MINI-REVIEW

Transcription factor EB and TFE3: new metabolic coordinators mediating adaptive responses to exercise in skeletal muscle?

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Markby GR, Sakamoto K. Transcription factor EB and TFE3: new metabolic coordinators mediating adaptive responses to exercise in skeletal muscle? Am J Physiol Endocrinol Metab 319: E763–E768, 2020. First published August 24, 2020; doi:10.1152/ajpendo.00339.2020.—In response to the increased energy demands of contractions, skeletal muscle adapts remarkably well through acutely regulating metabolic pathways to maintain energy balance and in the longer term by regulating metabolic reprogramming, such as remodeling and expanding the mitochondrial network. This long-term adaptive response involves modulation of gene expression at least partly through the regulation of specific transcription factors and transcriptional coactivators. The AMPK-peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) pathway has long been known to orchestrate contraction-mediated adaptive responses, although AMPK- and PGC1α-independent pathways have also been proposed. Transcription factor EB (TFEB) and TFE3, known as important regulators of lysosomal biogenesis and autophagic processes, have emerged as new metabolic coordinators. The activity of TFEB/TFE3 is regulated through posttranslational modifications (i.e., phosphorylation) and spatial organization. Under nutrient and energy stress, TFEB and TFE3 are dephosphorylated and translocate to the nucleus, where they activate transcription of their target genes. It has recently been reported that exercise promotes nuclear translocation and activation of TFEB/TFE3 in mouse skeletal muscle through the Ca2+-stimulated protein phosphatase calcineurin. Skeletal muscle-specific ablation of TFEB exhibits impaired glucose homeostasis and mitochondrial biogenesis with reduced metabolic flexibility during exercise, and global TFE3 depletion results in diminished endurance and abolished exercise-induced metabolic benefits. Transcriptomic analysis of the muscle-specific TFEB-null mice has demonstrated that TFEB regulates the expression of genes involved in glucose metabolism and mitochondrial homeostasis. This review aims to summarize and discuss emerging roles for TFEB/TFE3 in metabolic and adaptive responses to exercise and contractile activity in skeletal muscle.

AMPK; calcineurin; mTOR; PGC1α; transcription factor EB

INTRODUCTION

Cellular adaptation to nutrient and metabolic stresses, such as starvation and exercise, are critical for the maintenance of energy homeostasis in the body. Without robust and multifaceted short- and long-term adaptive processes, the efficiency of energy transfer in any given cell is reduced, which can lead to deleterious effects and cell death. In response to the increased energy demands of exercise/contractions, skeletal muscle adapts remarkably well by acutely regulating metabolic pathways to maintain energy balance and in the longer term by regulating metabolic reprogramming, such as remodeling and expanding the mitochondrial network to augment mitochondrial content and quality (4, 9, 10, 26). This long-term adaptive response involves modulation of gene expression, at least partly through regulation of specific transcription factors and transcriptional coactivators. The AMPK-mediated activation of peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) is known as one of the critical signaling nodes orchestrating exercise- and contraction-induced effects on mitochondria biogenesis and homeostasis through interaction with multiple transcription factors (6, 7, 11) [although AMPK- and PGC1α-independent pathways or compensatory mechanisms have also been proposed (12, 25)].

Emerging evidence points to a specific regulation of the transcription factor EB (TFEB) and TFE3 in control of metabolic processes in response to nutrient and energy stress. TFEB and TFE3 belong to the microphthalmia-associated transcription factor (MITF) family of the basic helix-loop-helix leucine zipper family of transcription factors, and activation of TFEB /TFE3 induces expression of several genes associated with lysosomal biogenesis and function (19, 24, 31). TFEB/TFE3 have been demonstrated to regulate expression of a network of lysosomal genes through binding to so-called coordinated lysosomal expression and regulation (or CLEAR) element [which is an E-
box (CANNTG) like 10-base-pair sequence (GTCACGTGAC) typically located within 200 base pairs of the transcription initiation site [28]. This permits TFEB and TFE3 to promote the expression of a wide range of lysosomal genes. TFEB and TFE3 also bind to the promoters of many other genes implicated in lysosome-related processes, such as endocytosis, phagocytosis, and autophagy [24, 30]. The activity of TFEB/TFE3 is tightly regulated via posttranslational modifications, protein-protein interactions, and spatial organization [19, 23]. In resting cells, under nutrient- and energy-rich conditions, TFEB is largely cytosolic and inactive. Upon starvation or energy stress, TFEB and TFE3 translocate to the nucleus and activate the transcription of their target genes. Interestingly, a growing body of evidence suggests that TFEB/TFE3 are involved in metabolic responses in various tissues and have been proposed to play a vital role in controlling metabolic homeostasis [5, 14, 29].

Upregulation of TFEB has been observed in multiple tissues following diet deprivation, including liver and skeletal muscle, and it has previously been shown that in the liver, TFEB regulates genes involved in lipid catabolism, fatty acid oxidation, and ketogenesis [29]. Some of these effects are regulated via TFEB-mediated induction of PGC1α [5, 29]. In addition, TFEB expression has also been linked to PGC1α expression with downregulation of PGC1α observed in the hepatocytes of TFE3-deficient mice [20], as well as in RNAi-mediated TFE3 knockdown in C2C12 muscle cells [27]. These studies implicate TFEB/TFE3 to be new regulators of mitochondrial and metabolic homeostasis in association with PGC1α. The aim of this mini-review is to summarize the metabolic and molecular responses to exercise and contractile activity that TFEB and TFE3 have recently been proposed to mediate in skeletal muscle. Although TFEB and TFE3 are also implicated in regulation of exercise-induced autophagy or mitophagy (selective autophagy of mitochondria) in skeletal muscle, this aspect is out of the scope of the current review, and the reader is referred to a recent review on this topic [10].

**EXERCISE/CONTRACTILE ACTIVITY PROMOTES NUCLEAR LOCALIZATION OF TFEB/TFE3 VIA ACTIVATION OF Ca²⁺- AND CALMODULIN-DEPENDENT PROTEIN PHOSPHATASE, CALCINEURIN, IN SKELETAL MUSCLE**

The mTOR kinase was demonstrated to phosphorylate specific serine residues (Ser-122, Ser-142, Ser-211) in TFEB and to play a key role in the regulation of TFEB subcellular localization in multiple cell types [15, 19, 32]. Notably, mTOR-mediated phosphorylation of TFEB on Ser-211 promotes interaction with the adaptor 14-3-3 proteins, leading to cytoplasmic sequestration [15]. While the mutations of either S211 or S142 into alanines (S142A, S211A) result in a constitutive retention of TFEB in the nucleus [23]. The nutrient dependence of mTOR-mediated TFEB phosphorylation suggested that mTOR complex 1 (mTORC1) is involved. Conversely, conditions that lead to mTOR inhibition, including amino acid and serum starvation and lysosomal stress, promote TFEB nuclear translocation and transcriptional activation of autophagic and lysosomal genes (15, 19). TFEB subcellular localization is also regulated by mTORC1-mediated phosphorylation and involves serine residues that are conserved between TFEB and TFE3 [17]. Although the regulation of TFEB/TFE3 phosphorylation mediated by mTORC1 and other kinases has been well studied, the identity and regulation of the phosphatase(s) involved in TFEB/TFE3 dephosphorylation were elusive. Through a high-content screening of 231 phosphatases followed by a series of cellular validation studies, Medina et al. [18] have identified the Ca²⁺- and calmodulin-dependent Ser/Thr protein phosphatase calcineurin as “TFEB phosphatase”. Mechanistically, they found that starvation triggers lysosomal Ca²⁺ release, which activates calcineurin, leading to dephosphorylation and nuclear localization of TFEB. Martina et al. [16] also have shown that calcineurin plays a role in dephosphorylation and nuclear translocation of TFE3 in response to cellular stress (e.g., starvation or endoplasmic reticulum stress). Medina et al. [18] then demonstrated that ectopic expression of constitutively active calcineurin and its regulatory subunit PPP3R1 (also known as calcineurin B) in adult mouse skeletal muscles resulted in a significant enrichment of nuclear TFEB. Interestingly, an acute bout of strenuous treadmill running exercise in mice (10% incline until exhaustion) promoted nuclear localization of TFEB in skeletal muscle [5, 18], and this effect was blunted when an endogenous calcineurin inhibitor, CAIN, was ectopically expressed in muscle cells [18]. Using the same exercise protocol, Pastore et al. [20] showed that the nuclear localization of ectopically expressed TFE3 in mouse skeletal muscle was also shown. In line with these observations, contractile activity (evoked via electrical stimulation) promoted TFEB nuclear translocation in C2C12 myotubes [5]. It has also been shown that running exercise training was associated with a higher proportion of TFEB in myonuclei compared with sedentary mice, although it seems it depends on intensity and/or duration of the exercise. For example, although 7 wk of progressively exercise-trained mice at increasing speeds on a treadmill resulted in an increase in TFEB nuclear localization, such effect was not observed in mice subjected to a shorter and milder exercise regimen (1 h at 15 cm/s for 4 days) [14].

Taken together, the studies to date suggest that energy stresses (e.g., nutrient deprivation and contractions) that induce elevation of cellular Ca²⁺ levels are the key cellular events, which promote dephosphorylation and nuclear translocation of TFEB/TFE3 via activation of calcineurin (and also likely inhibition of mTOR) [23] (Fig. 1). Further studies, including a detailed kinetics of calcineurin and mTORC1 activity in association with phosphorylation/subcellular localization of TFEB/TFE3 in skeletal muscle in response to exercise at different intensities and duration, are needed. Whether there is a fiber-type specific response to exercise in TFEB/TFE3 regulation would also be an interesting topic to study.

In addition to its effect on subcellular localization, contractile activity was shown to enhance transcription of TFEB in muscle cells. Using luciferase reporter assays, Erlich et al. [5] demonstrated that the activity of the 1,600 bp TFEB promoter increased by threefold following 2 h of contractions in C2C12 myotubes. Interestingly, this increase in transcriptional activity was significant in the 1,600-bp construct, but not the 1,200-bp proximal TFEB promoter. Currently, it is unknown what factor(s) triggers the TFEB promoter activity and whether a 400-bp DNA region is the “exercise-responsive” promoter region needs further investigation.

**GENETIC EVIDENCE THAT MUSCLE TFEB/TFE3 PLAY VITAL ROLES IN ENERGY AND GLUCOSE HOMEOSTASIS**

Since global deletion of murine Tcfeb (which encodes TFEB) resulted in embryonic lethality due to a defect in placental vascularization [33], conditional knockout (KO) mice have been
generated to delineate cell-/tissue-specific functions of TFEB. Skeletal muscle-specific TFEB-deficient mice, whereby TFEB floxed mice were crossed with myosin light chain 1f promoter-driven Cre mice, have been shown to have impaired muscle energy balance (14). Transcriptomic analysis of skeletal muscles from these mice indicated that genes enriched in metabolic processes are ablated, in particular, genes associated with lipid and glucose homeostasis, as well as mitochondrial function, were shown to be downregulated (14). These transcriptomic changes relayed into a series of metabolic changes in muscle tissue, including local insulin resistance, decreased capacity for glucose uptake, and an increased preponderance for anaerobic glycolysis for maintaining energy balance at rest. In addition, 10% of mitochondria showed malformations (assessed by electron microscopy) and increased proton leak from the mitochondria in the muscle was also detected. These phenotypes were shared to a degree in TFE3 global KO mice (20). Mitochondria in these mice also have malformations in skeletal muscle (and also in the liver), with an increased number and size observed in both the fed and fasted state in muscle tissue. Likewise, increased blood lactate levels compared with controls, indicative of higher glycolysis rates, were detected postexercise in these mice on top of lower resting glycogen stores in muscle and an impaired restoration of glycogen post-exercise (20). In the skeletal muscle TFEB KO model, exercise indicated a functional importance of TFEB in maintaining normal muscle function. Lack of TFEB resulted in a decrease in exercise tolerance compared with controls; this is shown to be due to the mice relying, as mentioned above, on glycolysis as opposed to utilizing full aerobic respiration on top of already depleted glycogen stores. This results in a faster change to employing muscle-based lipid stores, increased respiratory exchange rate, and fatty acid oxidation for the generation of ATP rather than glucose with rapid depletion of these stores affecting exercise capacity (14). However, it should be noted that muscle glycogen content is not a limiting factor for treadmill exercise performance/
tolerance in mice using skeletal muscle glycogen-deficient [glycogen synthase (GYs1) KO] model (21).

Global TFEB KO mice are viable and have previously been described to be indistinguishable from their WT littermates (34). However, interestingly, similar to skeletal muscle-specific TFEB KO, global TFEB KO mice showed a decreased capacity for exercise in comparison to WT littermates (20). Following a high-fat diet feeding, the TFEB KO mice were also shown to have a significantly reduced capacity to benefit from the exercise-induced metabolic adaptations (e.g., improved glucose tolerance and adiposity) following 8-wk exercise training regime. TFEB KO mice have severe mitochondrial abnormalities in hepatocytes and muscle cells, including accumulation of enlarged and dysfunctional mitochondria with impaired respiration (20), which may have resulted in compromised lipid catabolism and exercise intolerance. Given that the muscle TFEB KO mice also show local alterations, such as insulin resistance and metabolic adaptability to exercise (14), it would be interesting to perform similar molecular (e.g., transcriptomics) and metabolic analysis in these mice or ideally perform such analyses using skeletal muscle-specific TFEB KO mice. Indeed, TFEB has been implicated in rescuing the phenotype of liver-specific KO TFEB mice (20), and it has been suggested that under stress conditions, such as exercise, TFEB and TFE3 could work in cooperation to induce metabolic changes. Investigating how TFEB may be compensating for the lack of TFEB in these mice and what effect double KO of these proteins in skeletal muscle may be illuminating as to their role in metabolic adaptation to exercise.

Gain of function (i.e., overexpression) models have also been employed to investigate the role of TFEB in metabolic regulations and exercise adaptations in skeletal muscle. These include virus-mediated overexpression in C2C12 myotubes, in vivo adeno-associated virus (AAV)-mediated overexpression and a tamoxifen-inducible skeletal muscle-specific TFEB transgenic overexpression mouse line (5, 14). In support of the findings in the TFEB KO model, transcriptomic analysis of AAV-treated mice showed increases in the same metabolic pathways downregulated in KO mice (14). This corresponded with an increased number and size of mitochondria, an increase in several key mitochondrial proteins, increased mitochondrial respiration, and increased ATP levels, as well as an increased capacity for exercise in these mice. This mirrored many of the downregulated processes observed in the KO mice, as well as being supported by results in the overexpression mice; however, several other factors appeared to be altered as a result of overexpression that were not changed in the KO mice. Intriguingly, TFEB overexpression in muscles was associated with increased expression of mRNA/protein-involved glucose uptake, metabolism, and storage, including GLUT1, hexokinase 2, and GYS1 (14). This led to an increase in muscle glycogen content likely due to enhanced glucose uptake and allosteric activation of GYS1 (2) by glucose-6-phosphate. Although the phenotypic observations support a key role for TFEB in control of energy and glucose homeostasis, data from “overexpression” studies need to be cautiously interpreted. AAV-TFEB transduction resulted in a 20-fold increase in mRNA expression (no protein expression reported) (14), whereas an acute bout of 5-h contractile activity resulted in a less than twofold nonsignificant increase in TFEB mRNA expression in muscle cells (5). This level of constitutive overexpression (i.e., 20-fold at mRNA levels) is unlikely to mimic any physiological settings in skeletal muscle, and excess TFEB would be expected to disrupt expression dynamics of transcription factors leading to nonphysiologically relevant/aberrant gene expressions. Finally, it should be noted that TFEB overexpression was not sufficient to enhance autophagy flux and mitophagy gene expression (and TFEB deletion did not impair autophagy flux in the presence or absence of nutrients) (14). Whether TFEB plays a distinct or compensating role in controlling autophagy/mitophagy gene expression needs further investigation.

POtential cROS TALK BETWEEN PGC1A AND TFEB IN SKELETAL MUSCLE?

As a key factor (i.e., transcriptional coactivator) in the control of mitochondrial biogenesis, PGC1α has been implicated in the metabolic control and exercise adaptation of skeletal muscle (6, 10). PGC1α is induced in skeletal muscle in response to exercise (22), and its gene inactivation results in reduced exercise capacity, due most likely to mitochondrial content, turnover, and function (13, 35). Despite a consistent upregulation of PGC1α in TFEB overexpression models and downregulation of TFEB in PGC1α KO mouse models (5, 14), PGC1α’s subsequent role in downstream signaling is debated (Fig. 1). Mansueto et al. (14) showed that, despite TFEB being at a reduced expression level, exercise was still able to induce muscle TFEB nuclear localization and stimulate gene expression changes, including those involved in mitochondrial biogenesis in PGC1α-deficient mice. In addition, TFEB overexpression in the PGC1α KO mice was able to improve mitochondrial function and restore exercise tolerance. In contrast to these observations, another study by Erlich et al. (5) reported that exercise-induced TFEB nuclear localization in skeletal muscle was ablated in PGC1α KO mice. They speculated that it might be due to an increase in mTOR phosphorylation and activation upon exercise in PGC1α KO mice, although the phosphorylation status of TFEB on mTOR sites (Ser-142/Ser-211) was not assessed. Considering that one of the major outcomes of both muscle-specific TFEB KO and global TFEB KO is dysfunctional mitochondria and that PGC1α is a major contributor to the balance between mitochondrial biogenesis and mitophagy, it would be important for future research to clarify the influence that TFEB/TFE3 and PGC1α have on one another.

COncluding REMARKS AND PERSPECTIVE

Genetic loss-of-function and gain-of-function studies clearly demonstrate important roles that TFEB and TFE3 play in maintaining metabolic homeostasis and adaptive responses to exercise in mouse skeletal muscle. However, there are still missing pieces to substantiate whether TFEB/TFE3 are physiologically relevant key metabolic coordinators in skeletal muscle. For example, even though nuclear content of TFEB/TFE3 was significantly increased in response to exhaustive exercise in mouse skeletal muscle, exercise regulation of TFEB/TFE3 in human skeletal muscle has not been robustly explored. One study reported that muscle glycogen-depleting exercise resulted in a modest increase in TFEB in nuclear fraction in the postexercise period (2 h), but this was not associated with reduced levels of TFEB in cytosolic fraction (1). It would also be important to determine whether TFEB and TFE3 expression and function are decreased and impaired in people with metabolic disorders.
Mechanistically, although Ca$^{2+}$-mediated calcineurin activation has been proposed as a key mechanism to switch on TFEB/TFE3 in response to exercise, not much has been described of how TFEB and TFE3 are “switched off” following exercise in skeletal muscle. Since exercise/contraction regulates several protein kinases that are known to regulate phosphorylation/activation of TFEB/TFE3, it would be interesting to further investigate the mechanism of TFEB/TFE3 activation and inhibition. Recently, Collodet et al. (3) demonstrated that pharmacological activation of AMPK promoted dephosphorylation and nuclear localization of TFEB/TFE3 (Fig. 1) in multiple cell types, as well as skeletal muscle in a zebrafish model. It would be of interest to determine whether exercise- and contraction-mediated TFEB/TFE3 activation involves AMPK in muscle. Finally, it has been shown that the breadth of signaling pathways and kinases in skeletal muscle modulated by exercise is far greater than previously appreciated using a global phosphoproteomic approach (8). Investigating the effects of different exercise protocols in a temporal fashion, employing such a global approach, will further expand our knowledge on signaling pathways responsible for TFEB/TFE3 regulation and exercise responses beyond the AMPK-PGC1α pathway. Further studies to delineate molecular and physiological roles of TFEB/TFE3 will shed light on mechanisms underlying adaptive responses to exercise in skeletal muscle and may lead to the discovery of novel therapeutic targets for the treatment of metabolic disorders.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.S. prepared figures; G.R.M. and K.S. drafted manuscript; G.R.M. and K.S. edited and revised manuscript; G.R.M. and K.S. approved final version of manuscript.

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