Published in final edited form as:
*Cell Mol Life Sci*. 2014 July ; 71(13): 2483–2497. doi:10.1007/s00018-014-1565-8.

# Novel roles for the MiTF/TFE family of transcription factors in organelle biogenesis, nutrient sensing, and energy homeostasis

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## Abstract

The MiTF/TFE family of basic helix-loop-helix leucine zipper transcription factors includes MITF, TFEB, TFE3, and TFEC. The involvement of some family members in the development and proliferation of specific cell types, such as mast cells, osteoclasts, and melanocytes is well established. Notably, recent evidence suggests that the MiTF/TFE family plays a critical role in organelle biogenesis, nutrient sensing, and energy metabolism. The MiTF/TFE family is also implicated in human disease. Mutations or aberrant expression of most MiTF/TFE family members has been linked to different types of cancer. At the same time, they have recently emerged as novel and very promising targets for the treatment of neurological and lysosomal diseases. The characterization of this fascinating family of transcription factors is greatly expanding our understanding of how cells synchronize environmental signals, such as nutrient availability, with gene expression, energy production, and cellular homeostasis.

### Keywords

MITF; TFE3; TFEB; autophagy; lysosomes; nutrients; transcription; disease

## Introduction

Microphthalmia-associated transcription factor (MITF), transcription factor EB (TFEB), TFE3, and TFEC constitute the MiTF/TFE (Microphthalmia/TFE) subfamily of basic/helix-loop-helix/leucine zipper (bHLH-LZ) transcription factors [1–5]. All members of the MiTF/TFE family share a similar structure that includes three critically important regions. The basic motif binds to specific areas of DNA while the helix-loop-helix and leucine-zipper motifs are critical for protein interactions. Homodimerization and heterodimerization within members of the MiTF/TFE family is critical for binding to DNA and transcriptional activation of target genes; however, they do not heterodimerize with other bHLH-LZ-containing proteins such as MAX, USF, and MYC [6, 7]. In addition, it is well established that the MiTF/TFE family specifically binds to E-box (CANNTG) and/or M-box

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(AGTCATGTGCT) response elements present in the promoter region of their downstream target genes [8].

MITF is predominantly expressed in melanocytes, osteoclasts, mast cells, macrophages, NK cells, B cells, and heart; whereas TFEC expression is restricted to cells of myeloid origin [9]. In contrast, TFE3 and TFEB show a more ubiquitous pattern of expression and have been detected in multiple cell types [10, 11]. There are at least nine MITF isoforms currently described that differ in their amino-terminal regions and their expression (-or transcription-) is regulated in a tissue-dependent manner due to the usage of alternative promoters. In addition, many more isoforms are described as being generated by alternative splicing and posttranslational modifications [12, 13]. TFEB and TFEC contain multiple alternative first exons with restricted and differential tissue distributions, whereas the TFE3 gene seems to be regulated by a single promoter [14].

A large body of evidence supports the important role played by this family of transcription factors in many cellular and developmental processes. MITF is critical for development, survival, and differentiation of neural crest-derived melanocytes and retinal pigmented epithelium (RPE) [15, 4], and collaborates with TFE3 to regulate osteoclastogenesis [16, 17] and mast cell differentiation [18, 19]. TFE3 was first identified as a protein that binds to the mE3 motif within the immunoglobulin heavy-chain enhancer [10] and was implicated, together with TFEB, in humoral immunity [20]. Finally, TFEB was shown to be essential for placental vascularization [11]. Mutation and/or aberrant expression of several MiTF/TFE family members have been linked to different types of cancer in humans, such as renal carcinomas, alveolar sarcomas, and melanomas. Mutations in MITF are also the cause of the pigmentary and deafness disorder, Waardenburg syndrome type 2A [21].

Recent evidence suggests that some members of the MiTF/TFE family might function as critical factors in nutrient sensing and maintenance of cellular homeostasis. This review focuses on the role of TFEB in lysosomal homeostasis and energy metabolism. We also discuss the participation of other members of the MiTF/TFE family in the cellular response to nutrient deprivation.

## Role of TFEB in transcriptional regulation of lysosomal biogenesis

Lysosomes are the primary degradative organelle in all cells. A number of essential cellular processes are dependent upon normal lysosomal function, including the turnover of cellular constituents, cholesterol homeostasis, downregulation of surface receptors, inactivation of pathogenic organisms, antigen presentation, repair of the plasma membrane, and bone remodeling [22]. Lysosomes receive extracellular material destined for degradation through endocytosis, whereas intracellular components reach lysosomes mainly via autophagy [23].

Lysosomal biogenesis has long been considered as a housekeeping process. However, simultaneous expression of multiple lysosomal genes has been reported under certain conditions, such as sucrose-induced lysosomal stress [24]. These observations led to the prediction that formation of lysosomes might be transcriptionally regulated. The Ballabio group tested this possibility by analyzing the promoter region of different lysosomal genes [25]. Unexpectedly, they found that many lysosomal genes contain one or more repetitions

of a 10 base-pair motif (GTCACGTGAC) that in most cases localized within 200 base pairs of the transcription initiation site. This motif was named Coordinated Lysosomal Expression and Regulation (CLEAR) element and constitutes a type of E-box (CANNTG) known to be recognized by members of the basic helix-loop-helix (bHLH) family of transcription factors. Accordingly, over-expression of TFEB results in transcriptional activation of numerous lysosomal genes, including several subunits of the v-ATPase, lysosomal transmembrane proteins, and lysosomal hydrolases [26, 25]. TFEB directly binds to the CLEAR elements present in the promoter region of these lysosomal genes, thus increasing their expression. Consequently, TFEB over-expression leads to a significant increase in the total number of lysosomes. These findings indicate that TFEB is a master regulator of lysosomal biogenesis. Furthermore, through the regulation of TFEB activity, cells can monitor lysosomal function and adapt to degradation requirements and/or environmental signals.

## TFEB induces expression of autophagic and metabolic genes

Autophagy is a critical cellular process that allows cells to degrade their own components and recycle important molecules in situations of nutrient deprivation [27]. Many autophagy-related genes (ATG) encode protein complexes that act sequentially to regulate engulfment of portions of the cytosol into autophagosomes and subsequent delivery to lysosomes for degradation. Although the levels of ATG proteins are usually high in normal conditions, prolonged starvation can cause depletion of some key autophagy regulators. For this reason, it is important for cells to possess the capability of increasing transcription of autophagy genes when nutrients are scarce.

TFEB functions as a key transcriptional regulator of autophagy [28]. It directly binds to the TFEB-target sites present in the promoter regions of numerous autophagy genes and promotes their expression. These include *UVRAG*, *WIPI*, *MAP1LC3B*, *SQSTM1*, *VPS11*, *VPS18*, and *ATG9B*. In addition, its overexpression in various cell lines increases the number of autophagosomes, whereas depletion of endogenous TFEB by RNAi reduces autophagosome numbers. Activation of autophagy following TFEB over-expression has also been observed *in vivo* [28].

TFEB is not the only transcription factor implicated in autophagy regulation. FoxO3, HIF-1, and p53 have also been shown to activate the expression of autophagy genes in response to various stresses [29–31], whereas ZKSCAN3 functions as a negative regulator of both autophagy and lysosomal biogenesis [32]. However, while FoxO3, HIF-1, and p53 promote expression of genes implicated in the initial steps of autophagosome formation, TFEB appears to have a much broader role since it upregulates a more comprehensive network of autophagy genes. This network not only includes key regulators of autophagosome biogenesis, but also proteins required for fusion between autophagosomes and efficient degradation of the autophagic content. In addition, by simultaneously regulating autophagy induction and lysosomal formation, TFEB plays a unique role in the coordination of the two main degradative pathways in the cell. Therefore, modulation of cellular clearance requires a complex regulatory network that is, at least in part, controlled by TFEB.

A recent report revealed that TFEB also regulates lipid catabolism [33]. Over-expression of TFEB in mouse liver leads to increased expression of genes implicated in different types of lipid breakdown, including fatty acid oxidation, lipophagy, and ketogenesis. At least in part, this effect is the result of direct TFEB-mediated transactivation of *Pparα* (peroxisome proliferator-activated receptor-α) and *Pgc1α* (PPARγ co-activator 1α), two key regulators of liver lipid metabolism. TFEB over-expression is sufficient to revert obesity and metabolic syndrome in mice. However, this rescue is not observed in autophagy-deficient animals (*Atg7* $^{-/-}$), thus suggesting that functional autophagy is essential for the role of TFEB in lipid metabolism. Finally, liver-specific TFEB knockout results in defective degradation of lipids during starvation, further corroborating the important role played by TFEB in responding to the varying energetic demands of the cell.

## Mechanisms of activation of TFEB

### Nutrient deprivation

A key point in understanding the role of TFEB in lysosome formation and autophagy induction was the identification of the cellular stimuli that promote TFEB activation. In fully fed cells, TFEB remains in the cytosol and cannot access the promoter region of its target genes. In contrast, upon short times of starvation, TFEB rapidly translocates to the nucleus and induces expression of autophagy and lysosomal genes [34, 35, 28, 36]. Therefore, TFEB can sense and respond to fluctuations of nutrient levels in the cell.

Three different groups simultaneously identified the mechanism by which TFEB is retained in the cytosol under nutrient rich conditions [34–36]. In fully fed cells, the kinase complex mammalian target of rapamycin complex 1 (mTORC1) phosphorylates TFEB at several serine/threonine residues, including serine 211 (S211). Phosphorylated S211 functions as a binding site for the cytosolic chaperone 14-3-3, which keeps TFEB sequestered in the cytosol, probably through masking the TFEB nuclear import signal [34, 35]. When nutrient levels are low, mTORC1 is inactivated, the TFEB/14-3-3 complex dissociates, and TFEB is free to translocate to the nucleus and activate processes (such as autophagy and lipid degradation) that will assist in cellular survival during starvation conditions.

mTORC1 is a multi-subunit complex that includes the serine/threonine kinase mTOR. It couples energy and nutrient abundance to cell growth and proliferation by balancing anabolic (protein synthesis and nutrient storage) and catabolic processes (autophagy and the utilization of energy stores) [37]. Lysosomes play a critical role in the activation of mTORC1. When the levels of amino acids in the lysosomal lumen are high, the v-ATPase promotes activation of a lysosomal signaling complex that includes the small GTPases Rags and Ragulator [38–41]. Active Rags can then bind the mTORC1 component Raptor and redistribute mTORC1 to lysosomes where it is activated by the small GTPase Rheb [42, 43]. Rheb activity requires growth factors, thus suggesting that different stimuli must cooperate to regulate activation of mTORC1. Interestingly, TFEB is also recruited to lysosomes through direct interaction with active Rags [44]. This Rag-mediated redistribution of TFEB to the lysosomal surface facilitates the phosphorylation of TFEB by mTORC1 and constitutes an efficient mechanism to link nutrient availability to TFEB inactivation. Inhibition of the interaction between TFEB and Rags results in accumulation of TFEB in the

nucleus and constitutive activation of autophagy under nutrient rich conditions, thus indicating that recruitment of TFEB to lysosomes is critical for proper control of this transcription factor [44] (Figure 1).

The regulation of TFEB by mTORC1 reveals a novel and unexpected function of mTOR in lysosomal formation and further confirms the important role played by lysosomes in nutrient sensing and cellular metabolism. Interestingly, ZKSCAN3 shuttles from the nucleus to the cytosol under sustained starvation conditions or upon chemical inhibition of mTORC1 [32]. It is possible that TFEB, mTORC1, and ZKSCAN3 function in conjunction to orchestrate the response of cells to nutrient deprivation.

It has been well established that mTORC1 is a major regulator of autophagy. When nutrients are abundant, mTORC1 represses autophagy by directly phosphorylating and inhibiting specific Atg proteins, such as ATG13 and ATG1, required for autophagy induction [45, 46]. The ability of mTORC1 to control TFEB activation (as well as ZKSCAN3 inactivation) indicates that mTORC1 exerts a broader regulatory role in the autophagic process. Therefore, TFEB and mTORC1 work together as major regulators of cellular homeostasis by coordinating nutrient sensing with transcriptional regulation of lysosomal biogenesis, autophagy, and lipid catabolism (Figure 2).

In addition to mTORC1, other cellular kinases may contribute in regulation of TFEB activation. For example, phosphorylation of Ser142 by ERK2 helps promote cytosolic retention of TFEB [28], whereas TFEB becomes more resistant to degradation upon phosphorylation by PKCbeta (see below) [47]. Furthermore, cells in which mTORC1 is hyperactivated, such as TSC2-null murine embryonic fibroblasts, TFEB is phosphorylated in several serine residues located between amino acids 462–469, and, this modification promotes its nuclear translocation [48]. Consequently, TFEB modification by multiple kinases under different stress conditions may introduce a more dynamic type of regulation than previously appreciated. The capability of multiple modes of regulation is also evidenced by the self-regulatory mechanism attributed to TFEB. Under starvation conditions, TFEB translocates to the nucleus and binds to several CLEAR motifs present on its own promoter, thus enhancing its own expression [33]. It is thought that this auto-regulatory feedback loop is particularly relevant to achieve a sustained response under prolonged starvation conditions.

## Lysosomal stress

Activation of TFEB also occurs under conditions in which lysosomal function is compromised. Incubation with chloroquine, a lysosomotropic agent that prevents lysosomal acidification, induces rapid redistribution of TFEB to the nucleus [35, 36]. TFEB also shows a predominantly nuclear distribution in several different cellular models of Lysosomal Storage Disorders (LSDs) [25]. LSDs are metabolic disorders characterized by the progressive accumulation of undigested material in lysosomes that subsequently disrupts cellular physiology [49]. These observations suggest that TFEB may be implicated in a broader pathway of cellular response to lysosomal stress. Importantly, a crucial point to determine will be whether the same molecular machinery controls the activation of TFEB in response to either nutrient deprivation or lysosomal stress. Considering the important role

played by the v-ATPase in the activation of mTORC1, as well as the susceptibility of the former to the environment of the lysosomal lumen, it is very plausible that both pathways partially overlap.

## Cell differentiation

Some cell types require a profound reorganization of their endo-lysosomal system while undergoing differentiation. This is the case of osteoclasts, a specialized cell type that secrete lysosomal hydrolases at the site of bone resorption and whose function is critical for skeletal formation and remodeling. It was recently shown that TFEB induces lysosomal biogenesis in differentiated osteoclasts and is required for normal osteoclasts function *in vivo* [47] (Figure 2). Mice lacking TFEB in osteoclasts show decreased expression of lysosomal genes, reduced number of lysosomes, and defective resorption of the bone matrix. Interestingly, activation of TFEB in osteoclasts is not dependent on nutrient levels, but rather on the presence of specific cytokines. Incubation with the osteoclast differentiation factor RANKL induces phosphorylation of TFEB on several serine residues located in the C-terminal region [47]. This phosphorylation is mediated by PKCβ and causes stabilization of TFEB, thus resulting in a dramatic increase in the nuclear levels of TFEB and enhanced transcription of specific lysosomal genes.

Recent evidence suggests that TFEB may also play an important role linking lysosomal biogenesis to innate immunity and antiviral defense [50]. TREX1 is an exonuclease that digests host cytosolic DNA as a way to avoid autoimmunity. While the mechanism of TREX1 regulation of mTORC1 remains uncharacterized, the activity of mTORC1 decreases in the absence of TREX1, thus allowing transport of TFEB to the nucleus and promoting lysosomal biogenesis. The expansion of the lysosomal system results in the activation of an interferon-independent signaling pathway that involves STING, TBK1, IRF3 and IRF7, and leads to the expression of interferon-stimulated genes (ISGs). Accordingly, the activation of antiviral genes observed in TREX1-knockout cells is diminished upon TFEB depletion, whereas TFEB over-expression in wild-type cells is sufficient to induce expression of the ISG *Ifit1*, confirming the role of TFEB in antiviral defense. Since mutations in TREX1 have been linked to lupus erythematosus and other inflammatory disorders, it is possible that TFEB-induced lysosomal biogenesis also plays an important role in the pathogenesis of autoimmune diseases.

Finally, the levels of TFEB are significantly increased in primary $CD4^+$ mouse splenocytes upon TCR stimulation [20]. Similar to osteoclasts, the increase in TFEB levels seems to result from post-transcriptional modifications. TFEB directly binds to the promoter of the gene encoding CD40 ligand, a protein necessary for T cell-dependent antibody response, and promotes its expression thereby affirming the critical role of in T cell function and humoral immunity.

## Role of other members of the MiTF/TFE family in nutrient sensing, energy homeostasis, and organelle biogenesis

Recent evidence suggests that TFEB may not be the only member of the MiTF/TFE family involved in nutrient sensing and organelle biogenesis. Amino acid sequence alignment revealed that the domain that mediates binding between TFEB and Rag GTPases is also present in TFE3 and some MITF isoforms, including MITF-1 (also known as isoform MITF-A), MITF-2 (MITF-H), MITF-3 (MITF-C), and MITF-7 [44, 51] suggesting these transcription factors may share the same mechanism of activation. Accordingly, inactivation of mTORC1 by Torin-1 or induction of lysosomal stress by chloroquine causes a rapid translocation of TFE3 and MITF-1 to the nucleus [44, 35, 51]. Accumulation of TFE3 in the nucleus is also observed upon nutrient deprivation in several cell types [51]. Similar to TFEB, TFE3 interacts with active Rag GTPases in fully fed cells and is recruited to lysosomes. On the lysosomal surface, the mTORC1 complex phosphorylates TFE3, thus promoting interaction of TFE3 with 14-3-3 and retention of this transcription factor in the cytosol. Inactivation of mTORC1 by starvation releases TFE3, thereby allowing its redistribution to the nucleus and the TFE3-mediated expression of multiple target genes [51].

Among the genes regulated by TFE3 are critical metabolic and energetic regulators. In primary hepatocytes, TFE3 functions as a potent glucose lowering factor by activating the primary hepatic insulin signal cascade (IRS-2-PI3K-Akt), and stimulates glycogen synthesis through the induction *of Hk2* expression and the activation of GSK3β [52]. Conversely, depletion of TFE3 expression in livers of healthy mice increases plasma glucose and insulin levels, suggesting the appearance of insulin resistance [52]. TFE3 also plays an important role in glucose metabolism in mouse muscle. Iwasaki and coworkers demonstrated that TFE3 over-expression in skeletal muscle induced an increase in glycogen stores and enhanced endurance capacity through the upregulation of *Hk2*, *Glut4*, and *Gys* [53]. TFE3 over-expression also increased insulin sensitivity in transgenic animals challenged with exercise training [53]. In contrast, TFE3 has a negative effect on lipid synthesis as it markedly induces the expression of *Insig-1*, a key suppressor of the SCAP-SREBP complex involved in lipid synthesis [52, 54]. Recently, new evidence was provided suggesting that lipid metabolism and thermogenesis is regulated by TFE3 in adipose tissue [55]. Overall, these findings converge on the identification of TFE3 as an important player in nutrient sensing and energy-dependent metabolism *in vivo*.

Like TFEB, TFE3 appears to have a far-reaching regulatory role in the cell. Over-expression of TFE3 in ARPE-19 and HeLa cells is sufficient to induce expression of numerous lysosomal genes and significantly increases the number of LAMP1-positive structures, thereby implicating its role in lysosomal biogenesis [51]. Moreover, TFE3 binds to the CLEAR elements present in the promoter region of *MCOLN1*, thus suggesting that many lysosomal genes may be common targets for both, TFEB and TFE3. Depletion of TFE3 abolishes the enhanced expression of lysosomal genes induced by starvation, further confirming the role of TFE3 as a mediator that links nutrient sensing to lysosomal biogenesis [51]. TFE3 has been shown to have important regulatory functions in other

specialized cell types such as T and B cells [20, 56], as well as in mast cell-mediated allergic response [19]. In addition, TFE3 modulates the expression of genes implicated in bone resorption in well-differentiated osteoclasts [16]. Since all of these processes require the formation of specialized secretory lysosomes or granules, it appears that TFE3 regulation in biogenesis of lysosomes and lysosome related organelles (LROs) extends across many different cell types.

The role of MITF in the formation of LROs, particularly in the biogenesis of melanosomes, has been established in multiple cell types [57]. In B16 melanoma cells and normal human melanocytes, MITF functions as a critical signal transducer of the cAMP-dependent pigment production and melanogenesis pathways [58]. Also, some recently identified MITF targets, such as *HPS4*, *PSEN2* and *LYST*, are known to be important regulators of pigment biogenesis [59]. Interestingly, inhibition of mTORC1 activity by depletion of its components or by treatment with specific mTORC1 inhibitors such as rapamycin, causes upregulation of MITF and Tyrosinase with a concomitant increase in melanin production and late stage/ mature melanosome accumulation [60]. Similarly, Hah and coworkers reported that treatment of MNT1 melanoma cells with rapamycin led to increased MITF-4 protein levels, up-regulation of melanogenic enzymes, and melanogenesis stimulation [61].

Finally, over-expression of TFE3 or MITF-1 in ARPE-19 cells promotes transcriptional up-regulation of several critical regulators of the autophagic process, including *UVRAG*, *WIPI*, *ATG16L*, and *ATG9B* [51]. Therefore, the emerging view reveals fundamental similarities in the regulation of key cellular processes by TFEB, TFE3, and certain MITF isoforms (Figure 3). While future research will help to further discern the intricate correlations between nutrient sensing and organelle biogenesis, it is clear that the members of the MiTF/TFE family will play a major role in the control of these critical cellular functions.

## MiTF/TFE family and cancer

Many human cancers are characterized by highly specific chromosomal translocations [62]. Loss of transcriptional control has recently been implicated in renal cell carcinoma (RCC), a heterogeneous disease consisting of multiple subtypes classified by various morphological and genetic criteria [63, 64]. Translocation renal cell carcinoma (tRCC) or juvenile RCC was classified as one subtype of kidney cancer; and, while rare, it represents 15% of RCC in patients younger than 45 years [65]. This type of carcinoma is characterized by highly specific chromosomal translocations that often result in the fusion of a gene near the transcriptionally active promoter of another gene [64, 66]. This hybrid gene encodes an aberrant fusion protein that is both overexpressed and more active compared to its normal counterpart [62, 66]. The best-characterized tRCC involves translocations of the MiTF/TFE family of transcription factors.

TFEB associated kidney cancer is a more recently established form of juvenile kidney cancer that is characterized by a t(6:11)(p21;q13) translocation [67]. This translocation results in the fusion of the *TFEB* open reading frame on chromosome 6 with the 5’ regulatory region of the non-protein coding *Alpha* gene at chromosome 11 [67, 68]. While the *Alpha* gene does not contain an ORF of significant length and is transcribed in an

intronless manner, its promoter is more active and it resides near the multiple endocrine neoplasia type 1 locus that has previously been implicated in chromosomal abnormalities of numerous other tumors [69, 70]. Transcriptional studies revealed that the *Alpha/TFEB* mRNA levels were significantly upregulated in renal tumors compared to normal human kidney cells [68, 71]. These results were further corroborated by a dramatic upregulation of TFEB protein levels in the renal tumors [68, 71]. Importantly, transfection studies showed that TFEB protein encoded by *Alpha/TFEB* is efficiently targeted to the nucleus [68]. The dramatic transcriptional and translational overexpression of TFEB and its subsequent nuclear localization may disrupt proper downstream regulation of the MiTF/TFE family target genes that control normal cell growth, differentiation, and metabolism to ultimately drive renal tumorigenesis.

While misregulation of TFEB appears to be specific to one translocation locus, the tumorigenic effects resulting from aberrant activity of the other members of the MiTF/TFE family are more diverse. *TFE3* gene fusions have been implicated in two types of cancer. The hallmark of MiTF/TFE family misregulation in tRCC is the fusion of *TFE3* on the X chromosome to various gene targets including *PSF* (1p34), *PRCC* (1q21), *CLTC* (17q23), *NONO* (Xq12), *SFPQ* (1p34), and *ASPL* (17q25) [72, 73]. Also, the *ASPL/TFE3* fusion is the characteristic modification found in alveolar soft part sarcoma (ASPS), a tumor that is usually involved in muscle and deep soft tissues of the extremities [74]. To date, the mechanistic differences between the various fusion genes have not been established. However, excessive activation of MET signaling has been observed in *ASPL-*, *PSF-*, and *NONO/TFE3* fusions and has been implicated in uncontrolled cell proliferation, invasion, and metastasis [75, 76]. More specifically, soft tissue sarcomas expressing the *ASPL/TFE3* fusion transcripts display mTOR pathway activation including elevated expression of pAKT T308, pp70S6K, and p4EBP1 [77] suggesting a greater potential for cell survival, proliferation, and metabolic alterations. Also, cells expressing the PRCC/TFE3 protein appear to have an impaired mitotic checkpoint that may be crucial in the development of renal cell carcinomas [78]. Other studies have also shown that *PRCC/TFE3* fusions deregulate cell-cycle proteins with the accumulation of cyclin D1, D3, and p21 [79, 80]. While these results appear to be antagonistic, it has been suggested that during the course of tumor development the delay is eventually minimized to allow full tumorigenic progression [79].

The third major member of the MiTF/TFE family, MITF, has not only been implicated in kidney cancer, but is a major target in understanding melanoma development. While MITF is expressed in most melanomas, the levels of this protein vary between melanoma specimens [81] and appear to present contradictory downstream effects. Several reports suggest that MITF overexpression may promote anti-melanoma activity. Indeed, high levels of MITF minimized proliferative activity, invasiveness, and tumor development [82–85]. Conversely, *MITF* amplification has been identified in 10–20% of melanomas [86]. Studies investigating chromosomal abnormalities in various human cancer cell lines using high-density single nucleotide polymorphism (SNP) arrays identified copy gains at the *MITF* locus in melanoma cell lines thus revealing the oncogenic potential of MITF [86]. Ultimately, this amplification of MITF activity promotes expression of its transcriptional

targets that are involved in cell proliferation (*CDK2*), cell survival (*BCL2*), invasiveness (*cMET*), and cell-cycle arrest (*p21, p16*) [87–91]. Carreira et al described that low MITF activity leads to a $p27^{KIP1}$-dependent cycle arrest with increased invasiveness properties. In contrast, intermediate levels of MITF activity result in proliferation, whereas high levels induce differentiation [92]. Tumor micro-environment may affect multiple signaling pathways that modulate MITF levels and activity, thus explaining the apparently contradictory effects of MITF in melanoma proliferation and invasiveness. Finally, recent work has revealed that impaired SUMOylation resulting from a missense mutation (Mi-E318K) enhanced MITF activity and occurred with high frequency in patients affected with melanoma, renal cell carcinoma, or both [93]. The Mi-E318K mutant also exhibited tumorigenesis by promoting melanocytic and renal cell invasion, migration, and colony formation. The oncogenic potential of MITF dysregulation was further corroborated in another cancer line in which clear cell sarcoma (CCS) tumors were found to harbor the chimera EWS-activating transcription factor 1 (*ATF1*) fusion gene that constitutively occupied the *MITF* promoter and induced MITF expression which subsequently permitted CCS survival [94]. Therefore, it will be critical to further characterize the contribution of the members of the MiTF/TFE family in tumor progression and survival.

## TFEB is a novel therapeutic target for the treatment of lysosomal diseases

Since the identification of TFEB as a master regulator of autophagy and lysosomal biogenesis [25], there has been a steady increase in the scientific interest to use this transcription factor as a therapeutic target for the treatment of human diseases. In particular, research has been focused on disorders in which the function of the lysosomal and/or autophagic compartments is compromised or in conditions where there is an intracellular accumulation of toxic proteins. These characteristics are common among neurodegenerative diseases. Using a murine model of Parkinson's disease (PD), Dehay and coworkers showed that lysosomal dysfunction induced by increased mitochondrial-derived reactive oxygen species resulted in defective clearance of the abnormally accumulated autophagosomes in dopaminergic cells. Interestingly, pharmacological induction of lysosomal biogenesis and TFEB-mediated upregulation of lysosomal genes ameliorated the PD-related dopaminergic neurodegeneration [95]. Furthermore, increased intracellular levels of the disease causing protein, SNCA/α -synuclein, prevented translocation of TFEB to the nucleus, thus effectively compromising the autophagy-lysosomal pathway [96]. Restoration of autophagy-lysosomal activity in rat neuronal cells by TFEB over-expression or pharmacological inhibition of mTORC1 had a neuroprotective effect in PD models [96]. Recently, over-expression of TFEB has also been used to promote intracellular clearance of huntingtin aggregates in a mouse model of Huntington's disease (HD). The restoration of a transcriptional program coordinated by PGC-1α improved symptoms in the brains of HD mice, mainly through the direct activation of TFEB [97]. These findings suggest that boosting lysosomal/autophagic activity and function though TFEB activation might be a potentially beneficial therapeutic approach for disease intervention in certain neurological and aging disorders. However, the therapeutic potential of TFEB is not restricted to neurodegenerative disorders, but can also contribute to the regression of other diseases as well. Alpha-1-anti-trypsin deficiency, the most common genetic cause of liver disease, is

characterized by the pathological aggregation of mutated Alpha- 1-anti-trypsin protein in the endoplasmic reticulum of hepatocytes. Pastore and coworkers demonstrated that the hepatic gene transfer of TFEB in the PiZ mouse model resulted in enhanced autophagic flux and allowed clearance of the hepatotoxic mutated protein, thus correcting the hepatic fibrosis and thus liver injury [98].

The central function of TFEB in cellular clearance has also been demonstrated in mouse models of Lysosomal Storage Disorders (LSDs). LSDs are characterized by the accumulation of undigested material in lysosomes due to the deficiency of specific lysosomal proteins. Multiple sulfatase deficiency (MSD) and mucopolysaccharidosis type IIIA (MPS-IIIA) are two LSDs characterized by severe neurodegeneration due to accumulation of glysosaminoglycans (GAGs) in neuron and glial cells [99, 100]. Over-expression of TFEB in glia-differentiated neuronal stem cells (NSCs) derived from MSD and MPS-IIIA mouse models results in a significant reduction in the amount of intracellular GAGs, increased GAG secretion, reduction in the number of enlarged lysosomes, and recovery of normal cell morphology [101]. Importantly, adenovirus-mediated over-expression of TFEB in a mouse model of MSD reverts not only GAG accumulation, but also secondary pathological processes associated with lysosomal storage such as inflammation and cell death [101]. Overexpression of TFEB also decreased intra-lysosomal accumulation of lipofuscin in fibroblasts derived from a patient with Batten disease [101] and dramatically reduced lysosomal size and intra-lysosomal glycogen accumulation in Pompe disease myotubes [102], thus suggesting that TFEB promotes clearance of different types of lysosomal substrates.

The mechanism by which TFEB mediates intra-lysosomal clearance not only includes increased lysosomal activity and biogenesis, but also TFEB-induced lysosomal exocytosis. Upon over-expression, TFEB promotes fusion of lysosomes with the plasma membrane by raising intracellular $Ca^{2+}$ levels through the increased expression and activation of the lysosomal $Ca^{2+}$ channel MCOLN1 [103, 101, 104]. Accordingly, transient or stable depletion of MCOLN1 impairs lysosomal exocytosis and the increase in intracellular $Ca^{2+}$ levels induced by TFEB over-expression. Moreover, over-expression of MCOLN1 alone is sufficient to significantly reduce GAG accumulation in glia-differentiated NSCs isolated from MSD mice [101]. Interestingly, the TFEB-mediated clearance of accumulated glycogen in a mouse model of Pompe disease was attributed to the exocytosis of autophagolysosomes, thus evidencing the important role of autophagy and TFEB in this process [102]. Finally, Song and colleagues recently reported that by upregulating the expression of genes implicated in folding and trafficking of lysosomal proteins, TFEB increased the lysosomal levels of glucocerebrosidase and β-hexosaminidase, two proteins associated with the development of Gaucher disease and Tay-Sachs disease, respectively [105]. This work suggests that TFEB can be used as a way to regulate lysosomal proteostasis and rescue lysosomal enzyme deficiencies in LSDs.

Considering the effect of prolonged TFEB over-expression in the development of renal carcinoma, it will be critical to develop therapeutic strategies aimed to modulate the activation of TFEB in a temporal and tissue specific manner. One could aim for approaches that combine reversible inhibition of mTORC1 with inducible ectopic expression of TFEB.

At the same time, the discovery of small molecules capable of activating endogenous TFEB independently of mTORC1 could avoid potential harmful side effects caused by prolonged mTORC1 inactivation. Finally, recent evidence suggests that over-expression of TFE3 also induces lysosomal exocytosis and promotes efficient clearance of intra-lysosomal glycogen in a cell model of Pompe disease [51]. Therefore, targeting of TFE3 either alone, or together with TFEB could potentially promote broader beneficial consequences for the treatment of these devastating diseases.

## Animal Models

To fully understand the physiological role of any transcription factor *in vivo*, the use of animal models is crucial. The expression pattern of *Tfeb* has previously been characterized in mouse by northern blot and *in situ* hybridization [106]. *Tfeb* is expressed at varying levels in most analyzed tissues in adult mice, including heart, striated muscle, smooth muscle, brain, and kidney tubules. During development *Tfeb* is mainly expressed in the heart and yolk sac at 9.5 dpc, while low level of expression are also detected in the embryonic liver, eye, and brain. While the knockouts of *Mitf* and *Tfe3* are viable [2, 17, 11], *Tfeb* null animals die between 9.5 and 10.5 days of embryonic development due to severe defects in placental vascularization, which is consistent with the high level expression of *Tfeb* in the labyrinthine trophoblast cells of the placenta [106]. Functional studies showed that although labyrinthine cells are present in the mutant *Tfeb* placenta, they fail to express VEGF, a potent mitogen required for normal vasculogenesis of the embryos and extraembryonic tissues, thus indicating that *Tfeb* plays a critical role in the signal transduction processes required for normal vascularization of the placenta. Unfortunately, the lethality associated with *Tfeb*-disrupted embryos in early development makes it impossible to study *Tfeb* function in organogenesis and later development. Therefore, tissue specific knockout have been generated to study the specific function of *Tfeb* in certain mouse tissues. As mentioned earlier, a mouse model lacking *Tfeb* specifically in osteoclasts, showed decreased expression of lysosomal genes, reduced number of lysosomes, and a decreased ability to resorb the bone matrix [47]. In addition, gain- and loss-of-function studies of *Tfeb* using both microinjection and a liver- specific knockout mouse line indicate that *Tfeb* transcriptionally regulates lipid catabolism. Absence of TFEB further promotes metabolic imbalance in obese animals, whereas TFEB overexpression causes the opposite effects and rescues obesity and associated metabolic syndromes [33].

HLH-30, the only member of the MiTF/TFE family in *Caenorhabditis elegans*, has been proposed to be the ortholog of either TFEB or TFE3 [107, 108, 33]. Interestingly, HLH-30 recognizes DNA sequences similar to the CLEAR motif and drives transcription of metabolic genes *in vivo* [107]. Lipid storage and metabolism were studied using *hlh-30* mutants in *C. elegans* intestinal cells, which perform similar metabolic functions to the vertebrate liver. The results suggest that HLH-30 is required to efficiently use lipid stores during starvation (Figure 2). Similar to TFEB, the levels of *hlh-30* mRNA are progressively increased in fasting worms and are quickly reduced after refeeding [108, 33]. Following fasting, HLH-30 accumulates in the nucleus and induces the expression of lysosome lipases, which are key enzymes in breaking down lipid-droplet fats through lipophagy [108]. In addition, autophagy is activated following nutrient deprivation as autophagy genes are

transcriptionally upregulated in fasted animals. However, *hlh-30* mutants fail to activate the transcription of essential autophagy genes, suggesting that HLH-30 coordinates the activation of lysosomal lipolysis and autophagy to meet the nutritional needs of the cell [108, 33]. In addition, the starvation-induced lifespan extension observed in wild-type *C. elegans* was lost in *hlh-30* mutants, suggesting a possible role of HLH-30 in longevity [33]. Lastly, loss of *hlh-30* function suppresses extended lifespan in several different longevity models [108, 109], whereas HLH-30 overexpression is sufficient to extend lifespan [109]. Therefore, the role of TFEB/TFE3 in energy metabolism and adaptation to starvation appears to be highly conserved during evolution.

Finally, the ex-uterus development of zebrafish makes it an exceptionally promising model for the study of TFEB function. Whole mount *in situ* hybridization analysis showed that the expression of *Tfeb* is first observed in the eye at 8-somite stage, and shortly thereafter in the paraxial and lateral plate mesoderm [110]. By the 18-somite stage *tfeb* expression is detected in pronephros and the medial portion of the posterior somites. The lens expression increases between 24 and 48 hpf and diminishes thereafter. Expression is also observed in the heart between 24 and 60 hpf, and in the kidney, which is robust at 60 hpf. In addition, there is variable expression of *Tfeb* in the brain, otic vesicle, pharyngeal arches, optic fissue, and jaw musculature [110]. All together, these observations suggest that TFEB may regulate crucial functions during early development.

## Concluding remarks

Cells must integrate multiple growth-stimulating and inhibitory signals to ultimately regulate a wide-array of key cellular functions, including gene expression and proliferation. Recent findings revealed that lysosomes function as signaling centers that link environmental cues, such as nutrient availability, to organelle biogenesis, energy production, and cellular homeostasis. The members of the MiTF/TFE family of transcription factors play a critical role as messengers in this lysosome-to-nucleus pathway, transducing the information from the cytosol to the nucleus and activating an integrated network of unsuspected complexity. So far we recognize that the cellular response to starvation (and possibly also to lysosomal stress) includes activation of the main degradative pathways in the cell (autophagy and lysosomes) as well as certain catabolic processes destined to provide energy from internal stores. However, it is possible that additional cellular pathways, such as endocytosis, biosynthetic transport, or mitochondrial function, are also modified in response to nutrient deprivation. Understanding the specific functions of the MiTF/TFE family, the mechanism of their expression, as well as the regulation of their target genes, will certainly improve our knowledge of how organisms adapt so that they can survive under starvation conditions. It will also help us to appreciate how alterations in this response may be critical in cancer and metabolic diseases.

## Acknowledgments

This work was supported by the Intramural Research Program of the National Institutes of Health, National Heart, Lung, and Blood Institute (NHLBI)

## Abbreviations

| | |
|---|---|
| **AKT** | v-akt murine thymoma viral oncogene |
| **ARPE-19** | retinal pigmented epithelium cell line |
| **ASPL** | alveolar soft part sarcoma chromosome region, candidate 1 |
| **ASPS** | alveolar soft part sarcoma |
| **ATF1** | activating transcription factor 1 |
| **ATG1** | unc-51 like autophagy activating kinase 1 |
| **ATG13** | autophagy related 13 |
| **ATG16L** | autophagy related 16-like (S. cerevisiae) |
| **ATG9B** | autophagy related 9B |
| **ATP6V** | ATPase, H+ transporting lysosomal |
| **BCL2** | B-cell CLL/lymphoma 2 |
| **bHLH-LZ** | basic/helix-loop-helix/leucine zipper |
| **c-MET** | receptor tyrosine kinase |
| **C. elegans** | Caenorhabditis elegans |
| **CCS** | Clear Cell Sarcoma |
| **CDK2** | cyclin-dependent kinase 2 |
| **CLEAR** | Coordinated Lysosomal Expression and Regulation |
| **CLTC** | clathrin, heavy chain (Hc) |
| **ERK2** | mitogen-activated protein kinase 1 |
| **FOXO3** | forkhead box O3 |
| **GAGs** | glycosaminoglycans |
| **GLUT4** | solute carrier family 2 (facilitated glucose transporter, member 4 |
| **GSK3β** | glycogen synthase kinase 3 beta |
| **GYS** | glycogen synthase |
| **HD** | Huntington’s disease |
| **HIF1** | hypoxia inducible factor 1 |
| **HK2** | hexokinase 2 |
| **HLH-30** | Caenorhabditis elegans TFEB orthologue |
| **HPS4** | Hermansky-Pudlak syndrome 4 |
| **IRF3** | interferon regulatory factor 3 |
| **IRF7** | interferon regulatory factor 7 |

| | |
|---|---|
| **IRS2** | insulin receptor substrate 2 |
| **ISGs** | interferon-stimulated genes |
| **LAMP1** | lysosomal-associated membrane protein 1 |
| **LROs** | Lysosome Related Organelles |
| **LSDs** | Lysosomal Storage Disorders |
| **LYST** | lysosomal trafficking regulator |
| **MAP1LC3B** | microtubule-associated protein 1 light chain 3 beta |
| **MAX** | MYC associated factor X |
| **MCOLN1** | mucolipin 1 |
| **MITF** | microphthalmia-associated transcription factor |
| **MPS-IIIA** | Mucopolysaccharidosis type IIIA |
| **MSD** | Multiple Sulfatase Deficiency |
| **MTORC1** | mechanistic target of rapamycin (serine/threonine kinase) complex 1 |
| **MYC** | v-myc avian myelocytomatosis viral oncogene homolog |
| **NONO** | non-POU domain containing, octamer-binding |
| **NSCs** | Neuronal Stem Cells |
| **p16** | cyclin-dependent kinase inhibitor 2A |
| **p21** | cyclin-dependent kinase inhibitor 1A (p21, Cip1) |
| **PD** | Parkinson’s disease |
| **RCC** | Renal Cell Carcinoma |
| **PI3K** | phosphatidylinositol-4,5-bisphosphate 3-kinase |
| **PPARA** | peroxisome proliferator-activated receptor alpha |
| **PPARGC1A** | peroxisome proliferator-activated receptor gamma, coactivator 1 alpha |
| **PRCC** | papillary renal cell carcinoma (translocation-associated) |
| **PKCβ** | protein kinase C, beta |
| **PSEN2** | presenilin 2 (Alzheimer disease 4) |
| **PSF** | splicing factor proline/glutamine-rich |
| **RANKL** | tumor necrosis factor (ligand) superfamily, member 11 |
| **RHEB** | Ras homolog enriched in brain |
| **SCAP** | SREBF chaperone |
| **SNP** | Single Nucleotide Polymorphism |
| **SQSTM1** | sequestosome 1 |

| | |
|---|---|
| **SREBP** | sterol regulatory element-binding protein |
| **STING** | stimulator of interferon genes |
| **TBK1** | TANK-binding kinase 1 |
| **TFE3** | transcription factor binding to IGHM enhancer 3 |
| **TFEB** | Transcription Factor EB |
| **TFEC** | Transcription Factor EC |
| **P53** | tumor protein p53 |
| **tRCC** | Translocation Renal Cell Carcinoma |
| **TREX1** | three prime repair exonuclease 1 |
| **TSC2** | tuberous sclerosis 2 |
| **USF** | upstream transcription factor |
| **UVRAG** | UV radiation resistance associated |
| **VPS11** | vacuolar protein sorting 11 homolog (*S. cerevisiae*) |
| **VPS18** | vacuolar protein sorting 18 homolog (S. cerevisiae) |
| **WIPI1** | WD repeat domain, phosphoinositide interacting 1 |
| **ZKSCAN3** | zinc finger with KRAB and SCAN domains 3 |

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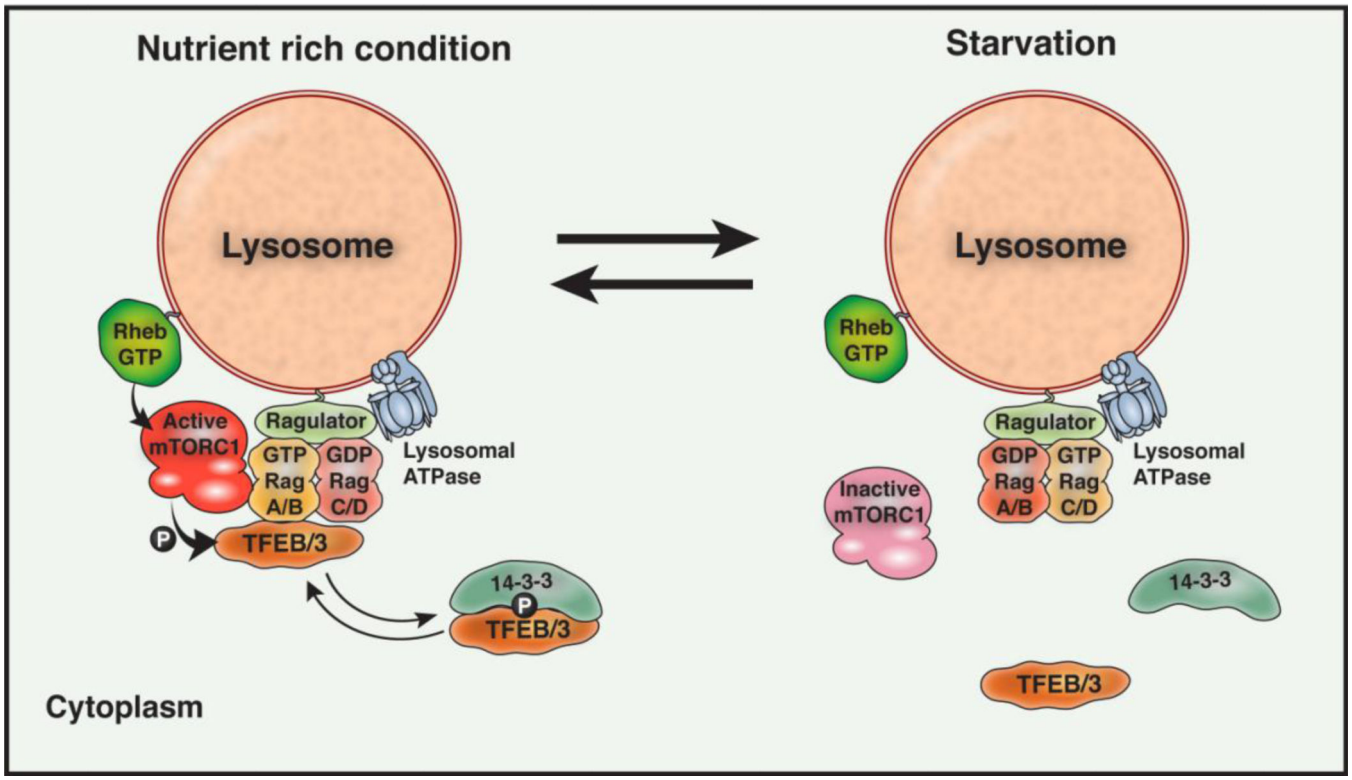


**Figure 1. Mechanism of TFEB regulation by Rag GTPases**

In fully fed cells, TFEB is recruited to lysosomes via direct interaction with active Rag GTPases. This recruitment is critical for mTORC1-dependent phosphorylation and the subsequent 14-3-3-mediated retention of TFEB in the cytosol. Inactivation of Rags and mTORC1 following starvation leads to dissociation of the TFEB/14-3-3 complex and rapid translocation of TFEB to the nucleus. The same regulatory mechanism controls the activity and intracellular distribution of TFE3.

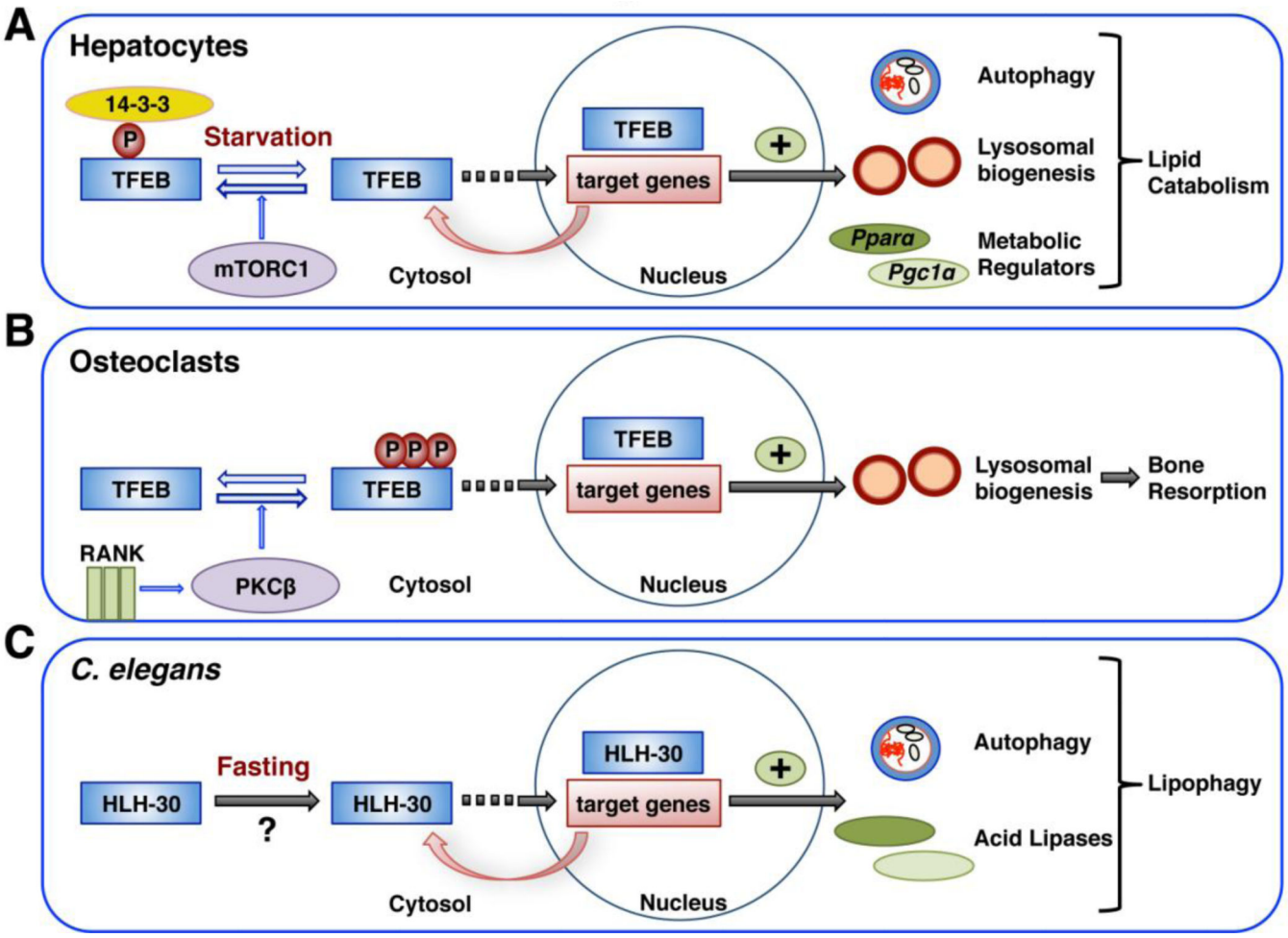


**Figure 2. Model of TFEB function and regulation in different cell types**

**(A)** TFEB plays a critical role in the adaptation of cells to nutrient deprivation. In hepatocytes TFEB translocates to the nucleus under starvation conditions to promote autophagy, lysosomal biogenesis, and expression of key metabolic regulators, thus ensuring efficient use of energy stores and cell survival. A TFEB auto-regulatory feedback loop (pink arrow) results in increased TFEB levels under prolonged starvation conditions. **(B)** Activation of TFEB in osteoclasts is not dependent on nutrient levels but RANKL-mediated signaling. Up-regulation of specific lysosomal genes by TFEB is essential for resorption of the bone matrix. **(C)** The role of TFEB in the cellular response to starvation is evolutionary conserved. In *C. elegans*, HLH-30 accumulates in the nucleus upon fasting and up-regulates expression of genes that mediate lipophagy. Starvation-induced activation of HLH-30 may also have important consequences in longevity.

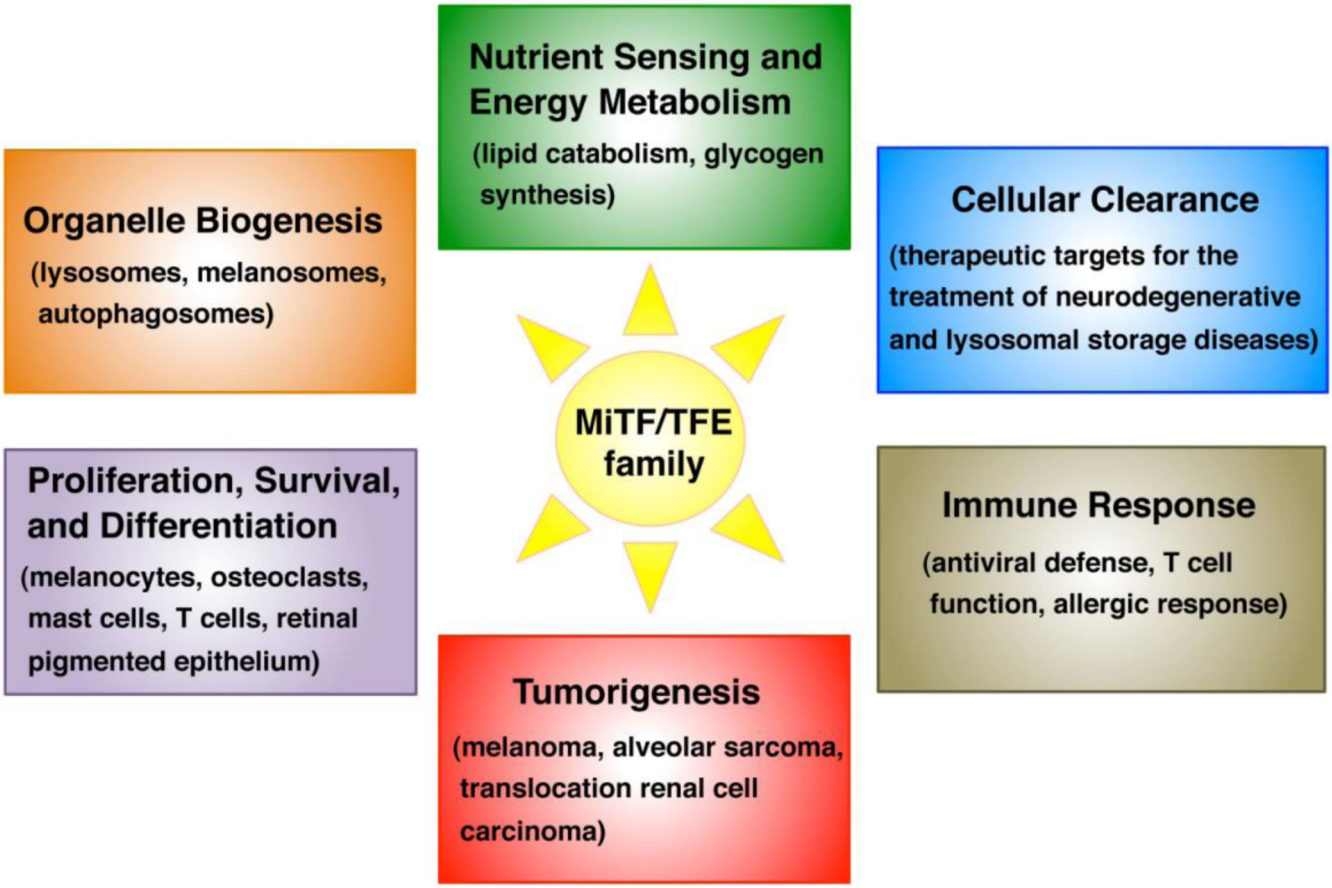


**Figure 3. The MiTF/TFE family of transcription factors regulates multiple cellular processes**

The members of the MiTF/TFE family share critical roles in organelle biogenesis, cell survival and differentiation, and tumorigenesis. In addition, TFEB and TFE3 participate in the regulation of nutrient sensing, energy metabolism, and cellular clearance.