Transcriptional and Epigenomic Regulation of Adipogenesis

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ABSTRACT Understanding adipogenesis, the process of adipocyte development, may provide new ways to treat obesity and related metabolic diseases. Adipogenesis is controlled by coordinated actions of lineage-determining transcription factors and epigenomic regulators. Peroxisome proliferator-activated receptor gamma (PPARγ) and C/EBPα are master "adipogenic" transcription factors. In recent years, a growing number of studies have reported the identification of novel transcriptional and epigenomic regulators of adipogenesis. However, many of these novel regulators have not been validated in adipocyte development *in vivo* and their working mechanisms are often far from clear. In this minireview, we discuss recent advances in transcriptional and epigenomic regulation of adipogenesis, with a focus on factors and mechanisms shared by both white adipogenesis and brown adipogenesis. Studies on the transcriptional regulation of adipogenesis highlight the importance of investigating adipocyte differentiation *in vivo* rather than drawing conclusions based on knockdown experiments in cell culture. Advances in understanding of epigenomic regulation of adipogenesis have revealed critical roles of histone methylation/demethylation, histone acetylation/deacetylation, chromatin remodeling, DNA methylation, and microRNAs in adipocyte differentiation. We also discuss future research directions that may help identify novel factors and mechanisms regulating adipogenesis.

KEYWORDS adipogenesis, epigenomic regulation, transcriptional regulation

Obesity, characterized by an excessive accumulation of adipose tissue (AT), is a major risk factor for type II diabetes and other metabolic diseases. There are two main types of adipose tissue with distinct functions in human body. White adipose tissue (WAT) stores excess energy in the form of triglycerides, while brown adipose tissue (BAT) burns energy through thermogenesis (1, 2). Promoting BAT development and suppressing WAT development may offer approaches for treating obesity. Therefore, understanding the molecular mechanisms behind adipocyte differentiation (adipogenesis) could provide new insights into a therapeutic strategy for combating obesity.

Adipocytes, which primarily compose adipose tissue, are derived from multipotent mesenchymal stem cells (MSCs). Two phases of adipogenesis are well characterized: commitment of MSCs to preadipocytes and terminal differentiation of preadipocytes toward mature adipocytes (3). Over the last few decades, there has been progress in understanding transcriptional and epigenomic regulation of adipogenesis. The major adipogenic transcription factors (TFs) peroxisome proliferator-activated receptor gamma (PPARγ) and C/EBPα work with other TFs and epigenomic regulators to activate genes required for terminal differentiation of preadipocytes (Fig. 1 and 2).

Many important findings in the adipogenesis field were obtained using immortalized cell lines such as 3T3-L1 preadipocytes for studying terminal differentiation (4) and C3H10T1/2 MSCs for studying adipogenic commitment (5). These cell lines cannot fully represent adipogenesis *in vivo*, as differentiation in culture is induced by a chemical
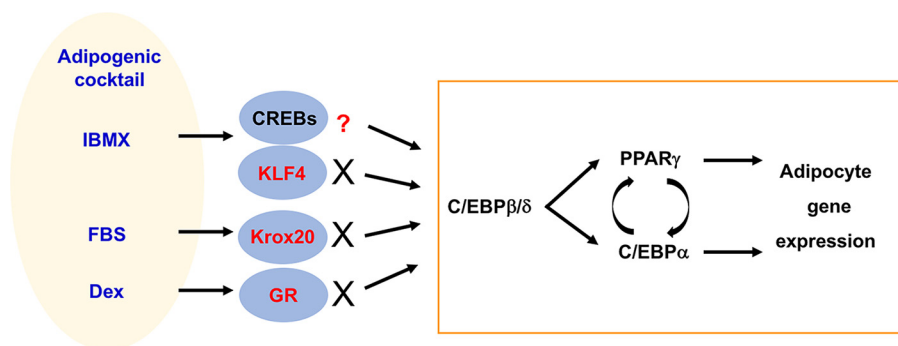
FIG 1 Activation or induction of TFs during adipogenesis in culture. The chemical cocktail of IBMX (3-isobutyl-1-methylxanthine), fetal bovine serum (FBS), and dexamethasone (Dex) has been routinely used to induce adipogenesis in culture. This cocktail induces expression of TFs KLF4 and Krox20 and activates CREBs and GR, which cooperate to activate C/EBPβ and C/EBPδ. It is generally accepted that C/EBPβ and C/EBPδ induce the expression of master adipogenic TFs PPARγ and C/EBPα, which directly activate adipocyte gene expression (highlighted by a rectangle). Recent studies indicated that induction of KLF4 and Krox20 and activation of GR are artifacts in cell culture. Neither KLF4 nor Krox20 nor GR is required for adipogenesis in culture and in mice. The role of CREBs in adipogenesis *in vivo* is unclear.

cocktail that is not present in physiological settings. To overcome this limitation, *in vivo* studies involving crossing conditional knockout mice with tissue-specific Cre-expressing mice are necessary. These Cre-expressing mouse lines include Myf5-Cre (6), Adipoq-Cre (7, 8), and Fabp4-Cre (9–11). Myf5-Cre deletes floxed genes in progenitor cells of BAT and a subset of skeletal muscles. It is particularly useful for studying adipogenesis during embryonic development. Adipoq-Cre and Fabp4-Cre delete floxed genes in mature adipocytes. However, Fabp4-Cre recombinase activity was also observed in nonadipose tissues such as heart, skeletal muscle, and testis and in multiple cell populations within adipose tissues (12, 13).

In this review, the focus is primarily on TFs and epigenomic regulators implicated in the terminal differentiation process that is common to white adipogenesis and brown adipogenesis (summarized in Tables 1 and 2). The factors that specifically regulate brown adipocyte functions such as Irf4, Zfp516, Jmjd3, and Jmjd1a have been extensively reviewed elsewhere (14–16).

TRANSCRIPTIONAL REGULATION OF ADIPOGENESIS

PPARγ and C/EBPs are required for adipogenesis. PPARγ and C/EBPα are considered master regulators of adipogenesis and have been shown to be essential for adipogenesis both in culture and *in vivo* (17–21). PPARγ, which has been extensively reviewed, controls terminal differentiation of adipocytes (1, 22–25) and is required for maintaining their differentiated state (26, 27). C/EBPα acts in concert with PPARγ to establish phenotypes of mature adipocytes (28, 29). It is generally accepted that adipogenesis is controlled by a transcriptional cascade (1, 22, 30) initiated through chromatin opening by C/EBPβ (31, 32). C/EBPβ and C/EBPδ are expressed in the early stage of adipogenesis in culture (33) and induce PPARγ and C/EBPα expression (34). However, C/EBPs cannot function efficiently without PPARγ.

C/EBPβ and C/EBPδ are essential for adipose tissue development, but their role in adipogenesis is not fully understood. C/EBPβ and C/EBPδ regulate adipogenesis synergistically as evidenced by lack of lipid droplets in BAT and significantly reduced epididymal WAT levels in double knockout ($Cebpb^{-/-}$ $Cebpd^{-/-}$) mice (35). Despite the reduced fat mass, $Cebpb^{-/-}$ $Cebpd^{-/-}$ mice express normal levels of PPARγ and C/EBPα, suggesting the presence of factors that enable cells to bypass the requirement for C/EBPβ and C/EBPδ *in vivo* (1). To clarify the role of C/EBPβ and C/EBPδ in adipogenesis, further studies using tissue-specific knockout mice will be necessary. In 3T3-L1 preadipocytes, opening on *Pparg* and other adipogenic genes occurs within 4 h after treatment with an adipogenic cocktail. Knockdown of C/EBPβ impairs chromatin
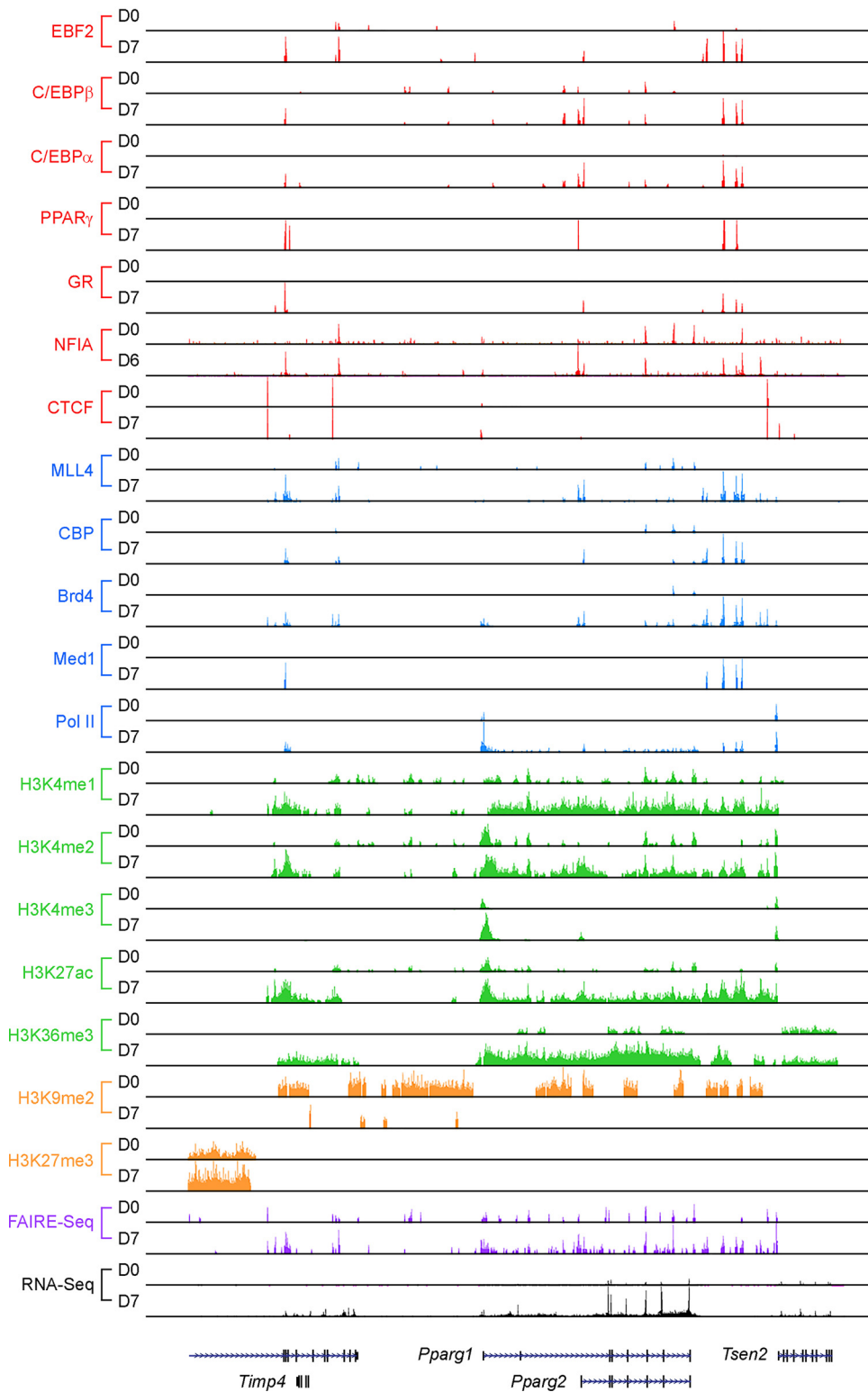
FIG 2 Transcriptional and epigenomic regulators enriched on *Pparg* locus in adipogenesis. A genome browser view of the *Pparg* locus before (day zero [D0]) and after (D7) adipogenesis of brown preadipocytes is shown. Published data were obtained from the GEO database and reanalyzed (NFIA data are from GSE83764, Brd4 data are from GSE99101, and all other data are from GSE74189) (41, 49, 97). CTCF, CCCTC-binding factor; RNA-Seq, transcriptome sequencing.

TABLE 1 Summary of transcription factors and coactivators implicated in adipogenesis

Factor	In vitro				In vivo		
	Effect[a]	Cells	Method(s)[b]	Reference	Mouse model	Phenotype in mice[c]	Reference
DNA binding transcription factors							
AP-1	NA[d]	NA	NA	NA	A-ZIP/F transgene	No WAT, ↓ BAT	72
ATF4	↑	hMSCs	KD	44	Atf4−/−	Lean, resistant to obesity	46
	↑	3T3-L1	KD	45			
C/EBPα	↑	3T3-L1	asRNA	21	Cebpa−/−; Albumin-Cebpa	No WAT, delayed BAT development	17
C/EBPβ/δ	↑	MEFs	KO	35	Cebpb−/−; Cebpd−/−	↓ WAT, no lipid in BAT	35
CREB	↑	MEFs, 3T3-L1	DN, KD	65	NA	NA	NA
EBF1	↑	NIH 3T3, 3T3-L1	OE, KD	37	Ebf1−/−	↓ WAT	42
EBF2	↑	NIH 3T3, 3T3-L1	OE, KD	37	Ebf2−/−	↓ BAT	39
	↑	Brown preadipocytes	OE, KD	39			
GATA2/3	↓	3T3-F442A, ES[e]	OE, KO	69	NA	NA	NA
GR	↑	3T3-L1	KD	31	GR f/f; Myf5-Cre	Normal BAT at E18.5 and 6 wks	57
	↑	Preadipocytes	KD, KO	57	LSL-luc; Adipoq-Cre; GR−/−	Normal BAT + WAT at E18	58
KLF2	↓	3T3-L1	OE	71	NA	NA	NA
KLF4	↑	3T3-L1	KD	53	Klf4 f/f; Myf5-Cre	Normal BAT	54
	—	Preadipocytes	KO	54			
KLF5	↑	MEFs, 3T3-L1	KD	66	Klf5+/−	↓ WAT at P3, normal at 4 wks	66
KLF15	↑	NIH 3T3, 3T3-L1	OE, KD	67	NA	NA	NA
Krox20	↑	3T3-L1	KD	52	Krox20 f/f; Myf5-Cre	Normal BAT	54
	—	Preadipocytes	KO	54			
NFIA/B	↑	3T3-L1	OE, KD	48	Nfia−/−	Normal BAT mass, ↓ Ucp1	49
	↑	Brown preadipocytes	KD	49			
PPARγ	↑	NIH 3T3	OE	18	Pparg−/− tetraploid	No BAT + WAT	19
	↑	Brown preadipocytes	KO	54	Pparg f/f; Myf5-Cre	No BAT	54
SREBP1c	↑	3T3-L1	DN	74	Srebp1−/−	Embryonic lethal, normal WAT in surviving mice	75
STAT5a	↑	3T3-L1	OE, DN	68	NA	NA	NA
Zfp423	↑	NIH 3T3, 3T3-L1	OE, KD	60	Zfp423−/−	↓ WAT and BAT at E18.5	60
					Zfp423 f/f; Adipoq-Cre	↓ fetal inguinal WAT	63
					Zfp423 f/f; Adipoq-rtTa; TRE-Cre	Browning of WAT	61
Coactivators							
Med1	↑	MEFs	KO	78	NA	NA	NA
Med14	↑	3T3-L1	KD	79	NA	NA	NA
Med23	↑	MEFs, 3T3-L1	KD, KO	80	NA	NA	NA
SRC-2	↑	NIH 3T3, 3T3-L1	OE	81	Src-2−/−	↓ interscapular WAT	81
SRC-3	↑	MEFs	KO	82	Src-3−/−	↓ WAT	82
TLE3	↑	10T1/2, 3T3-L1	OE, KD	84	Fabp4-Tle3 transgene	↑ WAT, whitening of BAT	84
	↑	Brown preadipocytes	OE	83	Tle3 f/f; Adipoq-Cre	↑ thermogenic genes in WAT + BAT	83

[a] ↑ and ↓ indicate promoting and inhibitory effects on adipogenesis, respectively; —, no effect on adipogenesis.
[b] KD, knockdown; asRNA, antisense RNA; KO, knockout; OE, overexpression; DN, overexpression of dominant-negative form.
[c] ↑, increase; ↓, decrease.
[d] NA, not available.
[e] ES, embryonic stem.

TABLE 2 Summary of epigenomic regulators implicated in adipogenesis

Factor	In vitro				In vivo		
	Effect[b]	Cells	Method(s)[a]	Reference(s)	Mouse model	Phenotype in mice[c]	Reference
Lysine methyltransferases							
Ehmt1	NA[d]	NA	NA	NA	Ehmt1$^{f/f}$, Myf5-Cre	↓ BAT	100
Ezh2	↑	Brown preadipocytes	KO	108	NA	NA	NA
G9a	↓	3T3-L1, brown preadipocytes	OE, KD, KO	98	G9a$^{f/f}$, Fabp4-Cre	↑ WAT + BAT	98
MLL3/MLL4	↓	3T3-L1, brown preadipocytes	KD, KO	91	Mll4$^{f/f}$; Myf5-Cre	↓ BAT	91
Nsd2	↑	Brown preadipocytes	KD	109	LSL-H3.3K36M; Myf5-Cre	↓ BAT	109
					Fabp4-H3.3K36M transgene	Whitening of BAT	NA
Setdb1	↓	3T3-L1	KD	103	NA	NA	NA
Lysine demethylases							
Kdm5	↑	3T3-L1, brown preadipocytes	KD	113	NA	NA	NA
Lsd1	↑	3T3-L1	KD	110	Lsd1$^{f/f}$; Myf5-Cre	↓ BAT	NA
					Lsd1$^{f/f}$; Ucp1-Cre	↑ body fat	112
Arginine methyltransferases							
Carm1	↑	3T3-L1	KD	115	Carm1$^{-/-}$	↓ lipid in BAT	115
Prmt5	↑	3T3-L1, C3H10T1/2	KD, OE, asRNA	118, 119	NA	NA	NA
Histone acetyltransferases							
CBP/p300	↑	3T3-L1	Ribozyme	125	Crebbp$^{+/-}$	↓ WAT	126
Gcn5/PCAF	↑	Brown preadipocytes	KO	124	Pcaf$^{-/-}$; Gcn5$^{f/f}$, Myf5-Cre	↓ BAT	124
Histone deacetylases and sirtuins							
Hdac1/2	↑	Fibroblasts	KO	130	NA	NA	NA
Hdac9	↓	3T3-L1, preadipocytes	OE, KO	131	NA	NA	NA
Sirt1	↓	MEFs, C3H10T1/2	KO, KD	133	Sirt1$^{f/f}$, Fabp4-Cre	↑ WAT + BAT HFD	132
Sirt2	↓	3T3-L1	KD	134, 135	NA	NA	NA
Sirt6	↑	MEFs, 3T3-L1	KD, KO	137	NA	NA	NA
Sirt7	↑	NA	NA	NA	Sirt7$^{-/-}$	↓ WAT	136
Epigenomic reader Brd4							
Brd4	↓	Brown preadipocytes	KO	97	Brd4$^{f/f}$, Myf5-Cre	↓ BAT	97
	↑	3T3-L1, C3H10T1/2	JQ1	140	NA	NA	NA
Chromatin remodeling complex SWI/SNF	↑	Fibroblasts	DN	145	NA	NA	NA
DNA methyltransferases/demethylases							
DNMT1	↓	3T3-L1	KD	149	NA	NA	NA
	↓		5-aza-dC	150	NA	NA	NA
Tet1/2	↑	MEFs	KO	153	NA	NA	NA

[a]Abbreviations are as defined for Table 1.
[b]↑ and ↓ indicate promoting and inhibitory effects on adipogenesis, respectively.
[c]↑, increase; ↓, decrease.
[d]NA, not available.

opening on these adipogenic gene loci (32). Whether C/EBPβ functions as a pioneering factor working upstream of PPARγ and C/EBPα *in vivo* needs to be validated.

EBF family TFs. Early B-cell factor (EBF) family TFs, including EBF1, EBF2, and EBF3, were initially suggested to be early regulators of adipogenesis. Ectopic expression of EBF1/2/3 stimulates adipogenesis in NIH 3T3 fibroblasts, while knockdown of EBF1/2 or expression of dominant-negative EBF1 represses 3T3-L1 adipogenesis (36, 37). EBF2 was recently shown to play a critical role in maintaining brown adipocyte identity (38–40). EBF2 recruits PPARγ to brown adipocyte-selective genes (39). Additionally, EBF2 regulates the expression of *Dpf3*, a gene encoding a brown adipocyte-specific subunit of chromatin remodeling complex, to activate thermogenic gene expression (40).

Although recent works focused primarily on the role of EBF2 in maintaining brown adipocytes, several lines of evidence suggest that EBF2 regulates adipogenesis and common adipogenic gene expression as well. It has been shown that knockdown of EBF2 in 3T3-L1 preadipocytes and in brown preadipocytes impairs adipogenesis (37, 39) and that *Ebf2*-null mouse embryos have reduced BAT mass compared with wild-type littermates at embryonic day 18 (E18) (39). In addition, reanalysis of published EBF2 chromatin immunoprecipitation sequencing (ChIP-Seq) data showed that EBF2 binding sites are enriched with motifs of adipogenic TFs C/EBPs and PPARγ (Fig. 3A) (41). Furthermore, EBF2 colocalizes with C/EBPs and PPARγ as well as with enhancer epigenomic regulators MLL4 and CBP on *Pparg* and *Cebpa* loci in addition to brown adipocyte marker *Ucp1* and *Prdm16* loci (Fig. 2 and 3B) (41). Therefore, it is possible that EBF2 cooperates with other adipogenic TFs to activate both general adipogenic gene expression and brown adipocyte-specific gene expression. There might be functional redundancy among EBF family members in regulating adipogenesis *in vivo*. For instance, *Ebf1*-null mice have reduced WAT mass but not reduced BAT mass, suggesting that EBF1 and EBF2 may have overlapping function in brown adipogenesis (42). Future studies using tissue-specific *Ebf* family knockout mice will be helpful to clarify their roles in general adipogenesis and fat depot-specific function. It would be informative to determine genomic localizations of EBF1 and EBF3 during adipogenesis.

There is some evidence to suggest that EBF family members may function as lineage-specific pioneering TFs. In B cell differentiation, EBF1 was suggested to be a pioneering TF that binds to naive chromatin and induces chromatin opening (43). In preadipocytes, EBF2 premarks a subset of adipogenic superenhancers prior to adipogenesis (41). Future study on whether EBF family members function as lineage-specific pioneering TFs and open chromatin during adipogenesis cooperatively and/or distinctively with C/EBPβ is necessary.

Atf4. ATF4 has been identified as a positive regulator of adipogenesis in human MSCs (hMSCs) (44). The enhancers with RNA polymerase II (Pol II) binding in confluent hMSCs are enriched with hybrid motifs of C/EBPs and ATF half-sites. While C/EBPβ binds to canonical C/EBP motifs upon induction with adipogenic cocktail, it binds to noncanonical hybrid motifs forming heterodimers with ATF4 in undifferentiated hMSCs. Therefore, ATF4 appears to play a role in the adipogenic commitment stage. ATF4 expression peaks in the confluent hMSCs and gradually decreases upon adipogenic induction. Knockdown of ATF4 reduces C/EBPβ and Pol II binding on enhancers and impairs adipogenesis in hMSCs.

A separate study using 3T3-L1 preadipocytes has shown that levels of ATF4 expression are very low in preadipocytes and peak at a later stage of differentiation and that knockdown of ATF4 inhibits adipogenesis (45). The difference between hMSCs and 3T3-L1 cells in ATF4 expression levels is likely a consequence of the fact that these two cell lines represent distinct stages of adipogenesis. Whole-body *Atf4*-null mice are lean and resistant to obesity (46). However, it remains unclear whether the leanness of *Atf4*-null mice is due to failure of adipogenesis or is secondary to growth retardation (47). Lineage-specific knockout of *Atf4* will provide insights on the role of ATF4 in adipogenesis *in vivo*. To understand how ATF4 regulates adipogenesis, genomic localization of ATF4 needs to be determined.

A

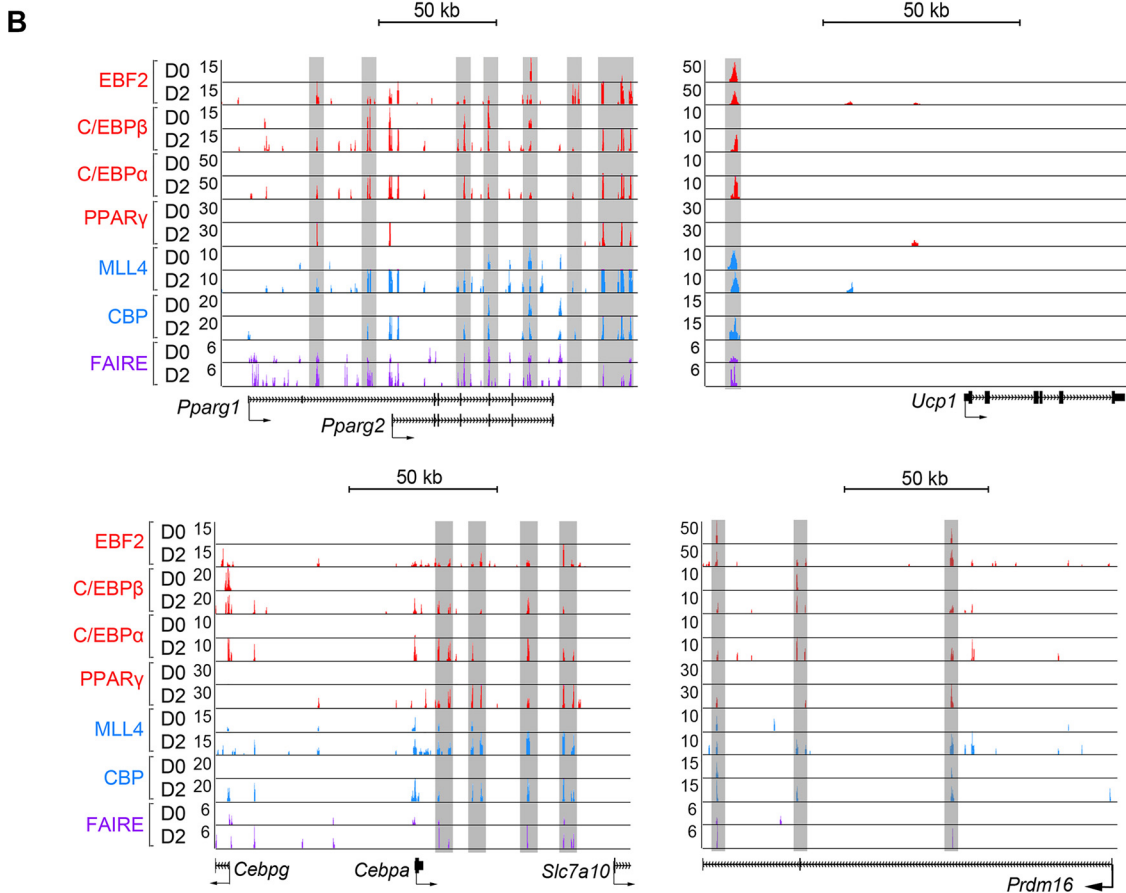| D-3			D0			D2			D7	
Factors	P value		Factors	P value		Factors	P value		Factors	P value
Ebf	<1E-300		Ebf	<1E-300		Ebf	<1E-300		Ebf	<1E-300
Jun	<1E-300		Jun	7E-97		Cebpa	4E-69		Cebpa	2E-75
Jdp2	2E-200		Jdp2	2E-59		Cebpb	2E-65		Nfic	1E-70
Jund	4E-140		Jund	8E-31		Nfic	1E-35		Cebpb	1E-67
Ctcf	2E-34		Tcf3	1E-18		Rela	4E-21		Jun	6E-27
Zfx	1E-23		Nfic	1E-18		Jun	5E-13		Rela	3E-23
Rela	8E-23		Runx1	2E-16		Atf4	1E-9		Pparg	4E-16
Nfic	3E-18		Batf	2E-14		Zfp423	6E-9		E2f4	1E-14
Tbx3	3E-17		Ctcf	5E-13		E2f4	1E-8		Zfx	1E-14
Runx1	2E-14		Zfx	5E-12		Zfx	3E-8		Ppara	1E-10
Etv5	3E-10		Rela	1E-10		Tbx3	4E-6		Snai1	1E-10
Tead4	9E-10		Tbx3	5E-10		Jdp2	1E-5		Atf4	3E-10
Ets1	2E-7		E2f4	3E-7		Pparg	1E-4		Jdp2	1E-9
Elk3	4E-6		Snai1	4E-6		Snai1	1E-4		Zfp423	2E-8
Snai1	4E-6		Tead4	2E-5		Ctcf	6E-4		Ctcf	6E-5
Atf4	6E-6		Hoxc9	3E-4					Tbx3	4E-4
Cebpb	2E-5		Egf2	4E-4						
Creb5	2E-5		Cebpb	6E-4						
Klf4	2E-5									
Creb1	6E-5									
Elf4	2E-3									

B

FIG 3 Genomic binding of EBF2 during brown adipogenesis. (A) Lists of significantly enriched motifs in EBF2 binding sites on enhancers at day −3 (D-3), D0, D2, and D7 of adipogenesis of brown preadipocytes. (B) EBF2 directly regulates expression of both common adipogenic genes *Pparg* and *Cebpa* and BAT-specific genes *Ucp1* and *Prdm16*. Highlighted regions indicate active enhancers bound by EBF2 at D2. Published ChIP-Seq data sets of adipogenic TFs EBF2, C/EBPs and PPARγ, and enhancer epigenomic writers MLL4 and CBP, as well as chromatin opening (formaldehyde-assisted isolation of regulatory element sequencing [FAIRE-Seq]) data sets, were obtained from GSE74189 (41) and reanalyzed.

NFI family TFs. Nuclear factor I (NFI) family TFs were initially identified as positive regulators of adipogenesis by motif analysis of open chromatin regions in differentiated 3T3-L1 cells (48). Knockdown of either NFIA or NFIB inhibits 3T3-L1 adipogenesis, while forced expression of NFIA induces adipogenic genes, including *Pparg*, *Cebpa*, and *Fabp4*, in undifferentiated 3T3-L1 cells. These data suggest that NFIs promote adipogenesis and adipogenic gene expression, although it was not shown whether they are required for adipogenesis *in vivo*.

A follow-up study using comparative genomic profiling showed that NFI motifs are enriched in BAT-specific open chromatin regions, suggesting that NFIs may play a role in regulating BAT-specific genes (49). Among the four members of NFI family, NFIA expression shows strong induction during adipogenesis of brown preadipocytes, while the expression levels of the others are comparable in brown and 3T3-L1 preadipocytes and remain steady during differentiation. In constrast to previous results in 3T3-L1 cells (48), knockdown of NFIA in brown preadipocytes had no effect on adipogenesis and adipogenic gene expression but decreased the levels of expression of BAT-specific genes. Furthermore, whole-body *Nfia*-null mouse embryos have normal BAT mass but show decreased expression of *Ucp1* and other thermogenic genes (49). In addition, PPARγ binding to the *Ucp1* enhancer is impaired in *Nfia*-null BAT, suggesting a role of NFIA in brown adipocyte-specific gene expression *in vivo*. Since genomic binding of NFIA is observed with enhancers of adipogenic genes, including *Pparg*, it is also possible that NFIs redundantly regulate adipogenesis and adipogenic gene expression (Fig. 2).

Krox20, KLF4, and glucocorticoid receptor are dispensable for adipogenesis *in vivo*. Our understanding of adipogenesis comes mainly from studies using preadipocyte cells such as 3T3-L1 (50). Adipogenesis is induced by adding chemical cocktail to confluent preadipocytes (Fig. 1). These chemicals activate the TFs CREB and glucocorticoid receptor (GR) and induce expression of Krox20, KLF4, C/EBPβ, and C/EBPδ within hours (51). Activation or ectopic expression of these TFs has been shown to promote adipogenesis in culture (1, 52, 53). However, it was unclear whether endogenous Krox20, KLF4, and GR are essential for adipogenesis *in vivo*.

A recent study demonstrated that endogenous Krox20 and KLF4 are dispensable for adipogenesis in culture and BAT development in mice (54). Deletion of *Krox20* or *Klf4* has little effect on either the induction of early adipogenic TFs, including C/EBPβ, C/EBPδ, KLF5, and KLF9, or the expression of adipogenesis markers, including PPARγ, C/EBPα, and Fabp4. Consistent with data determined *in vitro*, Myf5-Cre-mediated knockout of *Krox20* or *Klf4* does not affect BAT development in mice. Therefore, the adipogenesis defects observed in the previous knockdown experiments were likely due to off-target effects (52, 53). These recent findings on Krox20 and KLF4 caution us that we cannot solely rely on the knockdown approach and highlight the importance of studying adipogenesis *in vivo*. Whether Krox20 and KLF4 play specific roles in WAT development remains unclear.

It was believed that GR is critical for adipogenesis, since dexamethasone, a synthetic GR ligand, is commonly used to induce adipogenesis in mouse preadipocytes (Fig. 1), although it is not required for adipogenesis of rat preadipocytes (55, 56). Either knockdown or knockout of GR impairs adipogenesis of 3T3-L1 and primary or immortalized white and brown preadipocytes using a standard 7- to 8-day differentiation protocol (31, 57). Unexpectedly, GR-deficient preadipocytes eventually catch up to wild-type cells and fully differentiate in 3 weeks. In addition, mice that lack GR in Myf5$^+$ progenitors develop normal levels of BAT and are cold tolerant (57). Therefore, even though dexamethasone-bound GR accelerates adipogenesis in culture, endogenous GR is largely dispensable for adipogenesis in culture and BAT development in mice. GR expedites differentiation by recruiting histone acetyltransferase (HAT) CBP to activate C/EBPβ-primed enhancers in culture. An independent study further confirmed that GR is dispensable for adipogenesis *in vivo* using several approaches (58). First, both wild-type and GR-deficient mouse embryonic fibroblasts (MEFs) can form *de novo* fat

pads in mice. Second, using adipocyte-specific luciferase reporter mice, early inguinal white and brown adipocytes can be detected in GR-deficient embryos, demonstrating that GR is not required for embryonic development of WAT and BAT. Although GR is not required for adipogenesis, it is expressed and involved in lipogenesis in adipocytes (59).

Zfp423. A quantitative analysis of the transcriptome in clones of Swiss 3T3 fibroblasts identified Zfp423 as a determinant of preadipocytes (60). Compared to nonadipogenic sublines, adipogenic clones highly express Zfp423. Forced expression of Zfp423 in NIH 3T3 fibroblasts induces *Pparg2* expression and adipogenesis, while knockdown of Zfp423 in 3T3-L1 inhibits adipogenesis. Whole-body *Zfp423* knockout mouse embryos have been shown to have reduced levels of WAT and BAT mass at E18.5, although it is unclear whether this was due to the failure of adipogenesis or secondary to growth defects.

Studies using several tissue-specific *Zfp423* knockout mice identified a key role of Zfp423 in maintaining white adipocyte identity by antagonizing EBF2 and repressing thermogenic gene expression (61, 62). Data from a recent study using Adipoq-Cre-mediated *Zfp423* knockout mice suggest that Zfp423 is required for the later stage of fetal inguinal WAT development (63). It is worth noting that although Adipoq-Cre is mainly active in mature adipocytes in adult mice, it may target a subset of adipose stromal vascular cells, which likely represent actively differentiating or highly committed preadipocytes, in fetal inguinal WAT (64). It remains unclear whether Zfp423 regulates adipogenesis in general or in a fat depot-specific manner. To understand how Zfp423 regulates adipogenesis, profiling of Zfp423 genomic binding before and after adipogenesis is necessary.

Other TFs implicated in adipogenesis. Several additional TFs have been implicated in regulating adipogenesis (1, 3, 23). CREB (65), KLF5 (66), KLF15 (67), and STAT5a (68) have been suggested to be positive regulators of adipogenesis based on the finding that expression of dominant-negative form or RNA interference (RNAi)-mediated knockdown of these TFs inhibited adipogenesis in culture. GATA2/3 (69, 70) and KLF2 (71) have been suggested to be negative regulators of adipogenesis, as overexpression of these TFs inhibits 3T3-L1 adipogenesis and *Pparg* promoter activity in reporter assays. However, most of these TFs have not been validated in physiological settings using animal models and their direct target genes are largely unknown.

Mice with adipose-specific expression of A-ZIP/F, a dominant-negative protein that inhibits C/EBPs and AP-1 family TFs, have no WAT and severely reduced levels of BAT, indicating that either or both of C/EBPs and AP-1 are essential for adipose tissue development in mice (72). Since the AP-1 family includes Jun, Fos, ATF, and JDP, it remains unclear which specific TFs are important for adipogenesis *in vivo*.

SREBPs (including SREBP-1a, SREBP-1c, and SREBP-2) are key TFs that regulate fatty acid and cholesterol synthesis (73). In culture, SREBP-1c has been shown to promote adipogenesis by providing lipid ligands that mediate PPARγ activation (74). However, whole-body *Srebp1*-null mice showed no defects in adipose tissue development and adipocyte gene expression (75). Compensatory mechanisms may exist in *Srebp1*-null mice, as the majority of knockout mice show embryonic lethality. Therefore, phenotypes observed in surviving *Srebp1*-null mice may not reveal the roles of SREBP-1 in adipose tissues. Future studies using tissue-specific knockout mice are required to better evaluate the roles of SREBPs in adipogenesis.

Transcription coactivators in adipogenesis. Transcriptional coactivators, such as Mediator proteins and steroid receptor coactivator (SRC) family proteins, function as adaptors that connect TFs to Pol II or histone acetyltransferases to activate gene expression (76, 77). Several components of the Mediator complex, including Med1 (78), Med14 (79), and Med23 (80), and several components of the SRC family, including SRC-2 (81) and SRC-3 (82), have been shown to be required for adipogenesis in culture. However, cell-autonomous roles of these coactivators in adipogenesis have not been demonstrated using tissue-specific knockout mice.
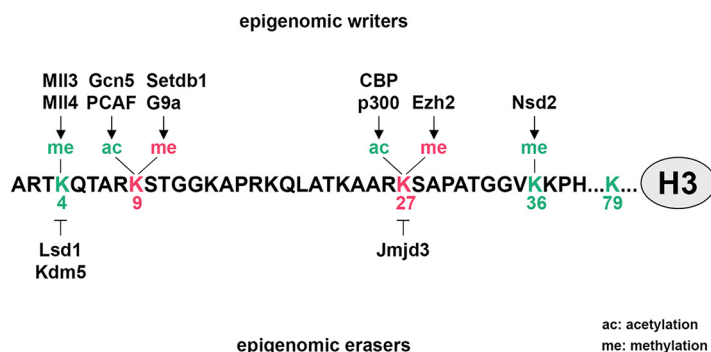
FIG 4 Epigenomic writers and erasers implicated in adipogenesis. A number of site-specific histone lysine methyltransferases, histone demethylases, and histone acetyltransferases play important roles in adipogenesis by adding or removing active (green) or repressive (red) histone marks on lysine residues. The resulting combination of epigenomic marks contributes to activation or repression of downstream target genes regulating adipogenesis. Enzymes that are responsible for methylation/demethylation and acetylation on histone H3 tail are shown.

Groucho family member TLE3 has been identified as a white adipocyte-selective PPARγ cofactor. TLE3 inhibits brown adipocyte-enriched gene expression by binding to Prdm16, a brown adipocyte-selective cofactor, and by competing for interactions between Prdm16 and PPARγ, while it promotes white adipocyte-selective gene expression (83). *Fabp4* promoter-driven expression of TLE3 in mice leads to an increase in WAT mass (84) and whitening of BAT (83). Adipoq-Cre-mediated knockout of *Tle3* induces thermogenic gene expression and improves the thermogenic function of BAT. Genomic binding of TLE3 during white adipogenesis and brown adipogenesis needs to be determined to better understand the fat depot-specific roles of TLE3.

EPIGENOMIC REGULATION OF ADIPOGENESIS

Epigenomic factors play important roles in regulating cell-type-specific gene expression during cell differentiation (85, 86). Key epigenomic regulators include but not limited to the following: histone methyltransferases (MTs)/demethylases, histone acetylases/deacetylases, epigenomic readers, chromatin remodeling factors, DNA methylases/demethylases, and microRNAs (miRNAs) (87, 88).

Lysine methyltransferases. Lysine residues on histone tails are modified by site-specific methyltransferases (Fig. 4). Histone methylation can be correlated with either gene activation or repression, depending on the methylation sites (89, 90). For example, methylation of histone H3 on lysine 4 (H3K4), lysine 36 (H3K36), or lysine 79 (H3K79) is correlated with gene activation, but methylation on lysine 9 (H3K9) or lysine 27 (H3K27) is correlated with gene repression. Studies have identified distinct roles of H3K4 methyltransferases MLL3 and MLL4 (MLL3/MLL4); H3K9 methyltransferases G9a, Setdb1, and Ehmt1; H3K27 methyltransferase Ezh2; and H3K36 methyltransferase Nsd2 in adipogenesis.

MLL3/MLL4 are members of the mammalian Set1-like H3K4 methyltransferases, a family which also includes MLL1, MLL2, SET1A, and SET1B (91–93). MLL3/MLL4 are major H3K4 monomethyltransferases enriched on enhancers and required for enhancer activation (91, 94). MLL3/MLL4 and associated PTIP and Ncoa6 have been shown to be required for induction of PPARγ and C/EBPα and subsequently for adipogenesis (91, 95, 96). MLL3/MLL4 are recruited by a pioneering TF C/EBPβ to activate the enhancers of *Pparg* and *Cebpa* and induce PPARγ and C/EBPα expression during adipogenesis. Once induced, PPARγ and C/EBPα recruit MLL3/MLL4 to further activate enhancers of downstream target genes (91). Deletion of *Mll3* and *Mll4* in preadipocytes impairs recruitment of histone acetyltransferases CBP/p300 and the epigenomic reader Brd4 on enhancers, thereby inhibiting induction of adipogenic genes (41, 97). Myf5-Cre-mediated knockout of *Mll4* in progenitor cells leads to severe defects in BAT development in mice (91). However, Adipoq-Cre-mediated knockout of *Mll4* in differentiated

adipocytes does not affect adipose tissue mass or gene expression (94). Therefore, MLL3/MLL4 act as major enhancer writers during adipogenesis, while they are largely dispensable for maintaining differentiated adipocytes. The roles of other H3K4 methyltransferases such as MLL1/MLL2 (MLL1/2) or SET1A/B in adipogenesis have not been reported (51).

H3K9 methyltransferases G9a and Setdb1 have been shown to repress adipogenesis, while Ehmt1 has been shown to promote brown adipogenesis (98, 100, 103). G9a is a major enzyme responsible for the repressive histone mark H3K9 dimethylation (H3K9me2) (101). In preadipocytes, the entire *Pparg* locus is covered by H3K9me2, where another repressive mark, H3K27me3, is absent (98). During adipogenesis, both H3K9me2 levels and G9a levels decrease, inversely correlating with PPARγ induction. Deletion of *G9a* in preadipocytes upregulates PPARγ expression by reducing H3K9me2 levels. Fabp4-Cre-mediated *G9a* knockout mice show increased adipose tissue mass. In addition, G9a promotes expression of Wnt10a, an inhibitor of adipogenesis, independently of its enzymatic activity. Therefore, G9a represses adipogenesis through both enzymatic activity-dependent and enzymatic activity-independent mechanisms. It remains unclear which DNA binding TFs are responsible for G9a recruitment to the *Pparg* locus. A recent study reported that AP-2α recruits G9a to the *Cebpa* promoter through sequential chromatin immunoprecipitation (ChIP-reChIP) assays (102). Future studies will be needed to assess whether AP-2α is also responsible for G9a recruitment to *Pparg* locus. In contrast to G9a, which represses both white and brown adipogenesis, Ehmt1 promotes brown adipogenesis (100). Myf5-Cre-mediated deletion of *Ehmt1* impairs BAT development and BAT-selective gene expression in mice. The role of Ehmt1 in white adipogenesis remains unclear. Setdb1, an H3K9 trimethyltransferase, has been shown to poise *Pparg* and *Cebpa* expression in preadipocytes by maintaining noncanonical H3K4me3/H3K9me3 bivalent domains (103). Lineage-specific DNA methylation on *Pparg* and *Cebpa* recruits the Setdb1-MBD1-MCAF1 complex to establish H3K4me3/H3K9me3 domains. Knockdown of Setdb1 inhibits H3K9me3 deposition on *Pparg* and *Cebpa* promoters, allows release of paused Pol II, and induces *Pparg* and *Cebpa* expression, which in turn facilitates adipogenesis. However, it remains to be determined whether Setdb1 represses adipogenesis *in vivo*.

Ezh2 is the catalytic subunit of the dominant H3K27 trimethyltransferase Polycomb repressive complex 2 (PRC2) in mammalian cells (104). PRC2 regulates development by repressing genes that need to be silenced during cell differentiation (105, 106). Secreted Wnt proteins activate the Wnt/β-catenin signaling pathway to inhibit adipogenesis (107). Ezh2 directly represses *Wnt* genes in a methyltransferase activity-dependent manner to facilitate adipogenesis (108). Deletion of *Ezh2* leads to decreases in H3K27me3 levels on *Wnt* genes and derepression of *Wnt* expression in preadipocytes and during adipogenesis. This is accompanied by an increase of global acetylation on H3K27 (H3K27ac) (108). With such a dramatic increase in H3K27ac levels, it is possible that other genes in addition to *Wnt* are activated in Ezh2 knockout cells, contributing to the phenotypes. Determining the genomic binding of Ezh2 or other subunits of PRC2 will be useful to identify genes directly repressed by H3K27me3. Future studies will be needed to identify TFs that recruit the Ezh2-containing PRC2 complex to *Wnt* genes. In addition, the roles of Ezh2 in adipogenesis *in vivo* have not been reported.

A recent study has identified Nsd2, an H3K36 dimethyltransferase, as a positive regulator of adipogenesis, based on findings showing that ectopic expression of K36M mutant form of histone H3 (H3K36M) inhibits Nsd2 enzymatic activity, depletes endogenous H3K36 dimethylation, and inhibits adipogenesis by increasing the levels of repressive H3K27me3 on *Cebpa* and other target genes of PPARγ (109). Knockout or knockdown of Nsd2, but not of other H3K36 methyltransferases (Nsd1 or Setd2), phenocopies the effect of H3K36M expression in adipogenesis. While H3K36M expression in Myf5+ progenitor cells leads to failure of BAT development in mice, *Fabp4* promoter-driven H3K36M expression results in whitening of BAT and induces insulin resistance in WAT, indicating that Nsd2-mediated H3K36me2 plays critical roles in

adipose tissue development and function. Future work will be needed to validate important roles of Nsd2 in adipogenesis and adipocyte function *in vivo*.

Lysine demethylases. Lsd1 is an H3K4 and H3K9 demethylase that has been implicated in both transcriptional activation and repression, depending on the biological context and the associated protein complex. Studies have identified Lsd1 as a positive regulator of adipogenesis, as knockdown or knockout of Lsd1 in preadipocytes inhibits adipogenesis (110, 111). Knockdown of Lsd1 increases the levels of the repressive histone mark H3K9me2 on the *Cebpa* promoter and impairs the induction of C/EBPα (110). A recent study suggested that Lsd1 is critical for BAT development and function (112). Knockout of Lsd1 in Myf5$^+$ progenitor cells leads to defects in BAT development in mice by increasing H3K9me2 on the *Ucp1* promoter and inhibiting Ucp1 expression. Ucp1-Cre-mediated *Lsd1* knockout in mice results in increased whole-body adiposity and cold intolerance, suggesting that Lsd1 is required for BAT function. Treating preadipocytes with a chemical inhibitor of Lsd1 prevents adipogenesis, suggesting that Lsd1 enzymatic activity is required for adipogenesis (111). These studies suggested that Lsd1 promotes *Cebpa* and *Ucp1* expression during adipogenesis by demethylating H3K9me2. The role of Lsd1 in WAT development *in vivo* remains to be determined.

A recent study reported that knockdown of the Kdm5 family of H3K4 demethylases in preadipocytes inhibits adipogenesis (113). Specifically, Kdm5A binds to and activates promoters of genes involved in cell cycle and proliferation. As a result, knockdown of Kdm5 impairs mitotic clonal expansion, which in turn leads to defects in adipogenesis. Reconstitution experiments suggested that the enzymatic activity of Kdm5A is required for the activation of cell cycle genes. Although mitotic clonal expansion is required for adipogenesis in culture, its role in adipose development *in vivo* is not clear. To better understand the role of Kdm5 family in adipogenesis *in vivo*, further study using conditional knockout mice will be necessary.

Arginine methyltransferases. Arginine methylation is mediated by nine protein arginine methyltransferases (PRMTs) in mammals (114). Among these PRMTs, Carm1 and Prmt5 have been implicated in regulating adipogenesis.

Carm1 is a transcriptional coactivator for PPARγ and is required for adipogenesis (115). Transcriptome analysis of whole-body *Carm1* knockout mouse embryos suggests that Carm1 is required for adipogenic and lipogenic gene expression downstream of PPARγ. Moreover, E18.5 *Carm1* knockout embryos have reduced lipid in BAT compared with wild-type littermates. *In vitro*, knockdown of Carm1 impairs 3T3-L1 adipogenesis. To prove a cell-autonomous role of Carm1 in adipogenesis, tissue-specific knockout mouse studies are needed. Although reporter assays suggest that Carm1 is a PPARγ coactivator, it needs to be determined whether Carm1 colocalizes with PPARγ on target gene loci. Carm1 has been shown to cooperate with other epigenomic factors, in particular, the enhancer regulators CBP and p300 (116, 117), suggesting the possibility that Carm1 regulates adipogenesis by participating in enhancer activation. Future studies are needed to determine whether the methyltransferase activity of Carm1 is required for adipogenesis.

Prmt5 has also been implicated in regulating adipogenesis (118). Knockdown of Prmt5 inhibits adipogenesis and adipogenic gene expression in 3T3-L1 and C3H10T1/2 cells. A recent study used chromosome conformation capture assay and identified a differentiation-dependent and Prmt5-dependent interaction between *Pparg2* promoter and kb −10 enhancer regions in C3H10T1/2 cells after differentiation (119). Prmt5 is also required for Med1 and Brg1 binding at the *Pparg2* promoter and the kb −10 enhancer. Given that Prmt5 is essential for cell growth (120), it remains unclear whether Prmt5 directly regulates adipogenic gene expression and whether the methyltransferase activity of Prmt5 is required for adipogenesis.

Histone acetyltransferases. Acetylation on histone lysine residues generally correlates with gene activation and is catalyzed by site-specific histone acetyltransferases (HATs). For example, Gcn5 and PCAF are major enzymes for acetylation on H3K9

(H3K9ac), while CBP and p300 are responsible for acetylation on H3K18 (H3K18ac) and H3K27 (H3K27ac) in mammalian cells (121–123).

Gcn5 and PCAF function redundantly to regulate adipogenesis (124). Double knockout of *Gcn5* and *Pcaf* by Myf5-Cre impairs BAT development in mice. *In vitro*, Gcn5/PCAF and their catalytic activities are required for adipogenesis. Interestingly, ectopic PPARγ can rescue adipogenesis but not brown adipocyte-enriched Prdm16 expression in *Gcn5 Pcaf* double knockout cells, suggesting that Gcn5 and PCAF regulate transcription of general adipogenic genes and brown adipocyte-enriched genes through different mechanisms. Determining genomic localization of Gcn5 and/or PCAF would provide a better understanding of the mechanism by which this functionally redundant pair of HATs regulates adipocyte genes and BAT-enriched genes in brown adipogenesis.

CBP and p300 are required for adipogenesis. Ribozyme-mediated targeting of either CBP or p300 inhibits adipogenesis and PPARγ target gene expression, suggesting that these two enzymes are not mutually compensating each other in regulating adipogenesis (125). *Crebbp*$^{+/-}$ mice have markedly reduced WAT mass compared to wild-type littermates, suggesting that CBP is essential for adipogenesis *in vivo* (126). CBP/p300-mediated H3K27ac marks active enhancers (127, 128). Consistently, profiling of CBP/p300 binding sites demonstrated that genomic binding of these HATs drives enhancer activation during adipogenesis (41).

Histone deacetylases and sirtuins. Histone deacetylation is mediated by classical zinc-dependent histone deacetylases (HDACs) and NAD$^+$-dependent sirtuins (SIRTs) (129). Studies have suggested diverse roles of HDACs and SIRTs in adipogenesis.

Among 11 HDACs, Hdac1, Hdac2, and Hdac9 have been shown to regulate adipogenesis. Hdac1 and Hdac2 positively regulate adipogenesis redundantly, since knockout of both, but not the individual knockout of each, inhibits adipogenesis of fibroblasts (130). In contrast, Hdac9 negatively regulates adipogenesis. Hdac9 expression is downregulated during adipogenesis. Preadipocytes isolated from *Hdac9* knockout mice show accelerated adipogenesis, while overexpression of Hdac9 represses 3T3-L1 adipogenesis independently of deacetylase domain (131). The roles of HDACs in adipogenesis *in vivo* and the genomic localization of HDACs remain to be determined.

Among seven SIRTs, Sirt1, Sirt2, Sirt6, and Sirt7 have been implicated in regulating adipogenesis. Fabp4-Cre-mediated *Sirt1* knockout mice show increased adipose tissue mass under conditions of a high-fat diet, suggesting a negative role of Sirt1 in adipose tissue development (132). In culture, *Sirt1* knockout enhances adipogenesis in MEFs (133). Sirt2 has also been implicated in negative regulation of adipogenesis. Two studies reported that stable knockdown of Sirt2 promotes adipogenesis in 3T3-L1 cells. Mechanistically, Sirt2 inhibits adipogenesis by deacetylating FOXO1, which represses PPARγ transcriptional activity (134, 135). In contrast, whole-body *Sirt7* knockout mice show reduced WAT mass, suggesting that Sirt7 positively regulates adipose tissue development (136). Sirt6 regulates mitotic clonal expansion in cells and is required for adipogenesis (137). Future studies are needed to identify direct target genes of SIRTs in adipogenesis and to demonstrate whether SIRTs play a cell-autonomous role in regulating adipogenesis *in vivo*.

Epigenomic reader Brd4. Brd4 is a member of the family of bromodomain and extraterminal domain (BET) proteins, which also includes Brd2 and Brd3 (138). Brd4 binds to acetylated histones and TFs and acts as an epigenomic reader (139). Brd4 is critical for adipogenesis in culture and in mice (97). Deletion of *Brd4* in preadipocytes prevents adipogenesis and adipogenic gene induction. During adipogenesis, lineage-determining transcription factors (LDTFs) cooperate with enhancer epigenomic writers MLL3/4 and CBP/p300 to recruit Brd4 to active enhancers, which facilitates the recruitment of Pol II, Mediator, TFIID, and p-TEFb. An independent study also showed that Brd4 is enriched on superenhancers in 3T3-L1 adipogenesis (140). Consistently, BET inhibitors block adipogenesis of 3T3-L1 and C3H10T1/2 cells (140, 141). In mice, Myf5-Cre-mediated knockout of *Brd4* impairs BAT development. Although Brd4 is essential for adipogenesis, it is largely dispensable for maintaining differentiated

adipocytes, as evidenced by the lack of the phenotype in mice with Adipoq-Cre-mediated *Brd4* knockout (97). Interestingly, another BET family protein, Brd2, has been suggested to inhibit adipogenesis (142, 143). Further study is necessary to clarify the shared and distinct functions of BET family members in adipogenesis *in vivo*.

Chromatin remodeling complex. SWI/SNF is a multisubunit ATP-dependent chromatin remodeling complex that uses Brg1 or Brm as the ATPase (144). C/EBPs initiate recruitment of SWI/SNF to *Pparg2* promoter in adipogenesis (145). Ectopic expression of dominant-negative Brg1 or Brm interferes with PPARγ-, C/EBPα-, or C/EBPβ-mediated adipogenesis in fibroblasts, suggesting that the SWI/SNF complex is required for adipogenesis. Whether the SWI/SNF complex is required for chromatin opening and adipogenesis *in vivo* and how it regulates adipogenic gene expression remain largely unclear. Recent studies in human cancer cells suggested that the SWI/SNF complex is critical for enhancer activation (146, 147). Whether the SWI/SNF complex is required for activation of enhancers critical for adipogenic gene expression may require further investigation.

DNA methylation. DNA methylation is catalyzed by DNA methyltransferases (DNMTs) and is removed by TET dioxygenases. There are five DNMTs responsible for establishing or maintaining DNA methylation (148). The role of DNMTs and DNA methylation in adipogenesis has been controversial. An earlier study showed that small interfering RNA (siRNA) knockdown of DNMT1 in 3T3-L1 cells reduces DNA methylation levels on the *Pparg* gene and accelerates adipogenesis (149). However, a recent study showed that pharmacological inhibition of DNA methylation by 5-aza-dC treatment suppresses adipogenesis and is accompanied by demethylation and upregulation of *Wnt10a* (150). To clarify the role of DNMTs in adipogenesis *in vivo*, tissue-specific knockout mouse studies are necessary.

DNA methylation in the form of 5-methylcytosine (5mC) can be reversed by TET1/2/3 (151). A study has shown that 5-hydroxymethylcytosine (5hmC), an intermediate of DNA demethylation process, marks a subset of PPARγ- and C/EBPα-bound enhancers during 3T3-L1 adipogenesis. Addition of 5hmC during adipogenesis coincides with increased levels of enhancer marks H3K4me2 and H3K27ac (152). In line with this finding, double knockout of *Tet1* and *Tet2* has been shown to block adipogenesis of mouse embryonic fibroblasts (153). The role of TETs in regulating adipogenesis should be investigated *in vivo* using tissue-specific knockout mice. In addition, whether Tet1 and/or Tet2 localizes on enhancers to regulate adipogenic gene expression needs to be determined.

microRNAs. MicroRNAs (miRNAs) are noncoding RNAs that posttranscriptionally regulate gene expression by degrading target mRNA or inhibiting protein synthesis (154). Studies have suggested that several miRNAs play a role in adipose tissue development and associated metabolism (155). A study used miRNA microarray analysis to profile PPARγ-regulated miRNAs in human adipocytes (156). Among 27 miRNAs whose expression was altered by treatment of pioglitazone, a synthetic PPARγ ligand, miR-378, has been shown to promote adipogenesis of subcutaneous adipocytes but not of visceral adipocytes. miR-378 is embedded in the first intron of *PPARGC1b*, which encodes PGC-1β. The target genes of miR-378 and its working mechanisms in adipogenesis have yet to be examined. Another important intronic miRNA is miR-33, which is embedded within the *SREBP-2* gene and targets ABCA1 to control cholesterol homeostasis (157, 158). Whole-body miR-33 knockout mice gradually become obese and develop hepatic steatosis through derepression of SREBP-1 in the liver (159). How miR-33 regulates adipogenesis and metabolism in adipocytes remains to be understood.

CONCLUSIONS AND FUTURE DIRECTIONS

In recent years, one major discovery in transcriptional regulation of adipogenesis is the identification of EBF2 as a major regulator of brown adipogenesis. Further study is necessary to clarify the role of EBFs in general versus in brown adipogenesis and their potential redundancy. This can be achieved by generating tissue-specific knockout of single and multiple EBFs in mice. Another important contribution to understanding transcriptional
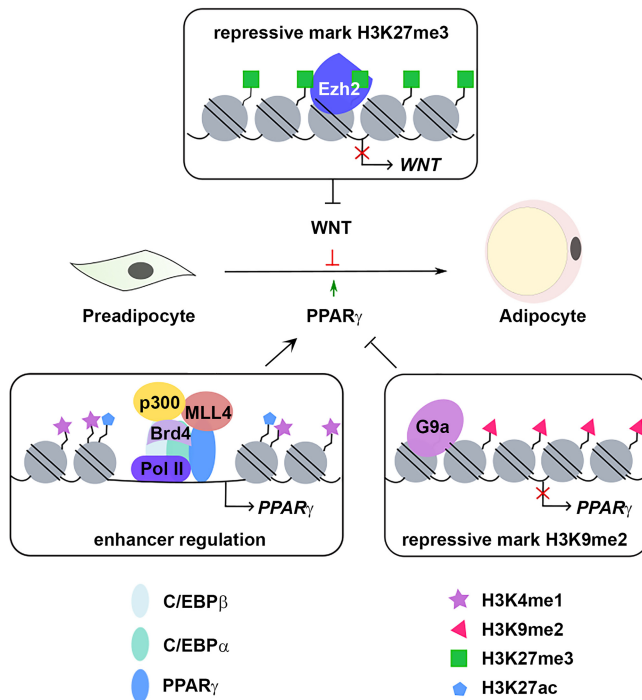
FIG 5 Epigenomic mechanisms control expression of master regulators of adipogenesis. Epigenomic factors such as MLL4, p300, and Brd4 bind to enhancers to activate expression of adipogenesis genes, including the master adipogenic TF PPARγ gene. Two repressive histone marks oppositely regulate adipogenesis. Ezh2-mediated H3K27me3 represses *Wnt* gene expression and promotes adipogenesis, whereas G9a-mediated H3K9me2 represses *Pparg* expression and inhibits adipogenesis.

regulation of adipogenesis is the exclusion of Krox20, KLF4, and GR from the list of adipogenic TFs, which underscores the importance of studying adipogenesis *in vivo* instead of drawing conclusions based on the knockdown approach in cell culture.

During the last decade, many epigenomic factors have been shown to regulate adipogenesis. These factors are involved in regulating histone modification, chromatin remodeling, and DNA methylation. Studies have revealed sequential actions of epigenomic factors on enhancers in adipogenesis. C/EBPβ recruits MLL3/MLL4 to prime enhancers, which facilitates binding of CBP/p300 to activate and label enhancers with H3K27ac. H3K27ac and acetylated TFs are recognized by Brd4, which recruits Mediator and Pol II to activate PPARγ and C/EBPα expression (41, 91, 97). The SWI/SNF complex may participate in these sequential events, leading to lineage-specific enhancer activation in adipogenesis (Fig. 5). The expression of adipogenesis master regulators is also under the control of two mutually exclusive repressive histone marks. G9a-mediated H3K9me2 represses PPARγ expression. Ezh2-mediated H3K27me3 represses Wnt expression (Fig. 5).

Despite the rapid progress in the study of transcriptional and epigenomic regulation of adipogenesis, several issues remain to be addressed and new techniques need to be employed. First, although cell culture systems have revealed important mechanisms regulating adipogenesis, they do not fully recapitulate adipogenesis *in vivo*. Many putative adipogenic TFs identified in cell culture need to be validated using mouse models, and specific and direct target genes of these TFs need to be identified by ChIP-Seq. Second, adipose tissues are heterogeneous and contain multiple cell types (160). Recently developed INTACT, TRAP, and NuTRAP transgenic mice allow isolation of mRNA and nuclei from specific cell types. These techniques will enable transcriptional and epigenomic profiling of adipogenesis *in vivo* using heterogeneous adipose tissues (161–163). Third, recent advances in single-cell technology provide opportunities for transcriptomic and epigenomic studies at single-cell resolution (164). This will help identify cell lineages and distinct developmental stages during adipogenesis. Fourth,

RNAi-mediated knockdown approach often lacks sufficient specificity and tends to create artifacts (165). Artifacts generated by RNAi are also found in the adipogenesis field. The recently developed revolutionary genome editing technique that uses the clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 system has enabled time- and cost-efficient gene knockout (166). By generating knockout cells by the use of the CRISPR-Cas9 approach, roles of poorly characterized, putative adipogenic factors can be reevaluated. Fifth, studies have implicated noncoding RNAs in regulating adipogenesis in culture (167, 168). Although knockdown of several candidate noncoding RNAs inhibits adipogenesis, more efforts are needed to determine the mechanism by which these noncoding RNAs regulate adipogenesis and to validate their roles with *in vivo* models. Sixth, higher-order chromatin interactions, in particular, promoter-enhancer looping, are involved in regulating gene expression during differentiation and development (169). It has been shown that promoter-anchored chromatin loops change dynamically during adipogenesis (170). Future efforts are needed to identify the factors that are responsible for dynamic promoter-enhancer looping during adipogenesis.

REFERENCES

1. Rosen ED, MacDougald OA. 2006. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 7:885–896. https://doi.org/10.1038/nrm2066.
2. Cypess AM, Kahn CR. 2010. Brown fat as a therapy for obesity and diabetes. Curr Opin Endocrinol Diabetes Obes 17:143–149. https://doi.org/10.1097/MED.0b013e328337a81f.
3. Cristancho AG, Lazar MA. 2011. Forming functional fat: a growing understanding of adipocyte differentiation. Nat Rev Mol Cell Biol 12:722–734. https://doi.org/10.1038/nrm3198.
4. Green H, Meuth M. 1974. An established pre-adipose cell line and its differentiation in culture. Cell 3:127–133. https://doi.org/10.1016/0092-8674(74)90116-0.
5. Reznikoff CA, Brankow DW, Heidelberger C. 1973. Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of division. Cancer Res 33:3231–3238.
6. Tallquist MD, Weismann KE, Hellstrom M, Soriano P. 2000. Early myotome specification regulates PDGFA expression and axial skeleton development. Development 127:5059–5070.
7. Eguchi J, Wang X, Yu S, Kershaw EE, Chiu PC, Dushay J, Estall JL, Klein U, Maratos-Flier E, Rosen ED. 2011. Transcriptional control of adipose lipid handling by IRF4. Cell Metab 13:249–259. https://doi.org/10.1016/j.cmet.2011.02.005.
8. Wang ZV, Deng Y, Wang QA, Sun K, Scherer PE. 2010. Identification and characterization of a promoter cassette conferring adipocyte-specific gene expression. Endocrinology 151:2933–2939. https://doi.org/10.1210/en.2010-0136.
9. Barlow C, Schroeder M, Lekstrom-Himes J, Kylefjord H, Deng CX, Wynshaw-Boris A, Spiegelman BM, Xanthopoulos KG. 1997. Targeted expression of Cre recombinase to adipose tissue of transgenic mice directs adipose-specific excision of loxP-flanked gene segments. Nucleic Acids Res 25:2543–2545. https://doi.org/10.1093/nar/25.12.2543.
10. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB. 2001. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature 409:729–733. https://doi.org/10.1038/35055575.
11. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM. 2003. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. Proc Natl Acad Sci U S A 100:15712–15717. https://doi.org/10.1073/pnas.2536828100.
12. Lee KY, Russell SJ, Ussar S, Boucher J, Vernochet C, Mori MA, Smyth G, Rourk M, Cederquist C, Rosen ED, Kahn BB, Kahn CR. 2013. Lessons on conditional gene targeting in mouse adipose tissue. Diabetes 62:864–874. https://doi.org/10.2337/db12-1089.
13. Jeffery E, Berry R, Church CD, Yu S, Shook BA, Horsley V, Rosen ED, Rodeheffer MS. 2014. Characterization of Cre recombinase models for the study of adipose tissue. Adipocyte 3:206–211. https://doi.org/10.4161/adip.29674.
14. Loft A, Forss I, Mandrup S. 2017. Genome-wide insights into the development and function of thermogenic adipocytes. Trends Endocrinol Metab 28:104–120. https://doi.org/10.1016/j.tem.2016.11.005.
15. Wang W, Seale P. 2016. Control of brown and beige fat development. Nat Rev Mol Cell Biol 17:691–702. https://doi.org/10.1038/nrm.2016.96.
16. Inagaki T, Sakai J, Kajimura S. 2016. Transcriptional and epigenetic control of brown and beige adipose cell fate and function. Nat Rev Mol Cell Biol 17:480–495. https://doi.org/10.1038/nrm.2016.62.
17. Linhart HG, Ishimura-Oka K, DeMayo F, Kibe T, Repka D, Poindexter B, Bick RJ, Darlington GJ. 2001. C/EBPalpha is required for differentiation of white, but not brown, adipose tissue. Proc Natl Acad Sci U S A 98:12532–12537. https://doi.org/10.1073/pnas.211416898.
18. Tontonoz P, Hu E, Spiegelman BM. 1994. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell 79:1147–1156. https://doi.org/10.1016/0092-8674(94)90006-X.
19. Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, Koder A, Evans RM. 1999. PPAR gamma is required for placental, cardiac, and adipose tissue development. Mol Cell 4:585–595. https://doi.org/10.1016/S1097-2765(00)80209-9.
20. Wu Z, Rosen ED, Brun R, Hauser S, Adelmant G, Troy AE, McKeon C, Darlington GJ, Spiegelman BM. 1999. Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity. Mol Cell 3:151–158. https://doi.org/10.1016/S1097-2765(00)80306-8.
21. Lin FT, Lane MD. 1992. Antisense CCAAT/enhancer-binding protein RNA suppresses coordinate gene expression and triglyceride accumulation during differentiation of 3T3-L1 preadipocytes. Genes Dev 6:533–544. https://doi.org/10.1101/gad.6.4.533.
22. Farmer SR. 2006. Transcriptional control of adipocyte formation. Cell Metab 4:263–273. https://doi.org/10.1016/j.cmet.2006.07.001.
23. Lee JE, Ge K. 2014. Transcriptional and epigenetic regulation of PPAR-gamma expression during adipogenesis. Cell Biosci 4:29. https://doi.org/10.1186/2045-3701-4-29.
24. Lefterova MI, Haakonsson AK, Lazar MA, Mandrup S. 2014. PPARgamma and the global map of adipogenesis and beyond. Trends Endocrinol Metab 25:293–302. https://doi.org/10.1016/j.tem.2014.04.001.
25. Siersbæk R, Mandrup S. 2011. Transcriptional networks controlling adipocyte differentiation. Cold Spring Harbor Symp Quant Biol 76:247–255. https://doi.org/10.1101/sqb.2011.76.010512.
26. Tamori Y, Masugi J, Nishino N, Kasuga M. 2002. Role of peroxisome

proliferator-activated receptor-gamma in maintenance of the characteristics of mature 3T3-L1 adipocytes. Diabetes 51:2045–2055. https://doi.org/10.2337/diabetes.51.7.2045.

27. Imai T, Takakuwa R, Marchand S, Dentz E, Bornert JM, Messaddeq N, Wendling O, Mark M, Desvergne B, Wahli W, Chambon P, Metzger D. 2004. Peroxisome proliferator-activated receptor gamma is required in mature white and brown adipocytes for their survival in the mouse. Proc Natl Acad Sci U S A 101:4543–4547. https://doi.org/10.1073/pnas.0400356101.

28. Nielsen R, Pedersen TA, Hagenbeek D, Moulos P, Siersbaek R, Megens E, Denissov S, Borgesen M, Francoijs KJ, Mandrup S, Stunnenberg HG. 2008. Genome-wide profiling of PPARgamma:RXR and RNA polymerase II occupancy reveals temporal activation of distinct metabolic pathways and changes in RXR dimer composition during adipogenesis. Genes Dev 22:2953–2967. https://doi.org/10.1101/gad.501108.

29. Lefterova MI, Zhang Y, Steger DJ, Schupp M, Schug J, Cristancho A, Feng D, Zhuo D, Stoeckert CJ, Jr, Liu XS, Lazar MA. 2008. PPAR{gamma} and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. Genes Dev 22:2941–2952. https://doi.org/10.1101/gad.1709008.

30. Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. 2000. Transcriptional regulation of adipogenesis. Genes Dev 14:1293–1307.

31. Steger DJ, Grant GR, Schupp M, Tomaru T, Lefterova MI, Schug J, Manduchi E, Stoeckert CJ, Lazar MA. 2010. Propagation of adipogenic signals through an epigenomic transition state. Genes Dev 24:1035–1044. https://doi.org/10.1101/gad.1907110.

32. Siersbæk R, Nielsen R, John S, Sung M-H, Baek S, Loft A, Hager GL, Mandrup S. 2011. Extensive chromatin remodelling and establishment of transcription factor 'hotspots' during early adipogenesis. EMBO J 30:1459–1472. https://doi.org/10.1038/emboj.2011.65.

33. Cao Z, Umek RM, McKnight SL. 1991. Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. Genes Dev 5:1538–1552. https://doi.org/10.1101/gad.5.9.1538.

34. Wu Z, Bucher NL, Farmer SR. 1996. Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. Mol Cell Biol 16:4128–4136. https://doi.org/10.1128/MCB.16.8.4128.

35. Tanaka T, Yoshida N, Kishimoto T, Akira S. 1997. Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene. EMBO J 16:7432–7443. https://doi.org/10.1093/emboj/16.24.7432.

36. Akerblad P, Lind U, Liberg D, Bamberg K, Sigvardsson M. 2002. Early B-cell factor (O/E-1) is a promoter of adipogenesis and involved in control of genes important for terminal adipocyte differentiation. Mol Cell Biol 22:8015–8025. https://doi.org/10.1128/MCB.22.22.8015-8025.2002.

37. Jimenez MA, Akerblad P, Sigvardsson M, Rosen ED. 2007. Critical role for Ebf1 and Ebf2 in the adipogenic transcriptional cascade. Mol Cell Biol 27:743–757. https://doi.org/10.1128/MCB.01557-06.

38. Wang W, Kissig M, Rajakumari S, Huang L, Lim HW, Won KJ, Seale P. 2014. Ebf2 is a selective marker of brown and beige adipogenic precursor cells. Proc Natl Acad Sci U S A 111:14466–14471. https://doi.org/10.1073/pnas.1412685111.

39. Rajakumari S, Wu J, Ishibashi J, Lim HW, Giang AH, Won KJ, Reed RR, Seale P. 2013. EBF2 determines and maintains brown adipocyte identity. Cell Metab 17:562–574. https://doi.org/10.1016/j.cmet.2013.01.015.

40. Shapira SN, Lim HW, Rajakumari S, Sakers AP, Ishibashi J, Harms MJ, Won KJ, Seale P. 2017. EBF2 transcriptionally regulates brown adipogenesis via the histone reader DPF3 and the BAF chromatin remodeling complex. Genes Dev 31:660–673. https://doi.org/10.1101/gad.294405.116.

41. Lai B, Lee JE, Jang Y, Wang L, Peng W, Ge K. 2017. MLL3/MLL4 are required for CBP/p300 binding on enhancers and super-enhancer formation in brown adipogenesis. Nucleic Acids Res 45:6388–6403. https://doi.org/10.1093/nar/gkx234.

42. Fretz JA, Nelson T, Xi Y, Adams DJ, Rosen CJ, Horowitz MC. 2010. Altered metabolism and lipodystrophy in the early B-cell factor 1-deficient mouse. Endocrinology 151:1611–1621. https://doi.org/10.1210/en.2009-0987.

43. Boller S, Li R, Grosschedl R. 2018. Defining B cell chromatin: lessons from EBF1. Trends Genet 34:257–269. https://doi.org/10.1016/j.tig.2017.12.014.

44. Cohen DM, Won KJ, Nguyen N, Lazar MA, Chen CS, Steger DJ. 2015. ATF4 licenses C/EBPbeta activity in human mesenchymal stem cells primed for adipogenesis. Elife 4:e06821. https://doi.org/10.7554/eLife.06821.

45. Yu K, Mo D, Wu M, Chen H, Chen L, Li M, Chen Y. 2014. Activating transcription factor 4 regulates adipocyte differentiation via altering the coordinate expression of CCATT/enhancer binding protein beta and peroxisome proliferator-activated receptor gamma. FEBS J 281:2399–2409. https://doi.org/10.1111/febs.12792.

46. Seo J, Fortuno ES, III, Suh JM, Stenesen D, Tang W, Parks EJ, Adams CM, Townes T, Graff JM. 2009. Atf4 regulates obesity, glucose homeostasis, and energy expenditure. Diabetes 58:2565–2573. https://doi.org/10.2337/db09-0335.

47. Masuoka HC, Townes TM. 2002. Targeted disruption of the activating transcription factor 4 gene results in severe fetal anemia in mice. Blood 99:736–745. https://doi.org/10.1182/blood.V99.3.736.

48. Waki H, Nakamura M, Yamauchi T, Wakabayashi K, Yu J, Hirose-Yotsuya L, Take K, Sun W, Iwabu M, Okada-Iwabu M, Fujita T, Aoyama T, Tsutsumi S, Ueki K, Kodama T, Sakai J, Aburatani H, Kadowaki T. 2011. Global mapping of cell type-specific open chromatin by FAIRE-seq reveals the regulatory role of the NFI family in adipocyte differentiation. PLoS Genet 7:e1002311. https://doi.org/10.1371/journal.pgen.1002311.

49. Hiraike Y, Waki H, Yu J, Nakamura M, Miyake K, Nagano G, Nakaki R, Suzuki K, Kobayashi H, Yamamoto S, Sun W, Aoyama T, Hirota Y, Ohno H, Oki K, Yoneda M, White AP, Tseng YH, Cypess AM, Larsen TJ, Jespersen NZ, Scheele C, Tsutsumi S, Aburatani H, Yamauchi T, Kadowaki T. 2017. NFIA co-localizes with PPARgamma and transcriptionally controls the brown fat gene program. Nat Cell Biol 19:1081–1092. https://doi.org/10.1038/ncb3590.

50. Rubin CS, Hirsch A, Fung C, Rosen OM. 1978. Development of hormone receptors and hormonal responsiveness in vitro. Insulin receptors and insulin sensitivity in the preadipocyte and adipocyte forms of 3T3-L1 cells. J Biol Chem 253:7570–7578.

51. Ge K. 2012. Epigenetic regulation of adipogenesis by histone methylation. Biochim Biophys Acta 1819:727–732. https://doi.org/10.1016/j.bbagrm.2011.12.008.

52. Chen Z, Torrens JI, Anand A, Spiegelman BM, Friedman JM. 2005. Krox20 stimulates adipogenesis via C/EBPbeta-dependent and -independent mechanisms. Cell Metab 1:93–106. https://doi.org/10.1016/j.cmet.2004.12.009.

53. Birsoy K, Chen Z, Friedman J. 2008. Transcriptional regulation of adipogenesis by KLF4. Cell Metab 7:339–347. https://doi.org/10.1016/j.cmet.2008.02.001.

54. Park YK, Wang L, Giampietro A, Lai B, Lee JE, Ge K. 4 January 2017, posting date. Distinct roles of transcription factors KLF4, Krox20, and peroxisome proliferator-activated receptor gamma in adipogenesis. Mol Cell Biol https://doi.org/10.1128/MCB.00554-16.

55. Deslex S, Negrel R, Ailhaud G. 1987. Development of a chemically defined serum-free medium for differentiation of rat adipose precursor cells. Exp Cell Res 168:15–30. https://doi.org/10.1016/0014-4827(87)90412-5.

56. Gregoire F, Todoroff G, Hauser N, Remacle C. 1990. The stroma-vascular fraction of rat inguinal and epididymal adipose tissue and the adipoconversion of fat cell precursors in primary culture. Biol Cell 69:215–222. https://doi.org/10.1016/0248-4900(90)90348-7.

57. Park YK, Ge K. January 4 2017, posting date. Glucocorticoid receptor accelerates, but is dispensable for, adipogenesis. Mol Cell Biol https://doi.org/10.1128/MCB.00260-16.

58. Bauerle KT, Hutson I, Scheller EL, Harris CA. 2018. Glucocorticoid receptor signaling is not required for in vivo adipogenesis. Endocrinology 159:2050–2061. https://doi.org/10.1210/en.2018-00118.

59. Wang JC, Gray NE, Kuo T, Harris CA. 2012. Regulation of triglyceride metabolism by glucocorticoid receptor. Cell Biosci 2:19. https://doi.org/10.1186/2045-3701-2-19.

60. Gupta RK, Arany Z, Seale P, Mepani RJ, Ye L, Conroe HM, Roby YA, Kulaga H, Reed RR, Spiegelman BM. 2010. Transcriptional control of preadipocyte determination by Zfp423. Nature 464:619–623. https://doi.org/10.1038/nature08816.

61. Shao M, Ishibashi J, Kusminski CM, Wang QA, Hepler C, Vishvanath L, MacPherson KA, Spurgin SB, Sun K, Holland WL, Seale P, Gupta RK. 2016. Zfp423 maintains white adipocyte identity through suppression of the beige cell thermogenic gene program. Cell Metab 23:1167–1184. https://doi.org/10.1016/j.cmet.2016.04.023.

62. Hepler C, Shao M, Xia JY, Ghaben AL, Pearson MJ, Vishvanath L, Sharma AX, Morley TS, Holland WL, Gupta RK. 2017. Directing visceral white adipocyte precursors to a thermogenic adipocyte fate improves insulin sensitivity in obese mice. Elife https://doi.org/10.7554/eLife.27669.

63. Shao M, Hepler C, Vishvanath L, MacPherson KA, Busbuso NC, Gupta RK. 2017. Fetal development of subcutaneous white adipose tissue is dependent on Zfp423. Mol Metab 6:111–124. https://doi.org/10.1016/j.molmet.2016.11.009.

64. Hong KY, Bae H, Park I, Park DY, Kim KH, Kubota Y, Cho ES, Kim H, Adams RH, Yoo OJ, Koh GY. 2015. Perilipin+ embryonic preadipocytes actively proliferate along growing vasculatures for adipose expansion. Development 142:2623–2632. https://doi.org/10.1242/dev.125336.

65. Zhang JW, Klemm DJ, Vinson C, Lane MD. 2004. Role of CREB in transcriptional regulation of CCAAT/enhancer-binding protein beta gene during adipogenesis. J Biol Chem 279:4471–4478. https://doi.org/10.1074/jbc.M311327200.

66. Oishi Y, Manabe I, Tobe K, Tsushima K, Shindo T, Fujiu K, Nishimura G, Maemura K, Yamauchi T, Kubota N, Suzuki R, Kitamura T, Akira S, Kadowaki T, Nagai R. 2005. Kruppel-like transcription factor KLF5 is a key regulator of adipocyte differentiation. Cell Metab 1:27–39. https://doi.org/10.1016/j.cmet.2004.11.005.

67. Mori T, Sakaue H, Iguchi H, Gomi H, Okada Y, Takashima Y, Nakamura K, Nakamura T, Yamauchi T, Kubota N, Kadowaki T, Matsuki Y, Ogawa W, Hiramatsu R, Kasuga M. 2005. Role of Kruppel-like factor 15 (KLF15) in transcriptional regulation of adipogenesis. J Biol Chem 280:12867–12875. https://doi.org/10.1074/jbc.M410515200.

68. Nanbu-Wakao R, Morikawa Y, Matsumura I, Masuho Y, Muramatsu MA, Senba E, Wakao H. 2002. Stimulation of 3T3-L1 adipogenesis by signal transducer and activator of transcription 5. Mol Endocrinol 16:1565–1576. https://doi.org/10.1210/mend.16.7.0862.

69. Tong Q, Dalgin G, Xu H, Ting C-N, Leiden JM, Hotamisligil GS. 2000. Function of GATA transcription factors in preadipocyte-adipocyte transition. Science 290:134–138. https://doi.org/10.1126/science.290.5489.134.

70. Tong Q, Tsai J, Tan G, Dalgin G, Hotamisligil GS. 2005. Interaction between GATA and the C/EBP family of transcription factors is critical in GATA-mediated suppression of adipocyte differentiation. Mol Cell Biol 25:706–715. https://doi.org/10.1128/MCB.25.2.706-715.2005.

71. Banerjee SS, Feinberg MW, Watanabe M, Gray S, Haspel RL, Denkinger DJ, Kawahara R, Hauner H, Jain MK. 2003. The Kruppel-like factor KLF2 inhibits peroxisome proliferator-activated receptor-gamma expression and adipogenesis. J Biol Chem 278:2581–2584. https://doi.org/10.1074/jbc.M210859200.

72. Moitra J, Mason MM, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B, Feigenbaum L, Lee E, Aoyama T, Eckhaus M, Reitman ML, Vinson C. 1998. Life without white fat: a transgenic mouse. Genes Dev 12:3168–3181. https://doi.org/10.1101/gad.12.20.3168.

73. Eberle D, Hegarty B, Bossard P, Ferre P, Foufelle F. 2004. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 86:839–848. https://doi.org/10.1016/j.biochi.2004.09.018.

74. Kim JB, Wright HM, Wright M, Spiegelman BM. 1998. ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand. Proc Natl Acad Sci U S A 95:4333–4337. https://doi.org/10.1073/pnas.95.8.4333.

75. Shimano H, Shimomura I, Hammer RE, Herz J, Goldstein JL, Brown MS, Horton JD. 1997. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. J Clin Invest 100:2115–2124. https://doi.org/10.1172/JCI119746.

76. York B, O'Malley BW. 2010. Steroid receptor coactivator (SRC) family: masters of systems biology. J Biol Chem 285:38743–38750. https://doi.org/10.1074/jbc.R110.193367.

77. Malik S, Roeder RG. 2005. Dynamic regulation of pol II transcription by the mammalian Mediator complex. Trends Biochem Sci 30:256–263. https://doi.org/10.1016/j.tibs.2005.03.009.

78. Ge K, Guermah M, Yuan CX, Ito M, Wallberg AE, Spiegelman BM, Roeder RG. 2002. Transcription coactivator TRAP220 is required for PPAR gamma 2-stimulated adipogenesis. Nature 417:563–567. https://doi.org/10.1038/417563a.

79. Grontved L, Madsen MS, Boergesen M, Roeder RG, Mandrup S. 2010. MED14 tethers mediator to the N-terminal domain of peroxisome proliferator-activated receptor gamma and is required for full transcriptional activity and adipogenesis. Mol Cell Biol 30:2155–2169. https://doi.org/10.1128/MCB.01238-09.

80. Wang W, Huang L, Huang Y, Yin JW, Berk AJ, Friedman JM, Wang G. 2009. Mediator MED23 links insulin signaling to the adipogenesis transcription cascade. Dev Cell 16:764–771. https://doi.org/10.1016/j.devcel.2009.04.006.

81. Picard F, Gehin M, Annicotte J, Rocchi S, Champy MF, O'Malley BW, Chambon P, Auwerx J. 2002. SRC-1 and TIF2 control energy balance between white and brown adipose tissues. Cell 111:931–941. https://doi.org/10.1016/S0092-8674(02)01169-8.

82. Louet JF, Coste A, Amazit L, Tannour-Louet M, Wu RC, Tsai SY, Tsai MJ, Auwerx J, O'Malley BW. 2006. Oncogenic steroid receptor coactivator-3 is a key regulator of the white adipogenic program. Proc Natl Acad Sci U S A 103:17868–17873. https://doi.org/10.1073/pnas.0608711103.

83. Villanueva CJ, Vergnes L, Wang J, Drew BG, Hong C, Tu Y, Hu Y, Peng X, Xu F, Saez E, Wroblewski K, Hevener AL, Reue K, Fong LG, Young SG, Tontonoz P. 2013. Adipose subtype-selective recruitment of TLE3 or Prdm16 by PPARgamma specifies lipid storage versus thermogenic gene programs. Cell Metab 17:423–435. https://doi.org/10.1016/j.cmet.2013.01.016.

84. Villanueva CJ, Waki H, Godio C, Nielsen R, Chou WL, Vargas L, Wroblewski K, Schmedt C, Chao LC, Boyadjian R, Mandrup S, Hevener A, Saez E, Tontonoz P. 2011. TLE3 is a dual-function transcriptional coregulator of adipogenesis. Cell Metab 13:413–427. https://doi.org/10.1016/j.cmet.2011.02.014.

85. Ong CT, Corces VG. 2011. Enhancer function: new insights into the regulation of tissue-specific gene expression. Nat Rev Genet 12:283–293. https://doi.org/10.1038/nrg2957.

86. Reik W. 2007. Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 447:425–432. https://doi.org/10.1038/nature05918.

87. Chen T, Dent SY. 2014. Chromatin modifiers and remodellers: regulators of cellular differentiation. Nat Rev Genet 15:93–106. https://doi.org/10.1038/nrg3607.

88. Musselman CA, Lalonde ME, Cote J, Kutateladze TG. 2012. Perceiving the epigenetic landscape through histone readers. Nat Struct Mol Biol 19:1218–1227. https://doi.org/10.1038/nsmb.2436.

89. Li B, Carey M, Workman JL. 2007. The role of chromatin during transcription. Cell 128:707–719. https://doi.org/10.1016/j.cell.2007.01.015.

90. Kouzarides T. 2007. Chromatin modifications and their function. Cell 128:693–705. https://doi.org/10.1016/j.cell.2007.02.005.

91. Lee JE, Wang C, Xu S, Cho YW, Wang L, Feng X, Baldridge A, Sartorelli V, Zhuang L, Peng W, Ge K. 2013. H3K4 mono- and di-methyltransferase MLL4 is required for enhancer activation during cell differentiation. Elife 2:e01503. https://doi.org/10.7554/eLife.01503.

92. Ruthenburg AJ, Allis CD, Wysocka J. 2007. Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. Mol Cell 25:15–30. https://doi.org/10.1016/j.molcel.2006.12.014.

93. Rao RC, Dou Y. 2015. Hijacked in cancer: the KMT2 (MLL) family of methyltransferases. Nat Rev Cancer 15:334–346. https://doi.org/10.1038/nrc3929.

94. Wang C, Lee JE, Lai B, Macfarlan TS, Xu S, Zhuang L, Liu C, Peng W, Ge K. 2016. Enhancer priming by H3K4 methyltransferase MLL4 controls cell fate transition. Proc Natl Acad Sci U S A 113:11871–11876. https://doi.org/10.1073/pnas.1606857113.

95. Cho Y-W, Hong S, Jin Q, Wang L, Lee J-E, Gavrilova O, Ge K. 2009. Histone methylation regulator PTIP is required for PPAR gamma and C/EBP alpha expression and adipogenesis. Cell Metab 10:27–39. https://doi.org/10.1016/j.cmet.2009.05.010.

96. Qi C, Surapureddi S, Zhu Y-J, Yu S, Kashireddy P, Rao MS, Reddy JK. 2003. Transcriptional coactivator PRIP, the peroxisome proliferator-activated receptor {gamma} (PPAR{gamma})-interacting protein, is required for PPAR{gamma}-mediated adipogenesis. J Biol Chem 278:25281–25284. https://doi.org/10.1074/jbc.C300175200.

97. Lee JE, Park YK, Park S, Jang Y, Waring N, Dey A, Ozato K, Lai B, Peng W, Ge K. 2017. Brd4 binds to active enhancers to control cell identity gene induction in adipogenesis and myogenesis. Nat Commun 8:2217. https://doi.org/10.1038/s41467-017-02403-5.

98. Wang L, Xu S, Lee J-E, Baldridge A, Grullon S, Peng W, Ge K. 2013. Histone H3K9 methyltransferase G9a represses PPAR gamma expression and adipogenesis. EMBO J 32:45–59. https://doi.org/10.1038/emboj.2012.306.

99. Reference deleted.

100. Ohno H, Shinoda K, Ohyama K, Sharp LZ, Kajimura S. 2013. EHMT1 controls brown adipose cell fate and thermogenesis through the PRDM16 complex. Nature 504:163–167. https://doi.org/10.1038/nature12652.

101. Tachibana M, Sugimoto K, Nozaki M, Ueda J, Ohta T, Ohki M, Fukuda M, Takeda N, Niida H, Kato H, Shinkai Y. 2002. G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. Genes Dev 16:1779–1791. https://doi.org/10.1101/gad.989402.

102. Zhang ZC, Liu Y, Li SF, Guo L, Zhao Y, Qian SW, Wen B, Tang QQ, Li X. 2014. Suv39h1 mediates AP-2alpha-dependent inhibition of C/EBPalpha expression during adipogenesis. Mol Cell Biol 34: 2330–2338. https://doi.org/10.1128/MCB.00070-14.

103. Matsumura Y, Nakaki R, Inagaki T, Yoshida A, Kano Y, Kimura H, Tanaka T, Tsutsumi S, Nakao M, Doi T, Fukami K, Osborne TF, Kodama T, Aburatani H, Sakai J. 2015. H3K4/H3K9me3 bivalent chromatin domains targeted by lineage-specific DNA methylation pauses adipocyte differentiation. Mol Cell 60:584–596. https://doi.org/10.1016/j.molcel.2015.10.025.

104. Simon JA, Kingston RE. 2009. Mechanisms of Polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol 10:697. https://doi.org/10.1038/nrm2763.

105. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW, Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Volkert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R, Young RA. 2006. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell 125:301–313. https://doi.org/10.1016/j.cell.2006.02.043.

106. Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, Bell GW, Otte AP, Vidal M, Gifford DK, Young RA, Jaenisch R. 2006. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 441:349. https://doi.org/10.1038/nature04733.

107. Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, MacDougald OA. 2000. Inhibition of adipogenesis by Wnt signaling. Science 289:950–953. https://doi.org/10.1126/science.289.5481.950.

108. Wang L, Jin Q, Lee J-E, Su I, Ge K. 2010. Histone H3K27 methyltransferase Ezh2 represses Wnt genes to facilitate adipogenesis. Proc Natl Acad Sci U S A 107:7317–7322. https://doi.org/10.1073/pnas.1000031107.

109. Zhuang L, Jang Y, Park YK, Lee JE, Jain S, Froimchuk E, Broun A, Liu C, Gavrilova O, Ge K. 2018. Depletion of Nsd2-mediated histone H3K36 methylation impairs adipose tissue development and function. Nat Commun 9:1796. https://doi.org/10.1038/s41467-018-04127-6.

110. Musri MM, Carmona MC, Hanzu FA, Kaliman P, Gomis R, Parrizas M. 2010. Histone demethylase LSD1 regulates adipogenesis. J Biol Chem 285:30034–30041. https://doi.org/10.1074/jbc.M110.151209.

111. Duteil D, Metzger E, Willmann D, Karagianni P, Friedrichs N, Greschik H, Gunther T, Buettner R, Talianidis I, Metzger D, Schule R. 2014. LSD1 promotes oxidative metabolism of white adipose tissue. Nat Commun 5:4093. https://doi.org/10.1038/ncomms5093.

112. Sambeat A, Gulyaeva O, Dempersmier J, Tharp KM, Stahl A, Paul SM, Sul HS. 2016. LSD1 interacts with Zfp516 to promote UCP1 transcription and brown fat program. Cell Rep 15:2536–2549. https://doi.org/10.1016/j.celrep.2016.05.019.

113. Brier AB, Loft A, Madsen JGS, Rosengren T, Nielsen R, Schmidt SF, Liu Z, Yan Q, Gronemeyer H, Mandrup S. 2017. The KDM5 family is required for activation of pro-proliferative cell cycle genes during adipocyte differentiation. Nucleic Acids Res 45:1743–1759. https://doi.org/10.1093/nar/gkw1156.

114. Bedford MT, Clarke SG. 2009. Protein arginine methylation in mammals: who, what, and why. Mol Cell 33:1–13. https://doi.org/10.1016/j.molcel.2008.12.013.

115. Yadav N, Cheng D, Richard S, Morel M, Iyer VR, Aldaz CM, Bedford MT. 2008. CARM1 promotes adipocyte differentiation by coactivating PPAR-gamma. EMBO Rep 9:193–198. https://doi.org/10.1038/sj.embor.7401151.

116. Daujat S, Bauer UM, Shah V, Turner B, Berger S, Kouzarides T. 2002. Crosstalk between CARM1 methylation and CBP acetylation on histone H3. Curr Biol 12:2090–2097. https://doi.org/10.1016/S0960-9822(02)01387-8.

117. An W, Kim J, Roeder RG. 2004. Ordered cooperative functions of PRMT1, p300, and CARM1 in transcriptional activation by p53. Cell 117: 735–748. https://doi.org/10.1016/j.cell.2004.05.009.

118. LeBlanc SE, Konda S, Wu Q, Hu YJ, Oslowski CM, Sif S, Imbalzano AN. 2012. Protein arginine methyltransferase 5 (Prmt5) promotes gene expression of peroxisome proliferator-activated receptor gamma2 (PPARgamma2) and its target genes during adipogenesis. Mol Endocrinol 26:583–597. https://doi.org/10.1210/me.2011-1162.

119. LeBlanc SE, Wu Q, Lamba P, Sif S, Imbalzano AN. 2016. Promoter-enhancer looping at the PPARgamma2 locus during adipogenic differentiation requires the Prmt5 methyltransferase. Nucleic Acids Res 44: 5133–5147. https://doi.org/10.1093/nar/gkw129.

120. Karkhanis V, Hu YJ, Baiocchi RA, Imbalzano AN, Sif S. 2011. Versatility of PRMT5-induced methylation in growth control and development. Trends Biochem Sci 36:633–641. https://doi.org/10.1016/j.tibs.2011.09.001.

121. Jin Q, Yu L-R, Wang L, Zhang Z, Kasper LH, Lee J-E, Wang C, Brindle PK, Dent SYR, Ge K. 2011. Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation. EMBO J 30:249–262. https://doi.org/10.1038/emboj.2010.318.

122. Kasper LH, Qu C, Obenauer JC, McGoldrick DJ, Brindle PK. 2014. Genome-wide and single-cell analyses reveal a context dependent relationship between CBP recruitment and gene expression. Nucleic Acids Res 42:11363–11382. https://doi.org/10.1093/nar/gku827.

123. Lasko LM, Jakob CG, Edalji RP, Qiu W, Montgomery D, Digiammarino EL, Hansen TM, Risi RM, Frey R, Manaves V, Shaw B, Algire M, Hessler P, Lam LT, Uziel T, Faivre E, Ferguson D, Buchanan FG, Martin RL, Torrent M, Chiang GG, Karukurichi K, Langston JW, Weinert BT, Choudhary C, de Vries P, Van Drie JH, McElligott D, Kesicki E, Marmorstein R, Sun C, Cole PA, Rosenberg SH, Michaelides MR, Lai A, Bromberg KD. 2017. Discovery of a selective catalytic p300/CBP inhibitor that targets lineage-specific tumours. Nature 550:128–132. https://doi.org/10.1038/nature24028.

124. Jin Q, Wang C, Kuang X, Feng X, Sartorelli V, Ying H, Ge K, Dent SY. 2014. Gcn5 and PCAF regulate PPARgamma and Prdm16 expression to facilitate brown adipogenesis. Mol Cell Biol 34:3746–3753. https://doi.org/10.1128/MCB.00622-14.

125. Takahashi N, Kawada T, Yamamoto T, Goto T, Taimatsu A, Aoki N, Kawasaki H, Taira K, Yokoyama KK, Kamei Y, Fushiki T. 2002. Overexpression and ribozyme-mediated targeting of transcriptional coactivators CREB-binding protein and p300 revealed their indispensable roles in adipocyte differentiation through the regulation of peroxisome proliferator-activated receptor gamma. J Biol Chem 277:16906–16912. https://doi.org/10.1074/jbc.M200585200.

126. Yamauchi T, Oike Y, Kamon J, Waki H, Komeda K, Tsuchida A, Date Y, Li MX, Miki H, Akanuma Y, Nagai R, Kimura S, Saheki T, Nakazato M, Naitoh T, Yamamura K, Kadowaki T. 2002. Increased insulin sensitivity despite lipodystrophy in Crebbp heterozygous mice. Nat Genet 30:221–226. https://doi.org/10.1038/ng829.

127. Creyghton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, Hanna J, Lodato MA, Frampton GM, Sharp PA, Boyer LA, Young RA, Jaenisch R. 2010. Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci U S A 107:21931–21936. https://doi.org/10.1073/pnas.1016071107.

128. Rada-Iglesias A, Bajpai R, Swigut T, Brugmann SA, Flynn RA, Wysocka J. 2011. A unique chromatin signature uncovers early developmental enhancers in humans. Nature 470:279–283. https://doi.org/10.1038/nature09692.

129. Seto E, Yoshida M. 2014. Erasers of histone acetylation: the histone deacetylase enzymes. Cold Spring Harb Perspect Biol 6:a018713. https://doi.org/10.1101/cshperspect.a018713.

130. Haberland M, Carrer M, Mokalled MH, Montgomery RL, Olson EN. 2010. Redundant control of adipogenesis by histone deacetylases 1 and 2. J Biol Chem 285:14663–14670. https://doi.org/10.1074/jbc.M109.081679.

131. Chatterjee TK, Idelman G, Blanco V, Blomkalns AL, Piegore MG, Jr, Weintraub DS, Kumar S, Rajsheker S, Manka D, Rudich SM, Tang Y, Hui DY, Bassel-Duby R, Olson EN, Lingrel JB, Ho SM, Weintraub NL. 2011. Histone deacetylase 9 is a negative regulator of adipogenic differentiation. J Biol Chem 286:27836–27847. https://doi.org/10.1074/jbc.M111.262964.

132. Mayoral R, Osborn O, McNelis J, Johnson AM, Oh DY, Izquierdo CL, Chung H, Li P, Traves PG, Bandyopadhyay G, Pessentheiner AR, Ofrecio JM, Cook JR, Qiang L, Accili D, Olefsky JM. 2015. Adipocyte SIRT1 knockout promotes PPARgamma activity, adipogenesis and insulin sensitivity in chronic-HFD and obesity. Mol Metab 4:378–391. https://doi.org/10.1016/j.molmet.2015.02.007.

133. Zhou Y, Song T, Peng J, Zhou Z, Wei H, Zhou R, Jiang S, Peng J. 2016. SIRT1 suppresses adipogenesis by activating Wnt/beta-catenin signaling in vivo and in vitro. Oncotarget 7:77707–77720. https://doi.org/10.18632/oncotarget.12774.

134. Wang F, Tong Q. 2009. SIRT2 suppresses adipocyte differentiation by deacetylating FOXO1 and enhancing FOXO1's repressive interaction with PPARgamma. Mol Biol Cell 20:801–808. https://doi.org/10.1091/mbc.e08-06-0647.

135. Jing E, Gesta S, Kahn CR. 2007. SIRT2 regulates adipocyte differentiation through FoxO1 acetylation/deacetylation. Cell Metab 6:105–114. https://doi.org/10.1016/j.cmet.2007.07.003.

136. Fang J, Ianni A, Smolka C, Vakhrusheva O, Nolte H, Kruger M, Wietelmann A, Simonet NG, Adrian-Segarra JM, Vaquero A, Braun T, Bober E. 2017. Sirt7 promotes adipogenesis in the mouse by inhibiting autocatalytic activation of Sirt1. Proc Natl Acad Sci U S A 114:E8352–E8361. https://doi.org/10.1073/pnas.1706945114.

137. Chen Q, Hao W, Xiao C, Wang R, Xu X, Lu H, Chen W, Deng CX. 2017. SIRT6 is essential for adipocyte differentiation by regulating mitotic clonal expansion. Cell Rep 18:3155–3166. https://doi.org/10.1016/j.celrep.2017.03.006.

138. Wu SY, Chiang CM. 2007. The double bromodomain-containing chromatin adaptor Brd4 and transcriptional regulation. J Biol Chem 282:13141–13145. https://doi.org/10.1074/jbc.R700001200.

139. Shi J, Vakoc CR. 2014. The mechanisms behind the therapeutic activity of BET bromodomain inhibition. Mol Cell 54:728–736. https://doi.org/10.1016/j.molcel.2014.05.016.

140. Brown JD, Feldman ZB, Doherty SP, Reyes JM, Rahl PB, Lin CY, Sheng Q, Duan Q, Federation AJ, Kung AL, Haldar SM, Young RA, Plutzky J, Bradner JE. 2018. BET bromodomain proteins regulate enhancer function during adipogenesis. Proc Natl Acad Sci U S A 115:2144–2149. https://doi.org/10.1073/pnas.1711155115.

141. Hu YJ, Belaghzal H, Hsiao WY, Qi J, Bradner JE, Guertin DA, Sif S, Imbalzano AN. 2015. Transcriptional and post-transcriptional control of adipocyte differentiation by Jumonji domain-containing protein 6. Nucleic Acids Res 43:7790–7804. https://doi.org/10.1093/nar/gkv645.

142. Zang K, Wang J, Dong M, Sun R, Wang Y, Huang Y, Liu X, Li Y, Wang F, Yu M. 2013. Brd2 inhibits adipogenesis via the ERK1/2 signaling pathway in 3T3-L1 adipocytes. PLoS One 8:e78536. https://doi.org/10.1371/journal.pone.0078536.

143. Wang F, Liu H, Blanton WP, Belkina A, Lebrasseur NK, Denis GV. 2009. Brd2 disruption in mice causes severe obesity without Type 2 diabetes. Biochem J 425:71–83. https://doi.org/10.1042/BJ20090928.

144. Kadoch C, Crabtree GR. 2015. Mammalian SWI/SNF chromatin remodeling complexes and cancer: mechanistic insights gained from human genomics. Sci Adv 1:e1500447. https://doi.org/10.1126/sciadv.1500447.

145. Salma N, Xiao H, Mueller E, Imbalzano AN. 2004. Temporal recruitment of transcription factors and SWI/SNF chromatin-remodeling enzymes during adipogenic induction of the peroxisome proliferator-activated receptor gamma nuclear hormone receptor. Mol Cell Biol 24:4651–4663. https://doi.org/10.1128/MCB.24.11.4651-4663.2004.

146. Wang X, Lee RS, Alver BH, Haswell JR, Wang S, Mieczkowski J, Drier Y, Gillespie SM, Archer TC, Wu JN, Tzvetkov EP, Troisi EC, Pomeroy SL, Biegel JA, Tolstorukov MY, Bernstein BE, Park PJ, Roberts CW. 2017. SMARCB1-mediated SWI/SNF complex function is essential for enhancer regulation. Nat Genet 49:289–295. https://doi.org/10.1038/ng.3746.

147. Nakayama RT, Pulice JL, Valencia AM, McBride MJ, McKenzie ZM, Gillespie MA, Ku WL, Teng M, Cui K, Williams RT, Cassel SH, Qing H, Widmer CJ, Demetri GD, Irizarry RA, Zhao K, Ranish JA, Kadoch C. 2017. SMARCB1 is required for widespread BAF complex-mediated activation of enhancers and bivalent promoters. Nat Genet 49:1613–1623. https://doi.org/10.1038/ng.3958.

148. Lyko F. 2018. The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. Nat Rev Genet 19:81–92. https://doi.org/10.1038/nrg.2017.80.

149. Londono Gentile T, Lu C, Lodato PM, Tse S, Olejniczak SH, Witze ES, Thompson CB, Wellen KE. 2013. DNMT1 is regulated by ATP-citrate lyase and maintains methylation patterns during adipocyte differentiation. Mol Cell Biol 33:3864–3878. https://doi.org/10.1128/MCB.01495-12.

150. Yang X, Wu R, Shan W, Yu L, Xue B, Shi H. 2016. DNA methylation biphasically regulates 3T3-L1 preadipocyte differentiation. Mol Endocrinol 30:677–687. https://doi.org/10.1210/me.2015-1135.

151. Wu X, Zhang Y. 2017. TET-mediated active DNA demethylation: mechanism, function and beyond. Nat Rev Genet 18:517–534. https://doi.org/10.1038/nrg.2017.33.

152. Sérandour AA, Avner S, Oger F, Bizot M, Percevault F, Lucchetti-Miganeh C, Palierne G, Gheeraert C, Barloy-Hubler F, Péron CL, Madigou T, Durand E, Froguel P, Staels B, Lefebvre P, Métivier R, Eeckhoute J, Salbert G. 2012. Dynamic hydroxymethylation of deoxyribonucleic acid marks differentiation-associated enhancers. Nucleic Acids Res 40:8255–8265. https://doi.org/10.1093/nar/gks595.

153. Wiehle L, Raddatz G, Musch T, Dawlaty MM, Jaenisch R, Lyko F, Breiling A. 2016. Tet1 and Tet2 protect DNA methylation canyons against hypermethylation. Mol Cell Biol 36:452–461. https://doi.org/10.1128/MCB.00587-15.

154. Ambros V. 2004. The functions of animal microRNAs. Nature 431:350–355. https://doi.org/10.1038/nature02871.

155. Arner P, Kulyte A. 2015. MicroRNA regulatory networks in human adipose tissue and obesity. Nat Rev Endocrinol 11:276–288. https://doi.org/10.1038/nrendo.2015.25.

156. Yu J, Kong X, Liu J, Lv Y, Sheng Y, Lv S, Di W, Wang C, Zhang F, Ding G. 2014. Expression profiling of PPARgamma-regulated microRNAs in human subcutaneous and visceral adipogenesis in both genders. Endocrinology 155:2155–2165. https://doi.org/10.1210/en.2013-2105.

157. Najafi-Shoushtari SH, Kristo F, Li Y, Shioda T, Cohen DE, Gerszten RE, Näär AM. 2010. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. Science 328:1566–1569. https://doi.org/10.1126/science.1189123.

158. Rayner KJ, Suarez Y, Davalos A, Parathath S, Fitzgerald ML, Tamehiro N, Fisher EA, Moore KJ, Fernandez-Hernando C. 2010. MiR-33 contributes to the regulation of cholesterol homeostasis. Science 328:1570–1573. https://doi.org/10.1126/science.1189862.

159. Horie T, Nishino T, Baba O, Kuwabara Y, Nakao T, Nishiga M, Usami S, Izuhara M, Sowa N, Yahagi N, Shimano H, Matsumura S, Inoue K, Marusawa H, Nakamura T, Hasegawa K, Kume N, Yokode M, Kita T, Kimura T, Ono K. 2013. MicroRNA-33 regulates sterol regulatory element-binding protein 1 expression in mice. Nat Commun 4:2883. https://doi.org/10.1038/ncomms3883.

160. Lee MJ, Wu Y, Fried SK. 2013. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol Aspects Med 34:1–11. https://doi.org/10.1016/j.mam.2012.10.001.

161. Roh HC, Tsai LTY, Lyubetskaya A, Tenen D, Kumari M, Rosen ED. 2017. Simultaneous transcriptional and epigenomic profiling from specific cell types within heterogeneous tissues in vivo. Cell Rep 18:1048–1061. https://doi.org/10.1016/j.celrep.2016.12.087.

162. Zhou P, Zhang Y, Ma Q, Gu F, Day DS, He A, Zhou B, Li J, Stevens SM, Romo D, Pu WT. 2013. Interrogating translational efficiency and lineage-specific transcriptomes using ribosome affinity purification. Proc Natl Acad Sci U S A 110:15395–15400. https://doi.org/10.1073/pnas.1304124110.

163. Mo A, Mukamel EA, Davis FP, Luo C, Henry GL, Picard S, Urich MA, Nery JR, Sejnowski TJ, Lister R, Eddy SR, Ecker JR, Nathans J. 2015. Epigenomic signatures of neuronal diversity in the mammalian brain. Neuron 86:1369–1384. https://doi.org/10.1016/j.neuron.2015.05.018.

164. Shapiro E, Biezuner T, Linnarsson S. 2013. Single-cell sequencing-based technologies will revolutionize whole-organism science. Nat Rev Genet 14:618–630. https://doi.org/10.1038/nrg3542.

165. Kaelin WG, Jr. 2012. Molecular biology. Use and abuse of RNAi to study mammalian gene function. Science 337:421–422. https://doi.org/10.1126/science.1225787.

166. Shalem O, Sanjana NE, Zhang F. 2015. High-throughput functional genomics using CRISPR-Cas9. Nat Rev Genet 16:299–311. https://doi.org/10.1038/nrg3899.

167. Sun L, Goff LA, Trapnell C, Alexander R, Lo KA, Hacisuleyman E, Sauvageau M, Tazon-Vega B, Kelley DR, Hendrickson DG, Yuan B, Kellis M, Lodish HF, Rinn JL. 2013. Long noncoding RNAs regulate adipogenesis. Proc Natl Acad Sci U S A 110:3387–3392. https://doi.org/10.1073/pnas.1222643110.

168. Yi F, Yang F, Liu X, Chen H, Ji T, Jiang L, Wang X, Yang Z, Zhang LH, Ding X, Liang Z, Du Q. 2013. RNA-seq identified a super-long intergenic transcript functioning in adipogenesis. RNA Biol 10:991–1001. https://doi.org/10.4161/rna.24644.

169. Pombo A, Dillon N. 2015. Three-dimensional genome architecture: players and mechanisms. Nat Rev Mol Cell Biol 16:245–257. https://doi.org/10.1038/nrm3965.

170. Siersbæk R, Madsen JGS, Javierre BM, Nielsen R, Bagge EK, Cairns J, Wingett SW, Traynor S, Spivakov M, Fraser P, Mandrup S. 2017. Dynamic rewiring of promoter-anchored chromatin loops during adipocyte differentiation. Mol Cell 66:420–435.e5. https://doi.org/10.1016/j.molcel.2017.04.010.