ . *Science* 1996; **274**(5295): 2100–2103.
122. Puigserver P, Wu Z, Park CW *et al.* A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 1998; **92**(6): 829–839.
123. Pedraza N, Solanes G, Carmona MC *et al.* Impaired expression of the uncoupling protein-3 gene in skeletal muscle during lactation: fibrates and troglitazone reverse lactation-induced down-regulation of the uncoupling protein-3 gene. *Diabetes* 2000; **49**(7): 1224–1230.
124. Iizuka K, Wu W, Horikawa Y, Saito M, Takeda J. Feedback looping between ChREBP and PPAR $\alpha$  in the regulation of lipid metabolism in brown adipose tissues. *Endocr. J.* 2013; **60**(10): 1145–1153.
125. Arany Z, Foo S-Y, Ma Y *et al.* HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1 $\alpha$ . *Nature* 2008; **451**(7181): 1008–1012.
126. Cederberg A, Grønning LM, Ahre B, Enerba S. FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* 2001; **106**: 563–573.
127. Lidell ME, Seifert EL, Westergren R *et al.* The adipocyte-expressed forkhead transcription factor Foxc2 regulates metabolism through altered mitochondrial function. *Diabetes* 2011; **60**(2): 427–435.
128. Loft A, Schmidt SF, Mandrup S. Modulating the genomic programming of adipocytes. *Cold Spring Harb. Symp. Quant. Biol.* 2015; **80**: 239–248.



129. Park J, Kang H, Kang S *et al.* A multifunctional protein, EWS, is essential for early brown fat lineage determination. *Dev. Cell* 2013; **26**(4): 393–404.
130. Dempersmier J, Sambeat A, Gulyaeva O *et al.* Cold-inducible Zfp516 activates UCP1 transcription to promote browning of white fat and development of brown fat. *Mol. Cell* 2015; **57**(2): 235–246.
131. Kong X, Banks A, Liu T *et al.* IRF4 is a key thermogenic transcriptional partner of PGC-1 $\alpha$ . *Cell* 2014; **158**(1): 69–83.
132. Xu S, Chen P, Sun L. Regulatory networks of non-coding RNAs in brown/beige adipogenesis. *Biosci. Rep.* 2015; **35**(5): e00262.
133. Sharma BK, Patil M, Satyanarayana A. Negative regulators of brown adipose tissue (BAT)-mediated thermogenesis. *J. Cell. Physiol.* 2014; **229**(12): 1901–1907.
134. Hallberg M, Morganstein DL, Kiskinis E *et al.* A functional interaction between RIP140 and PGC-1 $\alpha$  regulates the expression of the lipid droplet protein CIDEA. *Mol. Cell. Biol.* 2008; **28**(22): 6785–6795.
135. Kiskinis E, Hallberg M, Christian M *et al.* RIP140 directs histone and DNA methylation to silence Ucp1 expression in white adipocytes. *EMBO J.* 2007; **26**(23): 4831–4840.
136. Powelka AM, Seth A, Virbasius JV *et al.* Suppression of oxidative metabolism and mitochondrial biogenesis by the transcriptional corepressor RIP140 in mouse adipocytes. *J. Clin. Invest.* 2006; **116**(1): 125–136.
137. Leonardsson G, Steel JH, Christian M *et al.* Nuclear receptor corepressor RIP140 regulates fat accumulation. *Proc. Natl. Acad. Sci. U. S. A.* 2004; **101**(22): 8437–8442.
138. Wang H, Zhang Y, Yehuda-Shnaidman E *et al.* Liver X receptor [alpha] is a transcriptional repressor of the uncoupling protein 1 gene and the brown fat phenotype. *Mol. Cell. Biol.* 2008; **28**(7): 2187–2200.
139. Hansen JB, Jørgensen C, Petersen RK *et al.* Retinoblastoma protein functions as a molecular switch determining white versus brown adipocyte differentiation. *Proc. Natl. Acad. Sci. U. S. A.* 2004; **101**(12): 4112–4117.
140. Scimè A, Grenier G, Huh MS *et al.* Rb and p107 regulate preadipocyte differentiation into white versus brown fat through repression of PGC-1 $\alpha$ . *Cell Metab.* 2005; **2**(5): 283–295.
141. Fajas L, Egler V, Reiter R *et al.* The retinoblastoma-histone deacetylase 3 complex inhibits PPAR $\gamma$  and adipocyte differentiation. *Dev. Cell* 2002; **3**: 903–910.
142. Pan D, Fujimoto M, Lopes A, Wang Y-X. Twist-1 is a PPAR $\delta$ -inducible, negative-feedback regulator of PGC-1 $\alpha$  in brown fat metabolism. *Cell* 2009; **137**(1): 73–86.
143. Ye L, Kleiner S, Wu J *et al.* TRPV4 is a regulator of adipose oxidative metabolism, inflammation, and energy homeostasis. *Cell* 2012; **151**(1): 96–110.
144. Ye L, Wu J, Cohen P *et al.* Fat cells directly sense temperature to activate thermogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 2013; **110**(30): 12480–12485.
145. Wijers SLJ, Saris WHM, Van Marken Lichtenbelt WD. Individual thermogenic responses to mild cold and overfeeding are closely related. *J. Clin. Endocrinol. Metab.* 2007; **92**(11): 4299–4305.
146. Kazak L, Chouchani ET, Jedrychowski MP *et al.* A creatine-driven substrate cycle enhances energy expenditure and thermogenesis in beige fat. *Cell* 2015; **163**(3): 643–655.