BCL6 regulates brown adipocyte dormancy to maintain thermogenic reserve and fitness

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Brown adipocytes provide a metabolic defense against environmental cold but become dormant as mammals habituate to warm environments. Although dormancy is a regulated response in brown adipocytes to environmental warmth, its transcriptional mechanisms and functional importance are unknown. Here, we identify B cell leukemia/lymphoma 6 (BCL6) as a critical regulator of dormancy in brown adipocytes but not for their commitment, differentiation, or cold-induced activation. In a temperature-dependent manner, BCL6 suppresses apoptosis, fatty acid storage, and coupled respiration to maintain thermogenic fitness during dormancy. Mechanistically, BCL6 remodels the epigenome of brown adipocytes to enforce brown and oppose white adipocyte cellular identity. Thus, unlike other thermogenic regulators, BCL6 is specifically required for maintaining thermogenic fitness when mammals acclimate to environmental warmth.

In mammals, brown and beige adipocytes generate heat (thermogenesis) through fuel oxidation and uncoupled respiration to defend core temperature in cold environments (1–4). As mammals acclimate to warm environments, their need for thermogenesis decreases, resulting in the entry of brown and beige adipocytes into a dormant state to conserve energy and prevent hyperthermia (1, 4). For example, depots of brown adipose tissue (BAT) in adult humans are largely dormant but can be activated by prolonged exposure to environmental cold or adrenergic agonists (5–9). Although entry of thermogenic adipocytes into dormancy is a well-appreciated adaptation to environmental warmth, the factors and mechanisms that regulate dormancy in BAT remain unknown.

While both brown and beige adipocytes undergo dormancy during acclimation to environmental warmth, their molecular, metabolic, and epigenetic states are quite distinct. For example, beige adipocytes lose virtually all of their thermogenic capacity during acclimation to environmental warmth, whereas brown adipocytes retain a significant portion of their thermogenic capacity even after remaining dormant for many weeks (10, 11). This distinction between beige and brown adipocytes is also observed at the level of the epigenome. For example, the enhancer landscape of beige adipocytes undergoes profound remodeling during adaptation to warmth, becoming almost indistinguishable from that of white adipocytes (10). In contrast, brown adipocytes largely maintain their chromatin state during dormancy, which allows them to maintain their thermogenic capacity (10). However, it is not known what factors contribute to maintenance of a stable epigenetic state during brown adipocyte dormancy.

B cell leukemia/lymphoma 6 (BCL6), a sequence-specific transcriptional repressor, is expressed in many cell types but has been best studied in the immune system. In germinal center B cells, it plays an essential role in the germinal center reaction by repressing genes involved in DNA damage response, apoptosis, cell activation, and plasma cell differentiation (12, 13). BCL6 is also required for the differentiation of follicular helper T cells and modulates the inflammatory activation of macrophages (14–17). Outside the immune system, BCL6 is expressed in metabolic tissues, such as adipocytes and liver, where its functions are just beginning to be investigated (18–22). For example, a recent report found that deletion of Bcl6 in all adipocytes increased de novo lipogenesis, resulting in selective expansion of subcutaneous white adipose tissue (WAT) and protection from obesity-induced insulin resistance (21). Although these studies suggest a potential role for BCL6 in fatty acid metabolism, the role of BCL6 in brown adipocyte biology has not been explored.

Here, we investigated the regulatory functions of BCL6 in both metabolically active and dormant brown adipocytes. We found that loss of BCL6 did not impair the commitment, differentiation, or adrenergic activation of brown adipocytes, but it selectively impaired their thermogenic capacity during dormancy. Thus, unlike other thermogenic regulators that act downstream of the sympathetic nervous system, our data suggest that BCL6 acts in parallel with the sympathetic nervous system to reinforce brown adipocyte cellular identity during dormancy.

\textbf{Results}

\textbf{BCL6 Maintains Thermogenic Capacity of BAT during Dormancy.} BCL6 is expressed in mature BAT and differentiating brown adipocytes (SI Appendix, Fig. S1 A and B), where its functions are not well

\textbf{Significance}

During exposure to environmental cold, brown adipocytes protect against hypothermia by generating heat (thermogenesis). In warm environments, brown adipocytes become inactive or dormant but still maintain their identity and thermogenic capacity, allowing rapid reactivation of thermogenesis upon subsequent cold exposure. Our understanding of the dormant state and its regulation is very limited. Here, we show that the transcription factor B cell leukemia/lymphoma 6 (BCL6) is specifically required for maintenance of thermogenic capacity during dormancy in brown adipocytes. Mechanistically, BCL6 drives a gene expression program that promotes survival, fatty acid oxidation, and uncoupled respiration. Thus, unlike other transcription factors that regulate cold-induced thermogenesis, BCL6 is specifically required for maintaining thermogenic fitness during adaptation to environmental warmth.

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Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (accession no. GSE122746).

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understood. To investigate the role of BCL6 in brown adipocyte biology, we crossed Bcl6f/f mice on C57BL6/J background with Ucp1Cre+/-, AdipoqCre+, and Myf5Cre to generate conditional knockouts of BCL6 in BAT (Bcl6f/fUcp1Cre+/-), pan-adipose (Bcl6f/fAdipoqCre+/-), and Myf5+ somatic brown adipocyte precursors (Bcl6f/fMyf5Cre+/-), respectively (Fig. 1A). In contrast to a previous study that implicated BCL6 in commitment and differentiation of adipocyte precursors (20), we found that BCL6 was not required in Myf5+ precursors for the development of the brown adipocyte lineage (Fig. 1B). Furthermore, in mice raised at the normal vivarium temperature of 22 °C, BCL6 was not required for cold-induced thermogenesis, as evidenced by normal core body temperature and survival of Bcl6f/fUcp1Cre+/- and Bcl6f/fAdipoqCre+/- mice upon acute exposure to 4 °C (Fig. 1C and D; SI Appendix, Fig. S1C). Since BCL6 was not required for BAT thermogenesis in cold-adapted mice (i.e., raised at 22 °C), we considered the alternative hypothesis that BCL6 is important for maintenance of thermogenic capacity in dormant BAT.

To investigate the role of BCL6 in BAT dormancy, we bred and reared mice at thermoneutrality (30 °C), a warm environment that minimizes thermogenesis and promotes entry of BAT into its dormant state. Although thermoneutral housing did not alter expression of the BCL6 protein in BAT (SI Appendix, Fig. S1D), loss of BCL6 had a profound effect on the thermogenic competence of dormant BAT. For example, during a cold challenge from 30 °C to 10 °C, core temperature rapidly declined in Bcl6f/fUcp1Cre+/- and Bcl6f/fMyf5Cre+/- mice, resulting in severe hypothermia (Fig. 1E and SI Appendix, Fig. S1E). In both conditional knockouts, the inability to maintain core temperature was uniformly lethal, whereas the majority of the control animals were able to survive the cold challenge after adaptation to thermoneutrality (Fig. 1F and SI Appendix, Fig. S1F).

![Diagram](https://example.com/diagram.png)

**Fig. 1.** BCL6 is required to maintain thermogenic capacity of dormant BAT. (A) Immunoblotting for BCL6 and lamin B1 protein in nuclear extracts of iBAT of Bcl6f/f, Bcl6f/fUcp1Cre+/-, and Bcl6f/fAdipoqCre+/- mice (n = 2 to 3 per genotype). (B) Gross morphology of iBAT isolated from Bcl6f/f, Bcl6f/fUcp1Cre+/-, Bcl6f/fAdipoqCre+/-, Bcl6f/fMyf5Cre+/- mice bred and housed at 22 °C. (C and D) Core temperature measurements (C) and survival curves (D) of Bcl6f/f and Bcl6f/fUcp1Cre+/- female mice bred at 22 °C and subjected to 4 °C cold challenge (n = 4 to 5 per genotype). (E and F) Core temperature measurements (E) and survival curves (F) of Bcl6f/f and Bcl6f/fUcp1Cre+/- female mice bred at 30 °C and subjected to 10 °C cold challenge (n = 8 to 10 per genotype). The P value for E was calculated using the Mann–Whitney U test at the 3 h time point. (G and H) Norepinephrine-stimulated changes in oxygen consumption rate (VO2) in Bcl6f/f and Bcl6f/fUcp1Cre+/- female mice housed at 30 °C (G, n = 6 per genotype) or 22 °C (H, n = 4 to 6 per genotype). The arrow indicates the time of norepinephrine (NE) injection. (I) Norepinephrine-stimulated changes in oxygen consumption rate (VO2) in Bcl6f/f and Bcl6f/fUcp1Cre+/- male mice initially housed at 22 °C (until 5 wk of age) followed by housing at 30 °C for 6 wk (n = 3 to 5 per genotype). (J and K) Representative infrared images (J) and quantified thermographic measurements of interscapular surface temperature (K) for Bcl6f/f and Bcl6f/fUcp1Cre+/- male mice subjected to 4 °C cold challenge from 30 °C (n = 5 to 6 per genotype). (L) Immunoblotting for phosphorylated PKA substrates, β3-adrenergic receptor (ADRB3), and HSP90 in whole-cell extracts of iBAT of Bcl6f/f and Bcl6f/fUcp1Cre+/- male mice that were housed at 30 °C and injected with CL-316,243 (1 mg/kg) or vehicle (Veh) for 30 min. Data are presented as mean ± SEM.
We next asked whether a decrease in thermogenic capacity of dormant BAT led to cold intolerance in Bcl6f/fUcp1Cre mice. Using norepinephrine-stimulated whole-body oxygen consumption, which reflects the organism’s thermogenic capacity (23, 24), we found that Bcl6f/fUcp1Cre mice had a blunted rise in the rate of oxygen consumption when they were bred or adapted at 30 °C but not at 22 °C (Fig. 1 G–I). In agreement with these data, thermographic imaging revealed lower surface temperature over mitochondria isolated from iBAT of Bcl6f/f mice housed at 22 °C or 30 °C (30 °C and exposed to 4 °C for 15 min (30 °C) and 22 °C) (SI Appendix, Fig. S1G), which contributed to lethal hypothermia and impaired survival in 30 °C-adapted Bcl6f/fAdipoqCre mice (SI Appendix, Fig. S1 H and I). This decrease in thermogenesis by iBAT of Bcl6f/fUcp1Cre mice could not be accounted for by changes in adrenergic signaling because expression of β3-adrenergic receptor and PKA-dependent phosphorylation in response to β3-adrenergic agonist CL-316,243 were preserved in iBAT of Bcl6f/fUcp1Cre mice (Fig. 1L). Together, these findings demonstrate that BCL6 is specifically required for the maintenance of thermogenic capacity in dormant (adapted to 30 °C) but not active iBAT (adapted to 22 °C).

**BCL6 Maintains Uncoupled Respiration in Dormant BAT.** We next asked whether loss of BCL6 affected respiratory capacity of dormant BAT. We found that after an acute cold challenge, oxygen consumption in isolated iBAT was reduced by ~40 to 50% in Bcl6f/fUcp1Cre and Bcl6f/fAdipoqCre mice adapted to environmental warmth (Fig. 24 and SI Appendix, Fig. S2A). A similar result was observed for isolated iBAT mitochondria (SI Appendix, Fig. S2B). This decrease in mitochondrial respiration was associated with nearly complete absence of UCPI mRNA and protein in iBAT of warm-adapted (30 °C), but not cold-adapted (22 °C), Bcl6f/fUcp1Cre mice (Fig. 2B and SI Appendix, Fig. S2C). A similar decrease in UCPI protein expression was also observed when Bcl6f/fUcp1Cre mice that were bred at 22 °C were adapted to 30 °C for 6 wk, independently verifying the importance of BCL6 in regulation of
BAT dormancy (SI Appendix, Fig. S2D). In contrast, mitochondrial abundance, cristae density, expression of respiratory chain complexes I to IV, and complex I activity were not significantly different between dormant iBAT of Bcl6−/− and Bcl6−/−Ucp1Cre mice (Fig. 2C and SI Appendix, Fig. S2E–I).

Because we observed that expression of ATP5A1, an F1 subunit of ATP synthase, was increased in iBAT of Bcl6−/−Ucp1Cre mice (Fig. 2C), we hypothesized that BCL6 might regulate switching between uncoupled (UCP1-mediated) and coupled (ATP synthase–mediated) respiration. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) analysis (described below) revealed BCL6 binding sites near the promoter region of the Atp5g1 gene (Fig. 2D), which encodes the rate-limiting component in the assembly of ATP synthase in brown adipocytes (25, 26). Consistent with our hypothesis, loss of BCL6 resulted in increased expression of Atp5g1 mRNA, resulting in higher ATP synthase activity in dormant iBAT of Bcl6−/−Ucp1Cre mice (Fig. 2E and F). These findings suggest that BCL6 suppresses coupled respiration while maintaining uncoupled respiration in BAT during dormancy.

In addition to cold-induced thermogenesis, previous studies have implicated UCP1 in diet-induced thermogenesis, which can mitigate the deleterious effects of high-fat diet (HFD) feeding (27). These observations prompted us to ask whether BCL6 in brown adipocytes might also regulate diet-induced thermogenesis. To test this hypothesis, we crossed Bcl6−/−mice with Ucp1Cre mice to generate Bcl6−/−/Ucp1Cre mice at thermoneutrality (30 °C) and fed them HFD for 13 wk. We found that body mass, body composition, glucose tolerance, and insulin sensitivity were not significantly different between the 2 genotypes after HFD feeding (Fig. 2G–J). Similar results were obtained in Bcl6−/−AdipoqCre mice that were fed HFD at thermoneutrality (SI Appendix, Fig. S3 A–E). In aggregate, these results suggest that while BCL6 is required for the maintenance of BAT’s thermogenic capacity during dormancy, it is dispensable for diet-induced thermogenesis.

BCL6 Regulates Survival of Dormant Brown Adipocytes. To further investigate the molecular basis for how BCL6 regulates thermogenic capacity in dormant brown adipocytes, we analyzed the iBAT transcriptome of Bcl6−/− and Bcl6−/−Ucp1Cre mice that were cold (22 °C) or warm (30 °C) adapted using RNA-seq. This analysis revealed that BCL6 regulates the iBAT transcriptome in a temperature-dependent manner. For example, ~42 and ~52% of all induced and repressed genes, respectively, were only significantly altered at 30 °C (Fig. 3A). Because BCL6 functions as a transcriptional repressor in other contexts, we first focused on genes induced in iBAT of Bcl6−/−Ucp1Cre mice. Gene ontology enrichment analysis revealed significant enrichment of immune, inflammatory, and apoptotic processes (Fig. 3B). For instance, a large group of genes involved in extrinsic and intrinsic pathways of apoptosis, including Bcl10, Bmf, Traf1, Dcl1, Lifat, Tnfrsf10b, Dapk1, Wdr92, and Egln3, was induced in iBAT of Bcl6−/−Ucp1Cre mice housed at 30 °C (Fig. 3C and SI Appendix, Table S1). While many of these genes were also up-regulated at 22 °C, their induction was much lower at 22 °C (Fig. 3C), indicating a greater dependence on BCL6 for their repression during dormancy at 30 °C. A gene signature of tissue inflammation was also induced in iBAT of warm-adapted (30 °C) Bcl6−/−Ucp1Cre mice (SI Appendix, Fig. S4A and Table S2); however, flow cytometric analysis did not reveal increased recruitment of immune cells into iBAT, indicating the absence of an overt inflammatory response (SI Appendix, Fig. S4B–D).

We next asked whether the induced genes were direct targets of BCL6 in brown adipocytes. ChIP-seq revealed 3,022 binding sites for BCL6 in dormant BAT, which localized primarily to intronic, intergenic, and promoter regions (Fig. 3D). De novo motif analysis revealed enrichment for the consensus BCL6 binding motif among the identified peaks (SI Appendix, Fig. S4E), confirming the specificity of the ChIP-seq. We next asked whether the genes closest to the BCL6 binding sites were differentially expressed in iBAT of Bcl6−/−Ucp1Cre mice. Using a fold change threshold of >1.5 and a false discovery rate threshold of <0.05, we identified 112 and 226 genes that exhibited positive and negative dependence on BCL6, respectively, for their expression in iBAT (Fig. 3E and SI Appendix, Table S3). Among these genes, we found specific binding of BCL6 near proapoptotic genes Bmf, Egln3, Dapk1, and Wdr92 (Fig. 3F and SI Appendix, Table S3), whose expression was induced in dormant iBAT of Bcl6−/−Ucp1Cre mice (Fig. 3G). Taken together, these data indicate that BCL6 represses expression of proapoptotic genes in brown adipocytes, which might enhance their survival during dormancy.

Previous studies have demonstrated that in addition to activating thermogenesis, adrenergic stimuli promote survival of brown adipocytes (28, 29). This led us to ask whether BCL6 might function in parallel with sympathetic signaling to promote survival of brown adipocytes during dormancy. In support of this hypothesis, we observed a higher frequency of apoptotic cells in dormant iBAT of Bcl6−/−Ucp1Cre mice, which over time resulted in reduced BAT mass and DNA content in the adipocyte fraction (Fig. 3H and SI Appendix, Fig. S4F). In contrast, during housing at 22 °C, the frequency of apoptotic cells was not increased, and BAT mass was not reduced in Bcl6−/−Ucp1Cre mice (Fig. 3H and I). Because mitochondrial maintenance of Ki67 proliferating cells was not significantly different between the genotypes (SI Appendix, Fig. S4 G and H), these data suggest that BCL6 promotes survival of brown adipocytes specifically during dormancy, when sympathetic input into BAT is minimal.

BCL6 Regulates Fatty Acid Metabolism in Dormant BAT. We next turned our attention to the genes that are down-regulated in dormant iBAT of Bcl6−/−Ucp1Cre mice. Gene ontology enrichment analysis of down-regulated genes revealed significant enrichment of fatty acid β-oxidation, tricarboxylic acid (TCA) cycle, and carbohydrate metabolism processes in iBAT of warm-adapted (30 °C) Bcl6−/−Ucp1Cre mice (Fig. 4A). For example, we observed that almost the entire program for β-oxidation of fatty acids was suppressed in dormant iBAT of Bcl6−/−Ucp1Cre mice (Fig. 4B). While a similar trend was observed at 22 °C, the magnitude of change was lower (Fig. 4B), indicating greater dependence on BCL6 in the dormant state (30 °C). This decrease in catabolism of fatty acids was accompanied by induction of acyl-CoA thioesterases (ACOTs), enzymes that hydrolyze fatty acyl-CoAs to the metabolically inactive fatty acid form (30). In particular, we observed that many of the cytosolic and peroxisomal ACOTs (Acot1, Acot3, Acot4, Acot7, and Acot8) were induced in iBAT of Bcl6−/−Ucp1Cre mice during dormancy, whereas expression of mitochondrial ACOTs (Acot2, Acot9, Acot13, and Them4) was more variable (Fig. 4C). In contrast, the expression of acyl-CoA synthetases, which catalyze the reverse reaction of synthesizing acyl-CoAs from fatty acids, was not significantly different between the genotypes (SI Appendix, Fig. S5A).

The importance of ACOTs in fatty acid metabolism prompted us to take a closer look at the regulation of these genes by BCL6. While ChIP-seq revealed that BCL6 bound near the promoter region of Acot1 (Fig. 4D), expression of Acot1, as well as the neighboring Acot3 and Acot4 genes, was increased in dormant iBAT of Bcl6−/−Ucp1Cre mice (Fig. 4E). While a similar trend was observed at 22 °C, the induction of Acot1 during dormancy was greater than that observed during warm-adaptation (30 °C) Bcl6−/−Ucp1Cre mice (Fig. 4F and G). These data together suggest a model in which BCL6 determines how fatty acids are apportioned between storage and oxidation in brown adipocytes.
adipocytes. In the absence of BCL6, activation and oxidation of fatty acids is decreased, favoring their storage into cytosolic lipid droplets (Fig. 4 H and I and SI Appendix, Fig. S5 B and C).

**BCL6 Reinforces Brown- and Opposes White-Specific Enhancers to Maintain Cellular Identity.** Because genes involved in thermogenic metabolism were suppressed in dormant BAT of Bcl6f/fUcp1Cre mice, we asked whether this represented a shift in brown adipocyte cellular state or identity. Analysis of the BATLAS gene set, which can robustly differentiate between brown and white adipocytes (32), revealed that loss of BCL6 led to up-regulation of white adipocyte (such as Lep, Nrat, Igf1r, Col6a1, and Gadd45a) and down-regulation of brown adipocyte (such as Acads, Cpt1b, Ech1, Hadha, Idh3a, Ndufs1, Sdha, Srucl, and Ucp1) markers in dormant iBAT of Bcl6f/fUcp1Cre mice (Fig. 5A and SI Appendix, Table S4). These changes in gene expression suggested that BCL6 might regulate brown adipocyte cellular identity during dormancy by maintaining a stable chromatin state. To test this hypothesis, we used ChIP-seq to measure the genome-wide abundance of acetylated histone 3 lysine 27 (H3K27ac), which marks active enhancers and promoters. We found that deletion of Bcl6 altered the chromatin state of brown adipocytes in a temperature-dependent manner. For example, H3K27 acetylation was increased at 224 sites and reduced at 1,011 sites in iBAT of warm-adapted Bcl6f/fUcp1Cre mice (Fig. 5B and SI Appendix, Fig. S6A), whereas acetylation was increased at 303 sites but reduced at only 228 sites in iBAT of cold-adapted Bcl6f/fUcp1Cre mice (SI Appendix, Fig. S6B).

We next asked whether these global changes in the chromatin state of Bcl6f/fUcp1Cre mice reflected a shift in adipocyte lineage-specific enhancers. For this analysis, we first constructed a list of brown...

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**Fig. 3.** BCL6 regulates survival of dormant brown adipocytes. (A) Venn diagrams showing differentially expressed genes in iBAT of Bcl6f/fUcp1Cre mice (fold change ≥ 1.5, adjusted P value < 0.05). Genes up-regulated (Left) and down-regulated (Right) in iBAT of Bcl6f/fUcp1Cre mice that were housed at different ambient temperatures. (B) Gene ontology enrichment analysis of up-regulated genes in iBAT of Bcl6f/fUcp1Cre mice at 30 °C. Enriched biological processes and corresponding P values are shown. (C) Heat map of up-regulated genes in the "apoptotic process" gene ontology category in iBAT of Bcl6f/f and Bcl6f/fUcp1Cre mice (n = 4 per genotype and temperature). The list of genes is provided in SI Appendix, Table S5. (D) Pie chart showing the distribution of BCL6 binding sites across the genome of iBAT. TTS, transcription termination site. (E) Heat map of BCL6-regulated genes (fold change ≥1.5) in iBAT of Bcl6f/f and Bcl6f/fUcp1Cre mice housed at 30 °C that are nearest to a BCL6 binding site. The list of genes is provided in SI Appendix, Table S6. (F) Genome browser tracks showing BCL6 binding sites near proapoptotic genes Bmf and Egln3 in iBAT isolated from Bcl6f/f and Bcl6f/fUcp1Cre (negative control) mice housed at 30 °C. (G) Quantitative RT-PCR measurement of Bmf and Egln3 mRNAs in iBAT of Bcl6f/f and Bcl6f/fUcp1Cre mice housed at 30 °C (n = 4 to 6 per genotype). (H) Quantification of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) apoptotic cells in iBAT of Bcl6f/f and Bcl6f/fUcp1Cre male mice housed at 22 °C or 30 °C for 1 wk (n = 3 to 5 per genotype). (I) Bmf mass in 8-wk-old Bcl6f/f and Bcl6f/fUcp1Cre male mice bred at 22 °C or 30 °C (n = 6 to 10 per genotype and condition). sBAT = subscapular BAT. (J) DNA content in adipocyte fraction of iBAT from Bcl6f/f and Bcl6f/fUcp1Cre mice bred at 30 °C (n = 4 to 5 per genotype). Data are presented as mean ± SEM.
and white adipocyte–specific enhancers (enriched more than 4-fold in either brown or white adipocytes) from the previously identified group of adipocyte enhancers (10, 33) (SI Appendix, Dataset S1). We then asked how loss of BCL6 affected the activation of these enhancers in dormant iBAT. We found that approximately half (∼55%) of BCL6-dependent enhancers were brown or white adipocyte–specific enhancers (Fig. 5C). Importantly, the direction of regulation by BCL6 at these enhancers clearly indicated a critical role for BCL6 in reinforcement of brown adipocyte cellular identity. For example, among the BCL6-regulated brown adipocyte–specific enhancers, 99% exhibited decreased acetylation in Bcl6f/fUcp1Cre iBAT (Fig. 5D). In contrast, 100% of the BCL6-regulated white adipocyte–specific enhancers exhibited increased acetylation in the Bcl6f/fUcp1Cre iBAT (Fig. 5E). Although the number of BCL6-dependent active enhancers was about 2-fold lower in iBAT of cold-adapted mice (SI Appendix, Fig. S6C), BCL6 acted in a similar manner at these enhancers (SI Appendix, Fig. S6D and E). In particular, we found that H3K27 acetylation was reduced at brown adipocyte–specific enhancers associated with Ucp1, Elovl3, Acaa1b, Cidea, Slc27a2, Gdf, and Ptk2b (Fig. 5F and SI Appendix, Fig. S6F). In contrast, we found that H3K27 acetylation was increased at white adipocyte–specific enhancers associated with Ccdc80, Lep, and Nnat in iBAT of Bcl6f/fUcp1Cre mice (Fig. 5G and SI Appendix, Fig. S6G). Similar enrichment of H3K27 acetylation was observed near BCL6 target genes in iBAT of Bcl6f/fUcp1Cre mice, including Atp5g1, acyl-CoA thioesterases (Acot1, Acot3, and Acot4), and Bmf (Fig. 5F and G, and SI Appendix, Fig. S6H), consistent with their direct repression by BCL6. In aggregate, these results indicate that BCL6 maintains thermogenic capacity of dormant BAT in part by reinforcing brown adipocyte–specific enhancers while concurrently suppressing white adipocyte–specific enhancers.

**BCL6 Regulates Brown Adipocyte Enhancers by Direct and Indirect Mechanisms.** Because BCL6 binding sites were not identified near the majority of altered enhancers in the iBAT of Bcl6f/fUcp1Cre...
mice, we postulated that BCL6 regulates brown adipocyte enhancers by both direct and indirect mechanisms. De novo sequence motif analysis revealed enrichment of the consensus binding motifs for hepatic leukemia factor (HLF) and BCL6 among the sites with increased H3K27 acetylation (Fig. 6A), whereas a nuclear receptor half site recognized by NR4A family members and estrogen-related receptors (ERRs) was enriched among the sites with reduced H3K27 acetylation in iBAT of Bcl6f/fUcp1Cre mice (Fig. 6B). This suggested that while BCL6 directly represses a subset of enhancers and promoters, including those near Tpt5g1, Aco1, Bmf, Egln3, and white adipocyte-specific genes Lep, Ccdc80, and Nnat (Figs. 2D, 3F, 4D, and 6C), it indirectly activates other regulatory sites, possibly by a mechanism involving ERRs, which are known to regulate fatty acid and oxidative metabolism in brown adipocytes (34, 35). Indeed, analysis of published ChIP-seq data for ERRα and ERRγ in BAT (36–39) revealed that the hypoacetylated H3K27 sites in iBAT of Bcl6f/fUcp1Cre mice were highly enriched for ERRα and ERRγ binding (Fig. 6D). For example, we observed decreased H3K27 acetylation near Ucp1 and genes involved in fatty acid oxidation, including Slc27a2, Hadha, Hadhb, and Acaa2, in iBAT of Bcl6f/fUcp1Cre mice housed at 30 °C, which corresponded to regions of ERRα and ERRγ binding (Fig. 6E). These results suggest that BCL6 might regulate the expression of these key metabolic genes indirectly by promoting the activity of ERRs at their nearby enhancers and promoters.

To investigate this idea further, we analyzed the expression and recruitment of ERRs to their binding sites in dormant BAT. We observed that expression of the 3 ERR subtypes was similar in dormant iBAT of Bcl6f/f and Bcl6f/fUcp1Cre mice (Fig. 6F). To determine if BCL6 modulated recruitment of ERRs to their respective binding sites near thermogenic genes, we performed ChIP-seq for ERRα in iBAT of Bcl6f/f and Bcl6f/fUcp1Cre mice.
housed at 30 °C. The cistromes of ERRα and BCL6 were largely nonoverlapping (Fig. 6G), indicating that they directly regulate distinct sets of genes. In addition, we found that recruitment of ERRα to the vast majority of its binding sites (98.2%) was not significantly different between the genotypes (Fig. 6H), suggesting that BCL6 does not regulate the recruitment of ERRα to its target genes. Although previous studies have implicated the coactivator protein PGC-1α and histone deacetylase HDAC3 in the transcriptional activation by ERRα (36, 40), the molecular and physiological phenotypes of mice lacking HDAC3 in brown adipocytes are distinct from those observed in Bcl6f/f Ucp1Cre mice. For example, loss of HDAC3 in brown adipocytes primarily affects genes involved in mitochondrial oxidative phosphorylation (36), metabolic pathways that are unaffected in brown adipocytes of Bcl6f/f Ucp1Cre mice. Thus, these findings suggest that BCL6 likely regulates the activity of ERRs at Ucp1 and fatty acid oxidation genes by a distinct mechanism, which will need to be investigated in the future.

Discussion

Several characteristics set BCL6 apart from the “classical” transcriptional regulators of brown adipocyte commitment, differentiation, and activation, such as early B cell factor 2 (EBF2), peroxisome proliferator-activated receptor γ (PPARγ), ERRα/γ, CCAAT/enhancer binding protein (CEBP), and IFN regulated factor 4 (IRF4) (41, 42). First, unlike transcription factors that act downstream of adrenergic stimuli, BCL6 operates in a parallel pathway, making it functionally more important during dormancy when sympathetic input into BAT is largely absent (Fig. 7). Second, unlike ERRα/γ, BCL6 is dispensable for expression of mitochondrial complexes involved in oxidative phosphorylation and electron transport but required for supporting uncoupled respiration and fatty acid oxidation in the mitochondria. Third, BCL6 directly binds to only a small number of genes linked to thermogenic metabolism, such as Aco1 and Atp5g1, where it acts as a transcriptional repressor to sustain uncoupled respiration during dormancy. Fourth, BCL6 positively regulates many thermogenic genes by an indirect mechanism, possibly by
activating ERRs. The potential regulation of ERR transcriptional activity by BCL6 is intriguing because it might represent a point of convergence with the adrenergic signaling pathway, which is known to induce expression and activity of ERRs (34). In this scenario, BCL6 might be important for maintaining basal activity of ERRs during dormancy, whereas full activation of ERRα/γ by adrenergic stimuli is necessary for adaptation to environmental cold. In support of this model, the molecular, histological, and physiological phenotypes of mice lacking ERRα/γ or ERRγ in their brown adipocytes partially overlap with those observed in dormant BAT of Bcl6+/Ucp1−/− mice (37, 43). Although these data suggest convergence of BCL6 and ERRs in regulation of thermogenic genes in dormant brown adipocytes, the precise mechanisms will require additional investigations in the future.

During adaptation to cold, BAT cellularity increases to support the higher demand for thermogenesis. This is due to the positive effect of adrenergic signaling on proliferation of brown adipocyte precursors and survival of differentiated brown adipocytes (28, 44). However, since the rate of apoptosis is low in dormant BAT of wild-type mice (45, 46), additional factors likely promote survival of brown adipocytes in the absence of adrenergic signals. Three distinct lines of evidence suggest that BCL6 is a critical factor that enhances survival of brown adipocytes when adrenergic input into this tissue is low. First, genetic deficiency of Bcl6 increased apoptosis of brown adipocytes, leading to reduction of BAT cellularity in thermoneutral mice. Second, loss of BCL6 increased expression of genes involved in promotion of apoptosis. Third, BCL6 directly bound to a subset of proapoptotic genes, including Bmf and Egln3, resulting in their repression in control animals. Thus, in the absence of adrenergic signals, BCL6 acts in a cell-autonomous manner to promote survival of brown adipocytes.

The chromatin state of differentiated cells is known to be highly stable and can be used to distinguish one differentiated cell type from another (47). For example, in response to changes in environmental temperature, brown adipocytes dramatically alter their morphology and gene expression but maintain a relatively stable chromatin state (10). Although this epigenetic state of brown adipocytes is established during differentiation in an EBF2-dependent manner (48), it was not known what factors might be involved in its stable maintenance across different thermal conditions. The data presented here demonstrate that BCL6 contributes to the maintenance of the stable epigenetic landscape of brown adipocytes. BCL6 accomplishes this by simultaneously reinforcing brown-specific enhancers while opposing white-specific enhancers in dormant BAT. Since ~17% of the active enhancers regulated by BCL6 were white specific, it suggests that BCL6, in part, maintains the cellular identity of brown adipocytes by repressing alternative cellular fates. In support of this hypothesis, profiling of genes comprising the BATLAS, which can be used to assess enrichment of white or brown adipocytes in adipose tissues (32), revealed a dramatic shift in the cellular identity of iBAT of Bcl6−/− Ucp1−/− mice. In particular, we found that expression of white adipocyte markers was increased, whereas expression of brown adipocyte selective genes was decreased in iBAT of Bcl6−/− Ucp1−/− mice. Together, these findings demonstrate that the transcriptional repressor BCL6 is critically important for the maintenance of cellular identity of brown adipocytes but not for their differentiation or thermogenic activation by adrenergic stimuli.

Previous studies have suggested that UCP1 not only provides a defense against environmental cold (49) but also contributes to diet-induced thermogenesis to mitigate the deleterious effects of obesogenic diets (27). This antiobesity effect of UCP1 was originally uncovered by housing Ucp1−/− mice at thermoneutrality and challenging them with a high-fat diet. Since thermoneutral mice do not engage in cold-induced thermogenesis, the increased weight gain observed in Ucp1+/− mice was attributed to increased metabolic efficiency stemming from the absence of diet-induced thermogenesis (27). In contrast, we found that while BCL6 was essential for maintenance of thermogenic fitness during transition from thermoneutrality to cold, it was dispensable for diet-induced thermogenesis in thermoneutral mice. Because a similar disconnect between cold- and diet-induced thermogenesis has been observed in other mouse models, including mice lacking Prdm16, ERRα/γ, Cpt2, and Hdac3 in their brown adipocytes (36, 43, 50–52), it suggests that increased metabolic efficiency and predisposition to diet-induced obesity might be a unique feature of Ucp1−/− mice. In this regard, recent studies demonstrate that in addition to the absence of UCP1, expression and activity of the mitochondrial respiratory chain are severely reduced in BAT of Ucp1−/− mice (53, 54). Together, these findings suggest that the metabolic phenotypes observed in Ucp1−/− mice might not accurately reflect the putative functions of BAT in diet-induced thermogenesis, an issue that remains controversial in the literature (55). Because there is immense clinical interest in therapeutic targeting of BAT for the treatment of obesity and metabolic diseases (56–59), additional studies will be necessary to validate the functions of BAT in diet-induced thermogenesis in both mice and humans.

Fig. 7. A model for regulation of brown adipocyte dormancy by BCL6. (A) During adaptation to cold, norepinephrine (NE) released by the sympathetic nervous system (SNS) activates β-adrenergic signaling in brown adipocytes, which supports their survival and increases their thermogenic capacity. Survival is mediated by the action of ERK1/2, while fatty acid oxidation (FAO), mitochondrial biogenesis, and mitochondrial uncoupling (UCP1) are stimulated at the transcriptional level by peroxisome proliferator-activated receptors (PPARs), estrogen-related receptors (ERRs), and the coactivator protein PGC-1α. (B) In contrast, during adaptation to warmth, when sympathetic tone is minimal and brown adipocytes become dormant, BCL6 renews survival and preserves thermogenic capacity. BCL6 promotes survival by repressing proapoptotic genes, such as Bmf and Egln3, and maintains reserve thermogenic capacity by repressing genes involved in hydrolysis of acyl-CoAs (Acot1, Acot3, Acot4) and mitochondrial coupling (Atp5g1), as well as white adipocyte-specific genes (Nnat, Lep, Ccdc80) and potentially by stimulating the activity of ERRs to promote expression of genes involved in FAO and uncoupled respiration (Ucp1).
Animal Studies

All experiments involving mice were conducted under an approved Institutional Animal Care and Use Committee (IACUC) protocol at University of California, San Francisco (UCSF).

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