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Transcriptional and epigenetic control of brown and beige adipose cell fate and function

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Abstract | White adipocytes store excess energy in the form of triglycerides, whereas brown and beige adipocytes dissipate energy in the form of heat. This thermogenic function relies on the activation of brown and beige adipocyte-specific gene programmes that are coordinately regulated by adipose-selective chromatin architectures and by a set of unique transcriptional and epigenetic regulators. A number of transcriptional and epigenetic regulators are also required for promoting beige adipocyte biogenesis in response to various environmental stimuli. A better understanding of the molecular mechanisms governing the generation and function of brown and beige adipocytes is necessary to allow us to control adipose cell fate and stimulate thermogenesis. This may provide a therapeutic approach for the treatment of obesity and obesity-associated diseases, such as type 2 diabetes.

Interscapular BAT
Brown adipose tissue (BAT) is a specialized organ that produces heat. BAT is localized in the interscapular and perirenal regions of rodents and infants.

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Adipose tissue has a central role in whole-body energy homeostasis. White adipose tissue (WAT) is the major adipose organ in mammals. It represents 10% or more of the body weight of healthy adult humans and is specialized for the storage of excess energy. Humans and rodents, however, possess an additional form of adipose tissue, known as brown adipose tissue (BAT), which is specialized to dissipate chemical energy in the form of heat. Evolutionarily, BAT functions as a defence mechanism against hypothermia, particularly in infants, small mammals and hibernating animals.

The best-known function of BAT is its thermogenic capacity, enabled by the BAT-selective expression of uncoupling protein 1 (UCP1), which stimulates thermogenesis by uncoupling cellular respiration and mitochondrial ATP synthesis. This thermogenic capacity of BAT has gained significant attention owing to its potential application in the amelioration of obesity and obesity-related diseases, such as insulin resistance, type 2 diabetes and fatty liver diseases (reviewed in REF. 1). Several human studies with ^{18}fluoro-labelled 2-deoxy-glucose positron emission tomography (^{18}FDG-PET) scanning indicate that the increased mass of ^{18}FDG-PET-positive BAT (which may result from, for example, increased BAT mass or thermogenic activity of existing BAT) is inversely correlated with body mass index (BMI), adiposity or fasting plasma glucose level in adult humans[2–5]. Recent studies in adult humans further demonstrated that chronic cold exposure stimulates the recruitment of new ^{18}FDG-PET-positive BAT even in subjects who had previously lacked detectable BAT depots before cold exposure, presumably owing to the emergence of new thermogenic adipocytes. This, then, leads to an increase in non-shivering thermogenesis and/or an improvement in insulin sensitivity[6–9]. These findings collectively support the significance of BAT in the regulation of energy expenditure and glucose homeostasis in adult humans.

Recent studies indicate that at least two distinct types of thermogenic adipocyte exist in mammals: a pre-existing form established during development, termed classical brown adipocytes; and an inducible form, termed beige (or brite) adipocytes. Classical brown adipocytes develop prenatally from a subset of dermomyotome cells and are localized predominantly in dedicated BAT depots, such as in the interscapular regions of rodents and human infants. The infant interscapular BAT depots eventually disappear in adult humans[10,11]. By contrast, beige adipocytes emerge postnatally from WAT, but the exact origin of these cells is much less well understood. A notable feature of beige fat is that beige adipocyte biogenesis is highly inducible by various environmental cues, such as chronic cold exposure, exercise and treatment with the agonist of the major regulator of adipogenesis, peroxisome proliferator-activated receptor-γ (PPARγ; discussed in more detail below), in a process referred to as the 'browning' or 'beige-ing' of white fat (reviewed in REF. 12). Notably, UCP1-positive adipocytes from adult human BAT in the supraclavicular region possess

molecular signatures that resemble murine beige adipocytes rather than classical brown adipocytes[11,13–15]. It has also been shown that human beige adipocytes can be derived from capillaries of subcutaneous WAT[16], further illuminating the inducible nature of beige adipocytes and indicating their relevance to adult humans. This is important, because promoting the browning of white fat may be applicable for the treatment of obesity and type 2 diabetes, especially in subjects who do not possess appreciable levels of existing BAT, including obese, diabetic and elderly individuals.

One of the major advances in the field of BAT biology was the identification of essential transcriptional regulators and cascades, which are involved in brown and beige adipocyte development and their thermogenic function. For instance, ectopic expression of a dominant determination factor of brown adipose cell fate, PRDM16 (PR domain zinc-finger protein 16), and its binding partners CCAAT/enhancer-binding protein-β (C/EBPβ) and PPARγ, is sufficient to convert non-adipogenic cells in humans and mice, including skin fibroblasts and myoblasts, into brown adipose lineage[17–19]. These studies open up a new opportunity to manipulate the amount or the thermogenic function of brown and beige adipocytes *in vivo*. A better understanding of such mechanisms will further enable us to gain control over this thermogenic organ with ease and safety, which may provide a novel therapeutic intervention against obesity and obesity-related diseases. In this Review, we describe recent advances in the elucidation of transcriptional and epigenetic regulation of brown and beige adipocyte development, and delineate emerging questions in the field.

Chromatin landscapes in adipocytes

Adipocyte differentiation occurs through commitment from multipotent stem cells to pre-adipocytes, and terminal differentiation from pre-adipocytes to mature adipocytes. These processes are regulated by the expression of lineage-specific transcriptional regulators and by changes in histone marks and chromatin conformation at specific developmental stages, which collectively generate unique chromatin landscapes associated with adipogenesis. Here, we describe the major features of chromatin in brown and beige adipocyte lineages.

Major transcriptional regulators of adipogenesis. In contrast to our relatively limited knowledge regarding the commitment phase, a large number of adipogenic transcription factors have been identified that control adipocyte differentiation (reviewed in REF. 20). PPARγ and C/EBPα, β and δ are the principal adipogenic regulators involved in the differentiation of adipocytes. In the mouse 3T3-L1 pre-adipocyte cell line (one of the best-characterized models of adipogenesis), expression of C/EBPβ and C/EBPδ is quickly induced within 4 hours post-differentiation and subsequently activates the transcription of PPARγ and CEBPα. Subsequently, PPARγ and C/EBPα activate each other's expression and coordinately induce a number of genes that define terminally differentiated adipocyte phenotypes. PPARγ and C/EBPα are also important regulators of the thermogenic programme of brown and beige adipocytes (reviewed in REF. 21). As PPARγ-deficient mouse models (tetraploid-rescued or chimeric PPARγ-null mice) possess no brown or white adipose tissues, PPARγ is required for the development of both white and brown adipocytes[22,23]. However, PPARγ overexpression in fibroblasts or mesenchymal cells drives them into white but not brown adipocyte cell lineage, leading to the hypothesis that additional transcriptional components that cooperate with PPARγ and C/EBPs are necessary to induce the brown fat-selective gene programme[21,24].

As described in more detail below, recent studies identified approximately 50 transcriptional and epigenetic regulators that positively or negatively regulate brown and beige adipocyte development (summarized in TABLE 1). It is notable that many, if not all, such regulators function through four transcriptional regulators: PPARγ, C/EBPβ, PPARγ co-activator-1α (PGC1α) and PRDM16. PPARγ and C/EBPβ function as transcription factors and bind to DNA directly. PRDM16, which is a 140 kDa zinc-finger nuclear protein that promotes brown adipocyte differentiation and represses myogenesis[17,19], was also initially considered to function primarily through direct binding to a specific DNA sequence via the zinc-finger domains[25]. However, a point mutation in PRDM16 that disrupts its DNA binding did not affect its ability to promote brown adipogenesis, indicating that PRDM16 is a transcriptional co-regulator[18]. Indeed, PRDM16 forms a transcriptional complex with canonical DNA-binding transcription factors such as PPARγ and C/EBPβ, through its zinc-finger domains, to specify and activate the BAT-selective gene programme[17,19]. More recently, analysis of PRDM16 by chromatin immunoprecipitation coupled to sequencing (ChIP–seq) showed that a large proportion of PRDM16 target genes are co-localized with binding sites of PPARγ and C/EBPs[26], further supporting its co-regulatory functions.

Chromatin features. In order to activate adipocyte-specific genes, master transcriptional regulators (such as PPARγ) are recruited to adipocyte-specific gene loci. This is coupled to the establishment of cell type-specific histone and DNA modifications, which coordinate the binding of transcriptional regulators[27,28] (BOX 1). In fact, only 4% of PPARγ-binding sites in 3T3-L1 adipocytes are found in mouse macrophages that also express PPARγ[29], underlining the importance of the establishment of distinct chromatin landscapes for correct fate specification during adipogenesis.

A genome-wide analysis of chromatin states in embryonic stem cells (ES cells), mesenchymal stem cells and 3T3-L1 pre-adipocytes revealed that the bivalent trimethyl marks on histone H3 Lys residues (H3K4me3 and H3K27me3) at the *Pparg* and *Cebpa* gene loci are specific to ES cells and are not observed in mesenchymal stem cells or pre-adipocytes[30]. In pre-adipocytes, on the other hand, the bivalent H3K4me3 and H3K9me3 marks are found at the *Pparg* and *Cebpa* gene loci and seem to establish a 'poised' chromatin state that is prepared for active transcription upon

Myoblasts
Myogenic progenitor cells that differentiate into myocytes (muscle cells).

Transcriptional co-regulator
Transcriptional regulator that acts through forming a complex with DNA-binding transcriptional factors.

induction (FIG. 1a). During the early phase of adipocyte differentiation (assessed with the use of the 3T3-L1 cell line), the bivalent H3K4me3 and H3K9me3 marks are resolved to a monovalent H3K4me3 mark, which activates transcription. Accordingly, phosphorylated C/EBPβ and its associated complexes with other transcription regulators, such as STAT5A (signal transducer and activator of transcription 5A), glucocorticoid receptor and Mediator complexes, are recruited to transcription factor binding sites during the early phase (~4 hours) of adipocyte differentiation and activate the expression of PPARγ[31].

DNase I hypersensitive site analysis coupled with next generation sequencing (DHS–seq; see BOX 2 for technical terms) also showed that chromatin opening peaks at 4 hours after initiation of adipocyte differentiation and gradually decreases towards the end of differentiation (6 days post induction of differentiation)[31]. These spatiotemporal dynamics in chromatin architecture may be attributed to the function of pioneer factors, which can bind to loci marked with activated histone marks and initiate chromatin opening. Subsequently, PPARγ forms a heterodimeric complex with retinoid X receptor (RXR) and activates the expression of adipocyte-specific genes (FIG. 1a). Formaldehyde-assisted isolation of regulatory elements (FAIRE) sequencing analysis (see BOX 2 for technical terms) found that many of the PPARγ-binding sites at enhancer regions are enriched with H3K27 acetylation (H3K27ac) and H3K4me3 active enhancer marks that are associated with open chromatin[32]. These studies demonstrated that the introduction of specific histone modifications at the correct time during differentiation plays an important part in defining the adipocyte-selective gene programme driven by PPARγ and C/EBPs.

As the factors discussed above are important for the specification of both WAT and BAT, how, then, are these two adipocyte linages differentially established? Several lines of evidence indicate that the chromatin state (including histone marks and chromatin opening) is also distinct between white adipocytes and brown adipocytes. Approximately 55% of PPARγ-binding sites are overlapping among adipocytes from interscapular BAT, epididymal WAT and inguinal WAT[33]. Notably however, 10% of PPARγ-binding sites are found to be specific to BAT when genome-wide PPARγ-binding sites are compared between interscapular BAT and epididymal WAT depots[34]. These data indicate that the BAT-selective chromatin structure defines the BAT-selective recruitment of PPARγ and C/EBPs to their target gene loci and drives the BAT-selective gene transcriptional programme. Of note, BAT-selective genes (for example, *Ucp1*) are demarcated from common adipocyte genes (for example, *Pparg*), partly by H3K27me3 marks (FIG. 1b). The promoter region of *Ucp1* contains H3K4me3 marks and is activated in brown adipocytes, whereas it contains H3K27me3 and is repressed in white adipocytes[35]. H3K27me3 marks on the BAT-selective genes are gradually removed during brown adipocyte differentiation. By contrast, the *Pparg* gene locus contains monovalent H3K4me3 marks at the promoter region both in white and brown adipocytes[28,35]. A recent study further compared the chromatin histone marks between cultured white adipocytes and beige adipocytes that were derived from human multipotent adipose-derived stem (hMADS) cells in the process of browning induced by a synthetic PPARγ ligand, rosiglitazone. Interestingly, this study showed that a relatively small subset (less than 10%) of all PPARγ-binding sites are altered during the rosiglitazone-induced browning[36]. It is therefore conceivable that this relatively small change in the PPARγ-binding sites may be sufficient to form beige-selective superenhancers and the chromatin opening state.

In addition to histone modifications, three-dimensional (3D) chromatin conformation changes have a key role in the control of adipocyte-selective gene expression. For example, a long-range connection between the fat mass and obesity-associated (*FTO*) locus (which exhibits strong genetic association with human obesity) and the homeobox gene *IRX3* encoding a transcription factor that negatively regulates beige adipocyte differentiation is achieved by 3D chromatin conformation changes[37,38]. Although the *FTO* and *IRX3* genes are separated by megabases, *FTO* controls IRX3 (and IRX5) expression. Notably, a sequence variant (rs1421085) with a T-to-C single-nucleotide substitution in the non-coding region of the *FTO* locus, which disrupts a binding motif for the transcriptional repressor ARID5B (AT-rich interactive domain-containing protein 5B), leads to transcriptional derepression of *IRX3* and *IRX5,* and thereby repression of beige adipocyte biogenesis. When this sequence variant is modified using CRISPR–Cas9-mediated genome editing, this phenotype can be reversed[38]. A recent study by chromatin conformation capture (3C; see BOX 2 for technical terms) also showed that a chromatin conformational change, mediated by a phosphorylated form of histone demethylase JMJD1A (also known as KDM3A), is required for an acute activation of *Ucp1* gene expression in brown adipocytes in response to cAMP stimuli (see below for details on the induction of the thermogenic programme)[39].

Altogether, brown and beige adipogenesis is associated with the establishment of a cell type-specific chromatin landscape, which then regulates the transcriptional programme driving differentiation. Notably, it seems that only very specific loci are distinctively regulated on a chromatin level during BAT as compared to WAT development, indicating that the acquisition of brown adipose cell fate is subject to precise regulation.

Regulation of brown adipogenesis

Development of classical brown adipocytes occurs through two phases: lineage commitment of precursors, followed by the differentiation from brown pre-adipocytes to mature brown adipocytes. In this section, we describe the major molecular players involved in activating the transcriptional programme controlling brown adipogenesis (FIG. 2a).

Brown adipose cell fate commitment. During prenatal development, classical brown pre-adipocytes arise primarily from cells residing in the dermomyotome that

Pioneer factors
Transcription factors that can access their target genomic sites in closed chromatin. Pioneer factors trigger enhancer competency and control cell fate determination.

Retinoid X receptor
(RXR). A nuclear receptor that heterodimerizes with other nuclear receptors, including peroxisome proliferator-activated receptors (PPARs). Endogenous ligands remain unknown.

Epididymal WAT and inguinal WAT
Inguinal white adipose tissue (WAT) is a major subcutaneous WAT depot in rodents. Epididymal WAT is a visceral WAT.

Human multipotent adipose-derived stem (hMADS) cells
Cells derived from the prepubic fat pad of a 4-month-old male donor. hMADS cells differentiate to white adipocytes using an adipogenic cocktail, and also to beige adipocytes as a result of chronic treatment with synthetic peroxisome proliferator-activated receptor (PPAR) agonists such as rosiglitazone.

Superenhancers
Enhancer domains that are densely occupied with transcriptional regulators and mediator complexes. Superenhancers have key roles in regulating expression of genes essential for cell fate.

Table 1 | **Transcriptional and epigenetic regulators of brown and beige adipocyte development**

Factors	Activity	Effectors and interacting partners	Role in brown adipocyte development	Role in beige adipocyte development	Refs
Blnc1	Long non-coding RNA (lncRNA)	EBF2	Yes (promoting in culture cells)	Yes (promoting in culture cells)	125
C/EBPβ	Transcriptional factor	PRDM16	Yes (promoting)	Yes (promoting)	17,48
CARM1	Arg methyltransferase	PPARγ	Yes (reduced BAT in knockout mice)	NA	126
CREB, ATF2	Transcription factor	CBP	Yes (promoting UCP1 expression)	Yes (promoting UCP1 expression)	69–71
CtBP1, CtBP2	Transcriptional repressor	PRDM16	NA	Yes (repressing WAT gene expression)	60,97
EBF2	Transcription factor	PRDM16	Yes (promoting)	Yes (promoting)	34,42,56
EHMT1	H3K9 methyltransferase	PRDM16	Yes (reduced BAT in knockout mice)	Yes (promoting)	43,44
ERRγ	Nuclear receptor	PGC1α	Yes (promoting UCP1 expression)	NA	127
EWS	Transcriptional repressor, RNA-binding protein	YBX1, BMP7	Yes (reduced BAT in knockout mice)	NA	46
FOXC2	Transcription factor	PKA	Yes (promoting)	Yes (promoting)	62
G9A (EHMT2)	H3K9 methyltransferase	PPARγ	Yes (inhibiting brown adipogenesis)	NA	128
GCN5	Acetyltransferase	PPARγ, C/EBPβ, PRDM16	Yes (reduced BAT in *GCN5/PCAF* double-knockout mice)	NA	52
HES1	Transcription factor (downstream of Notch)	PRDM16, PGC1α	No	Yes (inhibiting)	67
IRF4	Transcription factor	PGC1α	Yes (promoting)	Yes (promoting)	80
IRX3, IRX5	Transcription factor	PRDM16, PGC1α	NA	Yes (inhibiting)	38
JMJD1 (JHDM2A)	H3K27 demethylase	PPARγ	Yes (promoting)	NA	39,129
JMJD3	H3K9 demethylase	RREB1	Yes (promoting BAT gene expression)	Yes (promoting beige gene expression)	35
KLF11	Transcription factor	PPARγ	NA	Yes (promoting in culture cells)	36
LXR	Nuclear receptor	RIP140	Yes (inhibiting UCP1 gene expression)	Yes (inhibiting beige gene expression)	82
miR-133	microRNA	PRDM16	Yes (inhibiting)	Yes (inhibiting)	130–132
miR-155	microRNA	C/EBPβ	Yes (inhibiting)	Yes (inhibiting)	50
miR-27	microRNA	PRDM16, PPARα, PPARγ, CREB, PGC1β	Yes (inhibiting in culture cells)	Yes (inhibiting in culture cells)	133
miR-193b-365	microRNA	RUNX1T1	Yes (promoting in culture cells) or No (no change in BAT in knockout mice)	NA	134,135
miR-196a	microRNA	HOXC8, C/EBPβ	No	Yes (promoting)	51
miR-30	microRNA	RIP140	Yes (promoting)	Yes (promoting)	136
miR-34a	microRNA	FGFR1, SIRT1	Yes (inhibiting)	Yes (inhibiting)	137
miR-378	microRNA	PDE1B	Yes (promoting)	No	138
miR-455	microRNA	RUNX1T1, Necdin, HIF1AN	Yes (promoting)	Yes (promoting)	139
MLL4	H3K4 methyltransferase	PPARγ, C/EBPα, C/EBPβ	Yes (reduced BAT in knockout mice)	NA	53
MRTFA	Transcription factor	SRF, BMP7	No	Yes (inhibiting)	63
NUR77	Nuclear receptor (orphan)	cAMP	Yes (promoting UCP1 expression)	NA	140
p107	Pocket protein family	PRDM16, PGC1α	Yes (inhibiting)	Yes (inhibiting)	61
p53	Tumour suppressor	PGC1α	Yes (reduced BAT gene expression in knockout mice) or no change	Yes (inhibiting)	141,142
PGC1a	Transcriptional co-activator	PPARγ, PRDM16 and others	Yes (promoting)	Yes (promoting)	76,77, 89,90

Table 1 (cont.) | **Transcriptional and epigenetic regulators of brown and beige adipocyte development**

Factors	Activity	Effectors and interacting partners	Role in brown adipocyte development	Role in beige adipocyte development	Refs
PLAC8	Transcriptional co-activator	C/EBPβ	Yes (reduced BAT in knockout mice)	NA	49
PPARγ	Nuclear receptor	PRDM16, PGC1α and others	Yes (promoting)	Yes (promoting)	22,23, 58
PRDM16	Transcriptional co-activator/repressor	PPARγ, C/EBPβ, EHMT1 and others	Yes (promoting)	Yes (promoting)	17–19, 59
PRDM3	Transcriptional co-activator	C/EBPβ, EHMT1	Yes (reduced BAT gene expression in *PRDM16*/*PRDM3* double-knockout mice)	NA	44,45
Rb	Pocket protein family	PPARγ	Yes (increased BAT in knockout mice)	Yes (inhibiting)	85,143
REV-ERBα	Nuclear receptor	TGFβ	Yes (inhibiting UCP1 expression or promoting brown adipogenesis)	NA	144,145
RIP140	Transcriptional repressor	PGC1α, LXR	No	Yes (inhibiting beige gene expression)	86–88
SIRT1	Deacetylase	PPARγ	No	Yes (promoting)	103
SMAD3	Transcription factor (downstream of TGFβ)	PRDM16, PGC1α	Yes (inhibiting)	Yes (inhibiting)	65
SRC2 (TIF2)	Transcriptional co-regulator	PGC1α	Yes (inhibiting)	NA	83
TAF7L	TATA-binding protein-associated factor	PPARγ, PRDM16	Yes (reduced BAT in knockout mice)	NA	47
TLE3	Transcriptional repressor	PRDM16	Yes (inhibiting)	Yes (inhibiting)	55
TWIST1	Transcription factor	PGC1α	Yes (inhibiting brown thermogenesis gene)	Yes (inhibiting)	84
UTX	H3K27 demethylase	CBP	Yes (promoting in culture cells)	NA	146
USF1	Transcription factor	Unknown	Yes (increased BAT thermogenesis in knockout mice)	NA	147
ZFP423	Transcription factor	PPARγ, SMAD	Yes (reduced BAT in knockout mice)	NA	148
ZFP516	Transcription factor	PRDM16	Yes (promoting)	Yes (promoting)	54

BAT, brown adipose tissue; BMP7, bone morphogenetic protein 7; CBP, CREB-binding protein; C/EBP, CCAAT/enhancer-binding protein; CREB, cAMP-responsive element-binding; CtBP, carboxy-terminal binding protein; EBF2, early B cell factor 2; ERRγ, oestrogen-related receptor-γ; EWS, Ewing sarcoma; FGFR1, fibroblast growth factor receptor 1; FOXC2, forkhead box protein C2; H3K4, histone H3, Lys4; H3K27, histone H3, Lys27; HIF1AN, hypoxia-inducible factor-1α inhibitor; HOXC8, homeobox protein C8; IRF4, interferon regulatory factor 4; KLF11, Krüppel-like factor 11; LXR, liver X receptor; MLL4, myeloid/lymphoid or mixed-lineage leukaemia 4; MRTFA, myocardin-related transcription factor A; NA, not available; PCAF, P300/CBP-associated factor; PDE1B, calcium/calmodulin-dependent 3′,5′-cyclic nucleotide phosphodiesterase 1B; PGC1, PPARγ co-activator-1; PKA, protein kinase A; PLAC8, placenta-specific gene 8 protein; PPARγ, peroxisome proliferator-activated receptor-γ; PRDM16, PR domain zinc-finger protein 16; RIP140, receptor-interacting protein 140; RREB1, Ras-responsive element-binding protein 1; RUNX1T1, RUNX1 translocation partner 1; SIRT1, sirtuin 1; SRF, serum response factor; TAF7L, TATA-binding protein associated factor 7L; TGFβ, transforming growth factor-β; TLE3, transducin-like enhancer protein 3; TWIST1, Twist-related protein 1; UCP1, uncoupling protein 1; USF1, upstream stimulatory factor 1; WAT, white adipose tissue; YBX1, Y-box binding protein 1; ZFP, zinc-finger protein.

express engrailed 1 (EN1), myogenic factor 5 (MYF5), and paired-box protein 7 (PAX7), and can also give rise to skeletal muscle[19,40,41] (however, it is possible that other cell subpopulations may also be able to give rise to brown adipocyte lineages). The brown adipocyte versus muscle fate determination occurs through the activation of a set of genes that controls brown pre-adipocyte fate commitment, and through stable silencing of the muscle-selective gene programme (FIG. 2a). For instance, early B cell factor 2 (EBF2), a factor enriched in brown pre-adipocytes, is expressed in a distinct cell population from the population expressing muscle-specific transcription factor MYOD (myoblast determination protein) within the somitic mesoderm of early-stage mouse embryos (at embryonic day E12.5)[42]. Hence, it is likely that cell fate determination of brown pre-adipocyte versus myoblasts is already completed by mid-gestation. Ectopic expression of EBF2 in myoblasts induces brown adipogenesis, and EBF2 represses the expression of MYOD and another muscle-specific transcription factor, myogenin. EBF2 is abundantly expressed in adult human brown pre-adipocytes[15], indicating that EBF2 also has an important role during brown pre-adipocyte determination in humans.

As indicated with the EBF2 example above, by mis-expressing key lineage determination factors, it is possible to induce a switch between brown adipogenesis and myogenesis. Such experiments have been used to delineate various factors responsible for brown adipocyte cell fate commitment (FIG. 2a). For instance, genetic deletion of histone methyltransferase EHMT1, a crucial enzymatic component of the PRDM16 complex,

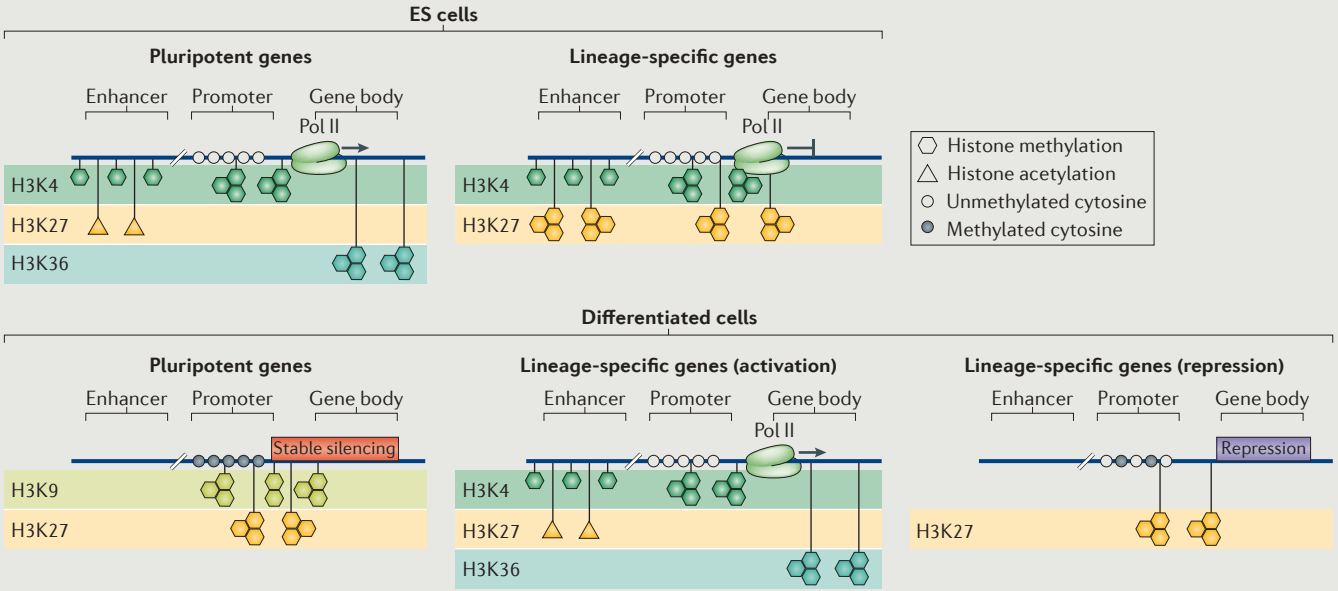
Somitic mesoderm
Mesodermal tissue, which in vertebrate embryos forms in concert with the neural tube during neurulation. This area develops into somites that give rise to skeletal muscle, bone, connective tissues and skin.

Box 1 | **The role of chromatin modifications in cell fate specification**

Pluripotent stem cells can differentiate into various types of somatic cell. Although stem cells share genome sequences with terminally differentiated cells, they gradually acquire cell type-specific characteristics by expressing lineage-specific genes (for example peroxisome proliferator-activated receptor-γ (PPARγ) for adipocytes) at an appropriate time point of differentiation. Stage-specific gene expression patterns, and thus cell fate determination, are tightly coupled to epigenetic chromatin changes such as DNA methylation, histone modifications (including acetylation and methylation), and chromatin remodelling regulated by chromatin remodelling proteins and transcription factors. Chromatin remodelling, such as mobilization, ejection and restructuring of nucleosomes, alters the recruitment of the transcriptional complex, thus regulating gene transcription.

In embryonic stem cells (ES cells), high expression of pluripotent genes is maintained owing to the ES cell-specific chromatin state on the pluripotent gene loci. The 'active' chromatin state is characterized by acetylated histone H3 Lys27 (H3K27ac) marks at enhancer regions, trimethylated H3K3 (H3K4me3) marks at promoter regions and DNA hypomethylation at promoter regions[118–120] (see the figure, top left panel; reviewed in REF. 121). By contrast, lineage-specific genes are stably silenced or expressed at very low levels in ES cells. The lineage-specific gene loci are associated with repressive H3K27me3 and active H3K4me3 marks (see the figure, top right panel). These bivalent histone marks keep the lineage-specific gene in a 'poised' state by pausing the promoter-proximal RNA polymerase II (Pol II)[122,123].

In differentiated cells, heterochromatin (transcriptionally inactive chromatin), as characterized by H3K9me3 and H3K27me3 marks and DNA methylation, is found at the promoter regions of pluripotent genes, leading to stable repression of pluripotent gene expression[119,124] (see the figure, bottom left panel). By contrast, bivalent histone marks at lineage-specific genes are resolved to a monovalent mark of either H3K4me3 or H3K27me3 upon induction of differentiation[124]. The activated lineage-specific genes are associated with H3K4me3 at promoter regions and H3K4me1 and H3K27ac at enhancer regions. On the other hand, silenced genes (that is, genes not expressed in the lineage in question) are associated with H3K27me3 and DNA methylation at promoter regions (see the figure, bottom middle and bottom right panels).



substantially impairs brown adipocyte differentiation but also leads to an ectopic activation of skeletal muscle-selective genes, including myogenin[43]. This gene silencing of the myogenic programme by EHMT1 is required for PRDM16 to initiate brown adipogenesis[43]. Of note, PRDM16 deletion in MYF5-positive precursors (including brown pre-adipocytes) does not affect embryonic BAT development, indicating the existence of compensatory factors[44]. One of these factors could be PRDM3 (also known as EVI1), a PRDM family member that has high homology with PRDM16, which also binds to EHMT1 and C/EBPß and is able to activate PPARγ expression[44,45]. Given the severe impairment of BAT development in *Ehmt1*-knockout mice, it can be concluded that EHMT1 probably functions as an enzymatic 'engine' for both PRDM3 and PRDM16 complexes and is a major determinant of the brown adipose fate by silencing the myogenic gene programme and by activating the BAT gene programme. Another important regulator of cell fate in dermomyotome precursors is Ewing sarcoma (EWS), the genetic deletion of which causes an ectopic activation of muscle genes in BAT[46]. EWS and its binding partner Y-box binding protein 1 (YBX1) activate the expression of bone morphogenetic protein 7 (BMP7) in brown pre-adipocytes, thereby promoting BAT development. Similarly, TATA-binding protein associated factor 7L (TAF7L) also plays a part in the transcriptional switch of BAT versus muscle lineages by forming a complex with PPARγ and PRDM16 (together with transcription factor IID (TFIID), a key multiprotein complex in the initiation of RNA polymerase II (Pol II)-dependent transcription). Consistent with the developmental cascade of BAT versus muscle lineage commitment, loss of TAF7L causes an ectopic activation of muscle-selective genes in BAT and impairs BAT development[47].

Brown adipocyte differentiation. PPARγ and the C/EBPs are the major regulators of adipocyte cell fate, and their activation drives the expression of adipocyte-linage specific genes. Despite the differences between BAT and

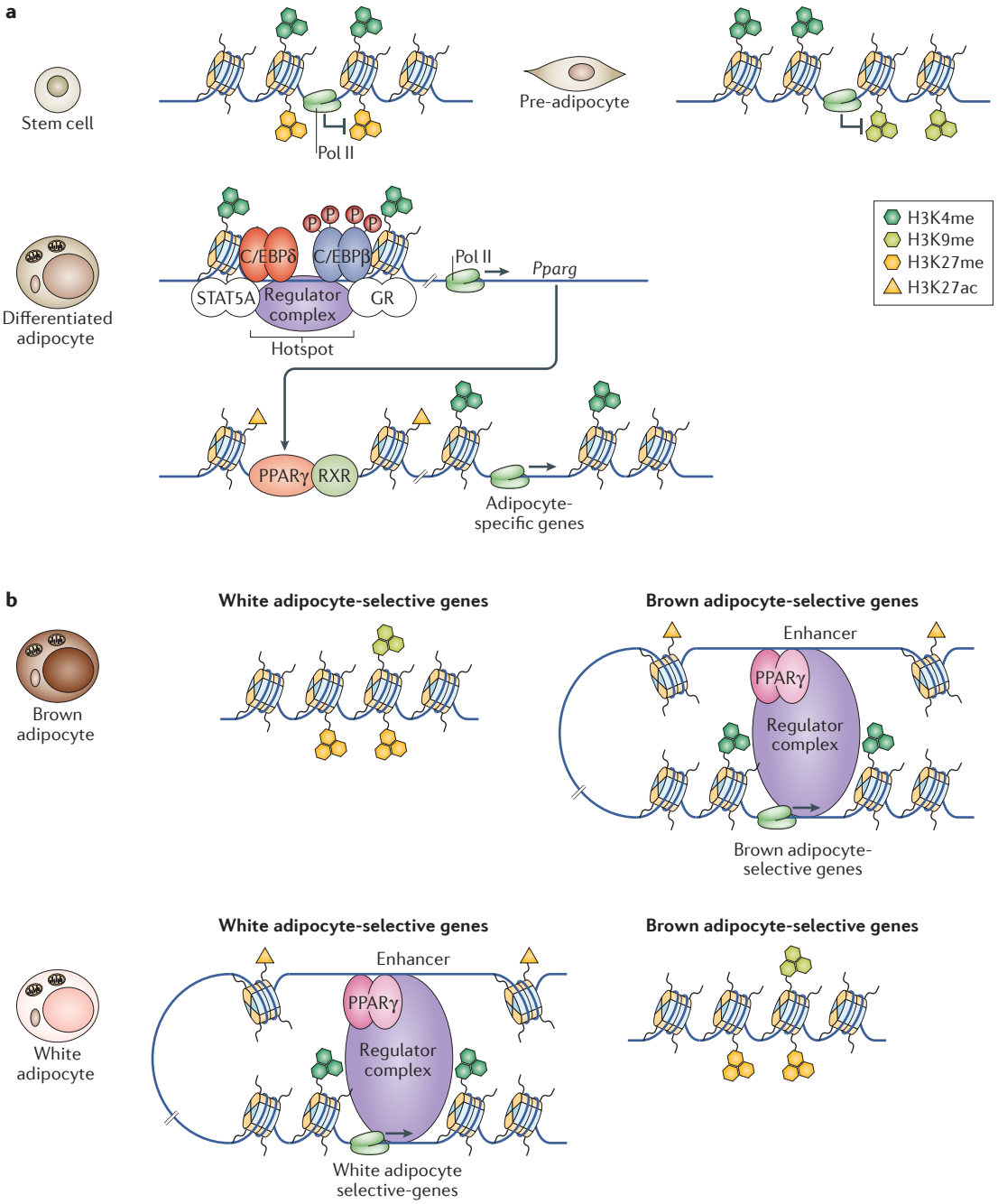


Figure 1 | **Cell type-specific chromatin states. a** | In stem cells, transcription of adipocyte lineage-specific genes, such as peroxisome proliferator-activated receptor-γ (*Pparg*), is paused by the presence of bivalent histone H3 Lys4 and Lys27 trimethyl marks (H3K4me3 and H3K27me3; top left). The bivalent H3K4me3 and H3K9me3 marks are observed in lineage-specified 3T3-L1 pre-adipocytes (top right). During adipocyte differentiation, the bivalent H3K4me3 and H3K9me3 marks are gradually resolved to a monovalent H3K4me3 mark, followed by recruitment of phosphorylated CCAAT/enhancer-binding protein-β (C/EBPβ) and other transcription regulators (for example, signal transducer and activator of transcription 5A (STAT5A), glucocorticoid receptor (GR) and Mediator complexes) to the transcriptional 'hotspot', leading to PPARγ and C/EBPα expression. Subsequently, PPARγ and its co-regulators, such as retinoid X receptor (RXR), induce adipocyte-specific gene expression (bottom). PPARγ-induced gene transcription occurs at active chromatin, as characterized by acetylated H2K27 (H3K27ac) marks at the enhancer regions and H3K4me3 marks at the promoter regions of adipocyte-specific genes. **b** | The chromatin landscape of cell type-selective gene loci in differentiated brown adipocytes (top) and white adipocytes (bottom). In brown adipocytes, recruitment of the PPARγ-containing transcriptional complex is associated with high levels of H3K27ac and abundant H3K4me3 marks at the enhancer and promoter regions of the brown adipose tissue (BAT)-selective genes, respectively. By contrast, white adipose tissue (WAT)-selective genes are in the heterochromatin state, as marked by H3K9me3 and H3K27me3. In white adipocytes, enhancer protein complexes at WAT-selective genes are associated with active histone marks, whereas BAT-selective genes are in the heterochromatin state. Pol II, RNA polymerase II.

Box 2 | **Technical approaches and methods to study chromatin and transcription**

Various experimental approaches have been developed to study epigenetic changes, recruitment of transcription regulators and 3D structure-based proximity of distal genes. Chromatin immunoprecipitation followed by deep sequencing (ChIP–seq) has been widely used to detect genome-wide distributions of a given transcription regulator. Histone modifications can also be determined by ChIP–seq, using specific antibodies raised against particular modified histones. For the detection of open chromatin, several techniques, including DNase I hypersensitive assay (DHS assay), formaldehyde-assisted isolation of regulatory elements (FAIRE) and assay for transposase-accessible chromatin sequencing (ATAC-seq), have been developed. DHS assay is able to detect DNA accessibility (that is, open chromatin regions bound by transcriptional regulators by performing limited DNase digestion of isolated nuclei, followed by PCR or deep sequencing. FAIRE is a method to detect open chromatin structure (nucleosome-depleted DNA) on the basis of a difference in crosslinking efficiency between DNA and DNA-binding proteins. ATAC-seq utilizes an engineered Tn5 transposon that integrates primer DNA sequences, allowing for fragmentation of open chromatin regions.

Changes in 3D chromatin structure can be analysed by chromatin conformation capture (3C), which also serves as the basis for several derivative techniques, such as chromatin conformation capture sequencing (Hi-C), circular chromatin conformation capture sequencing (4C-seq), chromatin conformation capture carbon copy (5C) and chromatin interaction analysis by paired-end tag sequencing (ChIA-PET). These protocols involve crosslinking between DNA and transcriptional regulator protein complexes followed by chromatin isolation and fragmentation. Ligated DNA fragments are subsequently amplified by PCR (3C) or deep sequenced (Hi-C). The DNA fragments obtained are further self-circularized and sequenced (4C-seq) or amplified using ligation-mediated PCR (5C). In ChIA-PET, crosslinked and fragmented DNA–protein complexes are immunoprecipitated to identify specific interactions with a given protein.

WAT in their physiological functions and developmental origins, as discussed above, many of the transcription cascades, including PPARγ and the C/EBPs, are important both in white and brown adipocyte differentiation[20,22,23]. However, the expression and transcriptional activity of these factors are differentially regulated by external stimuli and cell type-selective regulators, and various regulators and regulatory mechanisms of brown adipocyte differentiation have been identified (FIG. 2a). For example, C/EBPβ is expressed at higher levels in BAT than in WAT, and it is robustly induced by cold exposure in BAT[17,48]. C/EBPβ is also a major target of several transcriptional and epigenetic regulators of brown adipocyte development. One of these regulators is PLAC8 (placenta-specific gene 8 protein), which is a Cys-rich protein that directly binds to the regulatory region of the gene encoding C/EBPβ and activates its transcription, thereby promoting brown fat differentiation[49]. On the other hand, microRNA miR-155 represses C/EBPβ expression and impairs brown adipocyte differentiation[50]. Another microRNA, miR-196a, promotes brown adipocyte differentiation by repressing *HoxC8* (homeobox C8), which negatively regulates C/EBPβ by recruiting histone deacetylase 3 (HDAC3) on the 3′ untranslated region (UTR) of *Cebpb*[51], which then represses *Cebpb* transcription. Similarly, several co-regulators control brown adipogenesis through regulating the transcriptional activity of PPARγ. The acetyltransferase GCN5 (also known as KAT2A) and its homologue P300/CBP-associated factor (PCAF; also known as KAT2B) are required for brown adipogenesis, as they control transcription elongation during PPARγ expression and modulate Pol II recruitment to activate PRDM16 expression[52]. As described earlier, PRDM16 forms a transcriptional complex with C/EBPβ and PPARγ to powerfully promote brown adipogenesis[17,19]. EBF2 also promotes brown adipogenesis by recruiting PPARγ to its BAT-selective binding sites[34]. In addition, myeloid/lymphoid or mixed-lineage leukaemia 4 (MLL4; also known as KMT2D), a H3K4 mono- and dimethyltransferase, colocalizes with C/EBPβ and PPARγ to further stimulate the expression of genes regulated by these transcription factors, thereby promoting brown adipogenesis[53]. Recent studies also identified a zinc-finger protein, ZFP516, as an interacting partner of PRDM16, which promotes brown adipogenesis and thermogenesis[54]. Finally, transducin-like enhancer protein 3 (TLE3), a member of the Groucho family of transcriptional co-repressors, has been shown to antagonize PRDM16 and inhibit its action during brown adipogenesis by competing for its interaction with PPARγ[55].

Regulation of beige adipogenesis

Although much less is known regarding the cellular lineage specification of beige adipocytes as compared to classical brown adipocytes, it is clear that brown and beige adipocytes arise from distinct developmental lineages. Classical brown adipocytes are prenatally derived from a population of MYF5$^+$ dermatomes, whereas beige adipocytes emerge postnatally from a pre-adipocyte population positive for platelet-derived growth factor receptor-α (PDGFRα$^+$) and stem cells antigen 1 (SCA1$^+$), or from smooth muscle-like precursors positive for myosin heavy chain 11 (MYH11$^+$), and their emergence occurs in response to a variety of internal or external stimuli, including chronic cold exposure, PPARγ agonists, cancer cachexia, exercise and several endocrine hormones (reviewed in REF. 12). However, many of the currently identified transcriptional and epigenetic regulators have similar roles both in brown and beige adipocyte development (FIG. 2a,b). For instance, EBF2 is highly expressed in the PDGFRα$^+$ populations and promotes beige adipocytes in mouse inguinal WAT depots[42,56]. PRDM16 expression in white pre-adipocytes also potently activates a number of beige-selective genes and induces beige adipocyte biogenesis[57], whereas depletion of PRDM16 substantially impairs beige adipocyte development[58,59]. Of note, PRDM16 promotes beige adipocyte differentiation by functioning as an activator of the beige fat gene programme and a repressor of

Cancer cachexia
A wasting syndrome that leads to body weight loss and atrophy of WAT and skeletal muscle in response to a malignant growth.

Resistin
A Cys-rich secreted peptide from adipocytes that is associated with obesity and diabetes.

WAT-selective genes such as resistin. This repressive effect of PRDM16 on WAT-enriched genes occurs through a direct interaction between PRDM16 and carboxy-terminal binding proteins (CtBP1 and CtBP2)[60], which form a transcriptional repressor complex with PRDM16 and silences the WAT-selective genes. On the other hand, adipose-specific loss of retinoblastoma-like protein 1 (also known as p107), which antagonizes PRDM16 action or loss of TLE3, promotes beige adipocyte development *in vivo*[55,61]. Additionally, forkhead box protein C2 (FOXC2) expression promotes beige adipocyte biogenesis by upregulating the RIα subunit of protein kinase A (PKA)[62], which is a major downstream kinase activated by β-adrenergic receptor (β-AR) signalling (see the section on the regulation of adipocyte thermogenesis).

There are also a few examples of factors that are selectively involved in beige adipogenesis without affecting classical brown adipocyte development (TABLE 1). One such example is myocardin-related transcription factor A (MRTFA), which is a globular actin (G-actin)-regulated transcriptional co-regulator of serum response factor (SRF)[63]. The SRF–MRTFA transcriptional regulator complex is under the control of BMP7 (which also influences brown adipocyte cell fate commitment, as discussed above). Upon BMP7 treatment, the SRF–MRTFA complex is repressed, and this allows beige adipocyte differentiation. Genetic loss of MRTFA selectively promotes beige adipocyte biogenesis in WAT without affecting interscapular BAT. Conversely, overexpression of SRF or MRTFA inhibits beige adipocyte differentiation and induces smooth muscle differentiation in cultured mesenchymal cells[63]. Given the recent finding that some beige adipocytes are derived from smooth muscle-like precursors[64], SRF–MRTFA may control cell fate decisions between smooth muscle and beige adipocyte lineages. In contrast to BMP7, another signalling molecule, transforming growth factor-β (TGFβ) inhibits beige adipocyte differentiation and promotes smooth muscle-like differentiation. In line with this, genetic loss of SMAD3, a key mediator of TGFβ, or blockade of SMAD3

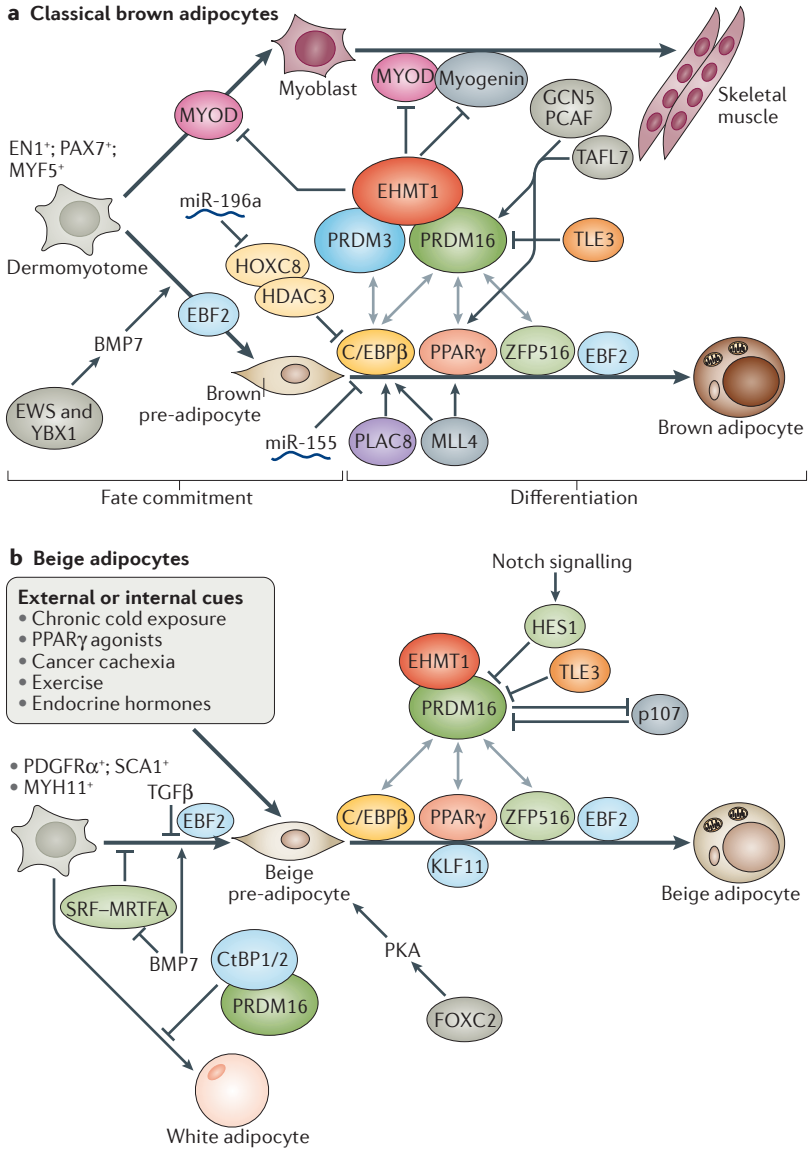


Figure 2 | **Transcriptional regulation of brown and beige adipocyte development. a** | During prenatal development, classical brown pre-adipocytes arise primarily from cells residing in the dermomyotome that express engrailed 1 (EN1), myogenic factor 5 (MYF5) and paired-box protein 7 (PAX7). MYOD (myoblast determination protein) and myogenin drive myogenesis in the dermomyotome, whereas a number of transcriptional regulators, together with their regulatory factors and regulatory signals, control brown pre-adipocyte fate commitment (see main text for details). **b** | Beige adipocytes emerge postnatally from a pre-adipocyte population that is positive for platelet-derived growth factor receptor-α (PDGFRα+) and stem cells antigen 1 (SCA1+), or from smooth muscle-like precursors positive for myosin heavy chain 11 (MYH11+). This cell population can give rise to both beige and white adipocytes. Various internal or external stimuli, such as chronic cold exposure, PPARγ agonists, cancer, exercise and several endocrine hormones are known to promote beige adipocyte differentiation. On a molecular level, beige adipogenesis, similarly to brown adipogenesis, is under the control of a plethora of transcriptional regulators and signalling pathways (see main text for details). BMP7, bone morphogenetic protein 7; C/EBPβ, CCAAT-enhancer-binding protein β; CtBP, carboxy-terminal binding protein; EBF2, early B cell factor 2; EWS, Ewing sarcoma; FOXC2, forkhead box protein; HDAC3, histone deacetylase 3; HOXC8, homeobox C8; KLF11, Krüppel-like factor 11; miR, microRNA; MLL4, myeloid/lymphoid or mixed-lineage leukaemia 4; MRTFA, myocardin-related transcription factor A; PCAF, P300/CBP-associated factor; PKA, protein kinase A; PLAC8, placenta-specific gene 8 protein; PPARγ, peroxisome proliferator-activated receptor-γ; PRDM, PR domain zinc-finger protein; SRF, serum response factor; TAF7L, TATA-binding protein associated factor 7L; TGFβ transforming growth factor-β; TLE3, transducin-like enhancer protein 3; WAT, white adipose tissue; YBX1, Y-box binding protein 1; ZFP, zinc-finger protein 16. Grey, double-headed arrows indicate protein interaction and complex formation, whereas black arrow-headed and bar-headed lines show stimulatory and inhibitory effects, respectively.

signalling by a neutralizing antibody against the activin receptor type IIB (ActRIIB), preferentially promotes beige adipocyte development[65,66]. As another example of beige-selective regulators, Krüppel-like factor 11 (KLF11) is induced in response to rosiglitazone treatment and selectively activates beige adipocyte-selective gene expression by maintaining the association of PPARγ with superenhancers of beige-selective genes[36]. It has also been shown that transcriptional repressor HES1, which is activated by Notch signal reception, represses *Prdm16* and *Pgc1a* gene expression during beige adipogenesis by directly binding to their proximal promoter regions. This effect seems to be specific to beige adipocytes, as inhibition of Notch signalling by deletion of *Hes1* selectively induces beige adipocyte biogenesis without affecting classical brown adipocyte development[67].

It is worth pointing out that each WAT depot possesses a different susceptibility to undergo browning. Cold exposure or chronic treatment with synthetic ligands of PPARγ (see below, under external cues regulating browning of WAT) strongly promotes beige adipocyte differentiation in subcutaneous WAT, whereas the browning effect is much more modest in visceral WAT[58,68]. This difference seems to be largely cell-autonomous, as much higher levels of browning can be achieved in cultured inguinal WAT-derived primary adipocytes as compared to epididymal-WAT derived adipocytes from identical mice[58]. Although high levels of PRDM16 have been shown to control a part of high browning propensity in inguinal WAT[57], ectopic expression of PRDM16 in visceral WAT is not sufficient to fully reprogramme the browning susceptibility to match that of the inguinal WAT[58]. At least in the context of browning induced by synthetic PPARγ ligands, it is possible that the cell-autonomous browning propensity depends, at least in part, on the differential chromatin opening on beige-selective gene promoters and/or enhancers in cells originating from different WAT depots, which would then differentially regulate the accessibility of PPARγ to its target genes.

Regulation of adipocyte thermogenesis

A unique feature of brown and beige adipocytes is their thermogenic function, which greatly depends on the expression and function of the BAT-specific mitochondrial protein UCP1. Studies to date have centred primarily on the regulatory mechanisms governing *Ucp1* transcription[69–71] (however, thermogenic transcriptional programmes are also responsible for the increase in the uptake of glucose and fatty acids, as well as enhanced uncoupling of mitochondrial respiration, leading to energy dissipation). The transcriptional network involved in the regulation of the thermogenic programme is depicted in FIG. 3a.

The thermogenic programme is triggered by cold stimuli and is largely (but not entirely) initiated by the activation of β-AR signalling (reviewed in REF. 72; see also the following section). The activated β-ARs increase intracellular levels of cAMP, which is followed by the activation of CREB (cAMP-responsive element-binding) and ATF2 transcription factors through their phosphorylation by PKA, leading to direct activation of *Ucp1* and *Pgc1a* transcription by these factors[70]. PPARγ and C/EBPβ are also known to directly activate *Ucp1* and *Pgc1a* transcription[17,19,73]. Adipose-selective transgenic expression of FOXC2 increases intracellular cAMP levels by increasing the expression of PKA (the RIα subunit) and promotes beige adipocyte thermogenesis in WAT[62]. By contrast, histone deacetylase HDAC1 (and HDAC2) inhibits thermogenesis through deacetylation of H3K27 on the regulatory regions of *Ucp1*, which then inhibits CREB and ATF2 mediated transcription[74]. Intriguingly it has been recently found that casein kinase 2 (CK2) activity in response to β-AR signalling is preferentially increased in WAT and inhibits the thermogenic programme in this tissue. Genetic or pharmacological inhibition of CK2 in white adipocytes promotes cAMP-induced thermogenesis, and this is mediated in part by lack of the stimulatory effect of CK2 on class I HDAC activity[75]. Additionally, β-AR signalling resulting from cold exposure activates histone demethylase JMJD1A, which, as discussed in more detail in the section below, promotes efficient transcription mediated by PPARγ[39].

PGC1α is a transcriptional co-activator that has a central role in controlling the cold-induced thermogenesis gene programme in differentiated brown and beige adipocytes[76,77]. PGC1α expression is highly induced in response to cold exposure followed by cAMP signalling, and it activates a number of thermogenic genes by interacting with various transcriptional regulators. For instance, thyroid hormone receptor, after binding its ligand triiodothyronine (T3), activates *Ucp1* transcription through forming an activator complex with PGC1α, PRDM16 and Mediator complex subunit 1 (MED1)[78,79]. Interferon regulatory factor 4 (IRF4) is activated by cAMP stimuli and interacts with PGC1α in brown adipocytes. The IRF4–PGC1α complex is recruited to the regulatory regions of the *Ucp1* gene locus to activate its transcription[80]. As described above, PRDM16 directly interacts with PGC1α and induces its transcriptional activity[18]. On the other hand, various proteins have been identified as negative regulators of PGC1α transcription or its activity. Receptor-interacting protein 140 (RIP140; also known as NRIP1) directly binds to PGC1α and represses its transcriptional activity[81]. Liver X receptor (LXR) interacts with RIP140 to dismiss the PGC1–PPARγ recruitment[82]. SRC2 (also known as TIF2 or GRIP1), a member of the steroid receptor co-activator family, and a helix–loop–helix-containing transcriptional regulator TWIST 1 (Twist-related protein 1) also repress PGC1α transcriptional activity by directly binding to PGC1α[83,84]. Similarly, retinoblastoma protein (Rb) and p107 negatively regulate PGC1α gene expression[61,85]. Genetic loss of these negative regulators (SRC2, TWIST1, RIP140, Rb and p107) induces thermogenic gene expression and adipose thermogenesis[61,83–88]. As described above, PGC1α is an essential transcriptional co-activator of BAT thermogenesis; however, brown adipogenesis and BAT mass are not affected by the genetic loss of PGC1α[89,90], indicating that PGC1α is dispensable for the fate determination of brown adipocytes.

Mediator complex subunit 1 (MED1). A core component of the Mediator complex that functions as a transcriptional co-activator. MED1 associates with general transcription factors and RNA polymerase II and has an essential role in activator-dependent transcription in all eukaryotes.

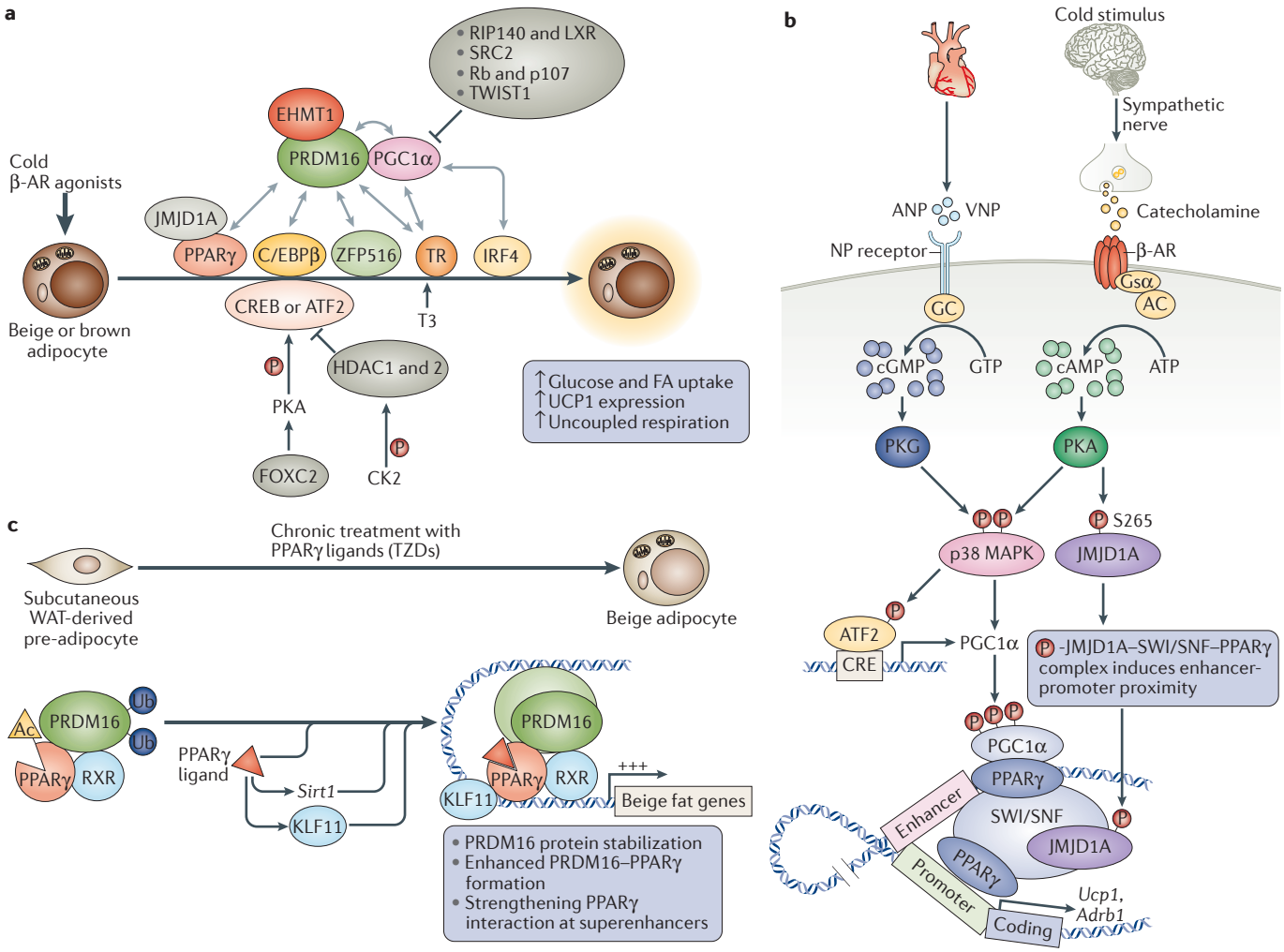


Figure 3 | **Molecular mechanisms governing thermogenesis and the role of external cues in beige adipocyte biogenesis and function. a** | Thermogenesis in differentiated brown and beige adipocytes is highly induced by cold exposure and β-adrenergic receptor (β-AR) signalling. Various transcriptional regulators are involved in this process (see main text for details) and, collectively, these molecules control the brown- and beige-selective thermogenic gene programme, including glucose and fatty acid (FA) uptake, *Ucp1* gene expression and uncoupled cellular respiration. Grey, double-headed arrows indicate protein interaction and complex formation, whereas black arrow-headed and bar-headed lines show stimulatory and inhibitory effects, respectively. **b** | Cold and natriuretic peptides (NP) induce expression of thermogenic genes. In response to cold stimuli, catecholamine is released from the sympathetic nerve endings. Released catecholamine binds to β-AR and activates adenylyl cyclase (AC), leading to an increase in cAMP levels and activation of protein kinase A (PKA). Activated PKA phosphorylates JMJD1A and p38 MAPK. p38 MAPK is also phosphorylated through activating guanylyl cyclase (GC)–cGMP–protein kinase G (PKG) signalling when atrial NP (ANP) or ventricular NP (VNP) bind to the cardiac NP receptor. Activated p38 MAPK phosphorylates peroxisome proliferator-activated receptor-γ (PPARγ) co-activator 1α (PGC1α) and transcription factor ATF2. ATF2 then binds to CRE elements on the *Pgc1a* gene promoter region and promotes *Pgc1a* transcription. Phosphorylated PGC1α functions as a co-activator for PPARγ. Phosphorylated JMJD1A forms a transcriptional complex with the SWI/SNF chromatin remodeller and PPARγ. The phosphorylated JMJD1A–SWI/SNF–PPARγ complex induces enhancer–promoter proximity through forming a three-dimensional long-range chromatin loop and activates the transcription of thermogenic genes (for example, *Ucp1* and *Adrb1* (adrenoceptor-β1)). **c** | Chronic treatment of subcutaneous white adipose tissue (WAT)-derived pre-adipocytes with PPARγ agonists (such as thiazolinedione (TZD)) promotes beige adipocyte differentiation. This is achieved partly through PR domain zinc-finger protein 16 (PRDM16) stabilization and enhanced formation of the PRDM16–PPARγ complex. Chronic TZD treatment probably exerts its effects through reducing PRDM16 protein ubiquitylation and inhibiting its degradation by the proteasome. In addition, TZD can induce sirtuin 1 (*Sirt1*), which induces deacetylation of PPARγ and enhances the formation of the PRDM16–PPARγ complex. Finally, TZD treatment induces Krüppel-like factor 11 (KLF11), which then maintains the association of PPARγ with superenhancers of beige-selective genes. This collectively promotes activation of the beige-selective gene programme. C/EBPβ, CCAAT-enhancer-binding protein-β; CK2, casein kinase 2; CREB, cAMP-responsive element-binding; FOXC2, forkhead box protein C2; Gsα, stimulatory G protein subunit-α; H3K27, histone H3, Lys27; HDAC, histone deacetylase; IRF4, interferon regulatory factor 4; LXR, liver X receptor; RIP140, receptor-interacting protein 140; RXR, retinoid X receptor; T3, triiodothyronine; TR, thyroid hormone receptor; TWIST1, Twist-related protein 1; *Ucp1*, uncoupling protein 1; ZFP, zinc-finger protein.

In addition to PGC1α, it has been shown that ZFP516 functions as a transcriptional regulator of thermogenesis. ZFP516 is induced by cold, and, after forming a complex with PRDM16, it is recruited to the *Ucp1* and *Pgc1a* gene loci and activates their expression, thereby promoting thermogenesis[54]. Moreover, C/EBPβ, as well as controlling white and brown adipogenesis, stimulates the transcription of cAMP-induced thermogenic genes (for example, *Ucp1* and *Pgc1a*) by forming a PRDM16 transcriptional complex[17,48].

Regulation by external cues

As well as intricate transcriptional networks, various external stimuli control brown and beige adipose tissue cell fate determination, maintenance and thermogenic function. This is particularly relevant for beige adipocytes, given the inducible characteristics of beige adipogenesis. As biogenesis of beige adipocytes bears strong relevance to the physiology of adult humans, it is particularly important to understand the molecular mechanisms by which external cues are integrated into chromatin state and how they control beige adipocyte development and functionality through a set of transcriptional and epigenetic machineries.

Among the many reported external cues (reviewed in REF. 12), here we primarily focus on the transcriptional mechanisms by which the brown and beige adipocyte thermogenic programme is regulated by cold-induced cAMP signalling. We then discuss how synthetic PPARγ ligands promote beige adipocyte biogenesis. In addition, we discuss possible roles of metabolites in adipocyte development, with a special emphasis on their roles as cofactors for epigenetic regulators.

Cold exposure. Cold is a potent stimulus of thermogenesis in brown and beige adipocytes. Under cold exposure, catecholamine is released from sympathetic nerve endings. Secreted catecholamine binds to β-AR on the cell surface adipocytes and subsequently enhances the interaction of the receptor with guanine nucleotide-binding protein (G protein), which in turn activates adenylyl cyclase to increase intercellular cAMP concentration (FIG. 3b). cAMP stimulates PKA and p38 MAPK (reviewed in REF. 72). P38 MAPK can also be activated by cardiac natriuretic peptides, such as atrial and ventricular natriuretic peptides released upon cold exposure. These peptides act through guanylyl cyclase-coupled receptors, inducing cGMP–protein kinase G (PKG) signalling in brown adipocytes[91] (FIG. 3b). Activated p38 MAPK phosphorylates ATF2, which, as discussed above, directly triggers the transcription of *Ucp1* and *Pgc1a*[70]. Besides the PKA–p38 MAPK signalling pathway, a recent study used phosphoproteomics in brown, beige and white adipocytes to identify previously unappreciated kinases, such as CK2, that are differentially regulated in a cell type-selective fashion in response to another β-AR agonist, noradrenaline[75].

Additionally, the enhancer–promoter proximity of thermogenic genes in brown adipocytes is dynamically regulated by cold (and cold stimulus-associated hormones). It has been demonstrated that PRDM16 alters the enhancer–promoter proximity by binding to and recruiting MED1 to the *Ucp1* and *Pgc1a* gene loci[26,78]. In addition, JMJD1A is phosphorylated at S265 in response to cAMP signalling via PKA[39] (FIG. 3b). The phosphorylated JMJD1A forms a transcriptional complex with the SWI/SNF chromatin remodeller and is recruited to the PPAR response elements of the thermogenic genes (for example, *Ucp1* and *Adrb1* (adrenoceptor-β1)). This recruitment induces demethylation of H3K9me2 and allows transcription factor-bound enhancers and thermogenic gene promoters to be brought into close proximity[39]. Intriguingly, the change in the JMJD1A-initiated promoter–enhancer proximity has been shown to occur within minutes and peaked 60 minutes after cAMP stimuli[39]. As covalent histone marks are generally more stable than protein phosphorylation, it is conceivable that cold-induced chromatin remodelling, as triggered by JMJD1A phosphorylation, serves as an 'epigenetic memory' that allows for rapid activation of thermogenic gene expression in response to a future bout of cold exposure.

PPARγ agonist treatment and PRDM16 protein stabilization. Chronic treatment with a synthetic ligand of PPARγ, such as thiazolinedione (TZD), strongly induces browning of WAT[58,92–98]. It was considered for a long time that such induction occurred simply by direct binding of ligand-activated PPARγ on the PPAR response elements on the regulatory regions of beige-selective genes[98–100]. However, this explanation is insufficient to explain in full the mechanism of TZD-induced browning, for the following two reasons. First, PPARγ is abundantly expressed both in WAT and BAT, and it is required for the formation of both cell types[22,23]; additionally, overexpression of PPARγ in white adipocytes does not induce WAT browning[101], whereas transactivation of PPARγ by synthetic ligands can do so. Second, chronic treatment with PPARγ ligands, at least for 3 days or longer, is required for the WAT browning effect[58]. The slow kinetics of PPARγ ligands are not consistent with a direct activation through the PPAR response elements, which can occur within 10 minutes of TZD treatment[102]. This implies the involvement of an alternative molecular mechanism of TZD-induced WAT browning.

It has been reported that PRDM16 in subcutaneous WAT is required for TZD-induced WAT browning[58]. Intriguingly, treatment with rosiglitazone, one of the members of the TZD class, stabilizes PRDM16 protein (without affecting *PRDM16* mRNA transcription) and extends its half-life from 5.9 hours to 17.5 hours[58]. This PRDM16 protein stabilization model explains well the slow kinetics of the TZD-induced browning, as three-day treatment with TZD can lead to the accumulation of PRDM16 protein by approximately 250-fold. In addition, TZD treatment has been shown to induce sirtuin 1 (SIRT1)-dependent deacetylation of PPARγ, which then enhances the formation of PRDM16 and PPARγ complex, further promoting WAT browning[103]. Furthermore, KLF11 is induced by TZD treatment and, as discussed above, cooperates with PPARγ to maintain the beige-selective histone modification in human white adipocytes[36] (FIG. 3c).

TZDs are pharmacological agents and may not necessarily well reflect physiological cues. However, it has recently been found that capsinoids (non-pungent analogues of capsaicin), through an activation of β2-AR, also induce WAT browning and PRDM16 protein stabilization in subcutaneous WAT[104]. It is conceivable that other hormonal stimuli — such as BMP7 (REFS 105,106), a newly identified myokine, irisin[107], fibroblast growth factor 21 (FGF21)[108,109] and natriuretic peptides[91] — may also induce WAT browning through this mechanism. The molecular mechanism by which chronic treatment with TZDs and capsinoids induces PRDM16 protein stabilization remains unknown. It is known, however, that PRDM16 stability is highly regulated by the ubiquitin–proteasome pathway, and therefore it is highly likely that deubiquitylation is the underlying mechanism responsible for the enhanced stabilization of PRDM16 (FIG. 3c). Although specific E3 ubiquitin-ligases for PRDM16 have not yet been identified, it is possible that the specific E3-ligases for PRDM16, once delineated, may serve as a plausible pharmacological target to induce the WAT browning without activating PPARγ transcriptional activity.

In addition to transcriptional activation of the beige adipocyte-specific programme, TZDs also repress WAT-selective genes in white adipocytes, as well as pro-inflammatory genes in macrophages. It has been shown that the repression of WAT-selective genes by TZDs requires C/EBPα and the recruitment of CtBP1 and CtBP2 on the promoter of the repressed genes[97]. In macrophages, the repression of inflammatory responses by PPARγ is achieved through ligand-dependent sumoylation of PPARγ, which promotes the recruitment of the PPARγ–nuclear receptor co-repressor (NCoR)–HDAC3 repressor complex to nuclear factor-κB (NF-κB) target gene promoters, resulting in their *trans*-repression[110,111]. A recent study proposed yet another mechanism of rosiglitazone-mediated transcriptional repression. In this study, enhancer transcription (that is, the synthesis of so-called enhancer RNAs (eRNAs)) in adipocytes was analysed[102]. It has been shown that rosiglitazone-induced activation of eRNA transcripts occurs at promoters and enhancers containing PPARγ-binding motifs and is largely mediated by the robust recruitment of co-activator complexes (including Mediator and p300–CREB-binding protein (CBP) complexes). At the same time, eRNA synthesis was downregulated at enhancers in the vicinity of genes not associated with PPARγ (but enriched for binding motifs of other transcriptional regulators such as AP1 and C/EBPs). It has been concluded that rosiglitazone-mediated repression results from the redistribution of co-activator complexes to sites strongly associated with PPARγ, thereby limiting the available co-activators that could function at sites bound by other transcription factors such as AP1 and C/EBPs.

Roles of metabolites. Several lines of evidence indicate that quantitative changes in levels of intracellular metabolites modulate the chromatin landscape (reviewed in REF. 112). For instance, NAD^+ is an essential cofactor for NAD^+-dependent histone deacetylases (sirtuins). Moreover, α-ketoglutarate (αKG), which is a metabolite of the tricarboxylic acid (TCA) cycle and glutamine metabolism, is a crucial co-factor for histone and DNA demethylases, such as JMJD histone demethylases and 5-methylcytosine hydroxylases (also known as TETs) (FIG. 4). In fact, αKG has been shown to maintain ES cell pluripotency by controlling H3K27me3 and DNA methylation[113]. αKG is mainly produced from isocitrate in the TCA cycle, in which the $NAD(P)^+$-dependent isocitrate dehydrogenases (IDH1 and IDH2) have key roles. When the *IDH* genes are mutated (as is frequently seen in several types of cancer cells), the mutant IDH produces 2-hydroxyglutarate (2HG) from αKG, which inhibits αKG-dependent enzymes such as JMJDs and TETs (FIG. 4). It is reported that mutation in the IDH2 is associated with elevated H3K9me3 and H3K27me3 levels at the promoter regions of *Pparg* and *Cebpa* in 3T3-L1 cells, leading to an inhibition of adipogenesis[114]. As another example, the glycolysis-derived metabolite acetyl-CoA, which can serve as a donor for histone acetylation, also controls differentiation of 3T3-L1 pre-adipocytes and ES cells by modulating histone acetylase activity[115,116].

What are the physiological implications of the metabolite-mediated epigenetic changes in adipose tissues? Although it is still a matter of speculation, a subtle change in intercellular metabolite concentrations may control differentiation or the thermogenic function of brown and beige fat. Reduced αKG levels may modulate JMJD and TET activities, thereby inducing hypermethylation (H3K9me3, H3K27me3 or DNA methylation) of their target genes. Given the role of JMJDs in the regulation of thermogenic gene expression, it is likely that a reduced concentration of αKG leads to a suppression of the BAT-selective gene programme and of thermogenic capability. As various metabolites are known to modulate epigenetic modifiers (FIG. 4), it would be now intriguing to examine the extent to which an alteration in intracellular metabolite levels affects adipocyte development and thermogenesis.

Conclusions and future perspectives

Brown and beige adipocyte development and thermogenic function rely greatly on the activation of brown and beige adipocyte-specific gene programmes that are coordinately regulated by adipose-selective chromatin architectures and by a set of unique transcriptional and epigenetic regulators. As discussed above, some of the transcriptional and epigenetic regulators, including PRDM16 and histone demethylases, are also required for establishing chromatin structures and regulating gene transcription to promote beige adipocyte biogenesis in response to various environmental stimuli.

A better understanding is now needed regarding the spatiotemporal regulation of chromatin conformation and transcriptional complex recruitment. It would be particularly compelling to unravel how these changes on the level of chromatin are induced and regulated in response to external stimuli. As discussed earlier, the cold-induced phosphorylation of JMJD1A changes the enhancer–promoter proximity of BAT-selective

Irisin
A secreted peptide from skeletal muscle (that is, a myokine) that regulates browning of WAT and thermogenesis.

Nuclear receptor co-repressor
(NCoR). A transcriptional repressor that recruits histone deacetylases to the regulatory elements of its target genes and represses their transcription.

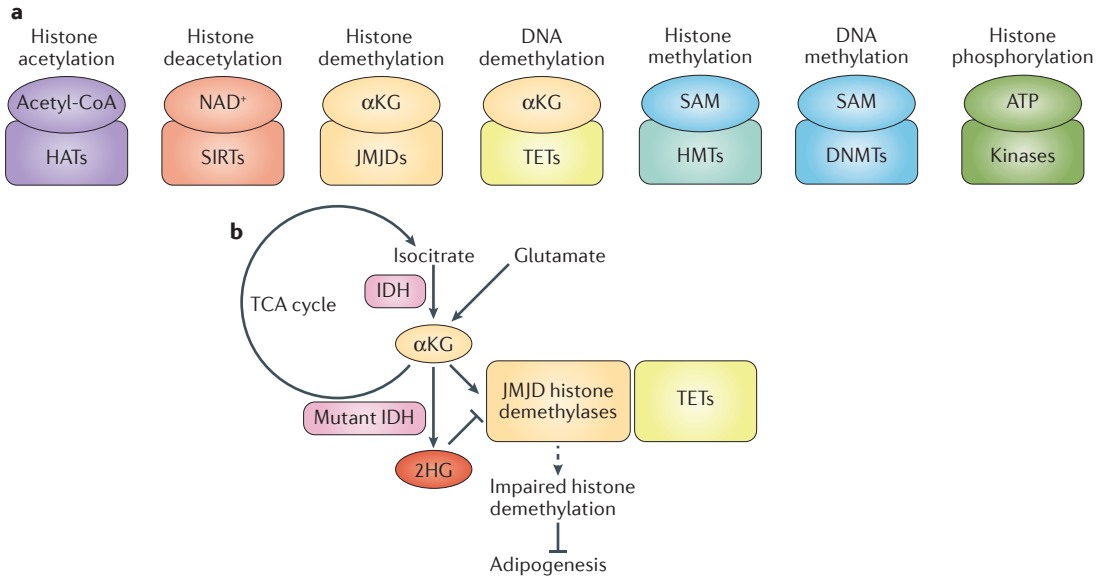


Figure 4 | **Regulation of epigenetic factors by metabolites. a** | Various metabolites can affect chromatin states by acting as cofactors for epigenetic regulators involved in adipogenesis. Acetyl-CoA and *S*-adenosyl methionine (SAM) are donors for histone acetyltransferases (HATs) and histone and DNA methyltransferases (HMTs and DNMTs), respectively. NAD^+ and ATP regulate sirtuin demethylases (SIRTs) and kinases, respectively. α-ketoglutarate (αKG) is a cofactor of JMJD histone demethylases (JMJDs) and 5-methylcytosine hydroxylases (also known as TETs). **b** | Metabolites and changes in metabolic pathways can affect adipogenesis. Isocitrate dehydrogenase (IDH) catalyses isocitrate to produce αKG in the tricarboxylic acid (TCA) cycle. However, mutations in the *IDH* gene lead to the production of 2-hydroxyglutarate (2HG) from αKG, which in turn inhibits enzymatic activities of the αKG-dependent histone and DNA demethylases, such as JMJD and TETs, thereby inhibiting adipogenesis.

thermogenic genes by forming a long-range chromatin loop with the SWI/SNF complex[39]. Another example is a functional long-range connection between the homeobox gene *IRX3* and the enhancer region of the *FTO* locus[37,38]. These studies suggest that locus-specific changes in 3D chromatin conformation are crucial for the establishment and maintenance of the beige-selective enhancers and promoters in response to a given cue. To this end, high-resolution chromatin conformation capture sequencing (Hi-C) or chromatin interaction analysis with paired-end tag sequencing (ChIA–PET; see BOX 2 for technical terms) may be useful to understand the genome-wide chromatin conformational changes.

Given the high cellular heterogeneity within adipose tissues, it is crucial to characterize the molecular signatures and chromatin structures of adipocytes, especially beige adipocytes, at the single-cell level resolution. By combining the CRISPR–Cas9-based DNA methylation reporter system and bisulfite sequencing, a recent study described time course changes in *de novo* DNA methylation at the promoter and superenhancers in single mouse ES cells[117]. Combined with single-cell RNA sequencing technology, single-cell resolution analyses would be useful to understand cellular heterogeneity within adipose tissues and further examine the epigenetic changes that control the cell-autonomous browning susceptibility.

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Competing interests statement

The authors declare no competing interests.