The Transcription Factor EB Links Cellular Stress to the Immune Response

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The transcription factor EB (TFEB\textsuperscript{†}) is the master transcriptional regulator of autophagy and lysosome biogenesis. Recent advances have led to a paradigm shift in our understanding of lysosomes from a housekeeping cellular waste bin to a dynamically regulated pathway that is efficiently turned up or down based on cellular needs. TFEB coordinates the cellular response to nutrient deprivation and other forms of cell stress through the lysosome system, and regulates a myriad of cellular processes associated with this system including endocytosis, phagocytosis, autophagy, and lysosomal exocytosis. Autophagy and the endolysosomal system are critical to both the innate and adaptive arms of the immune system, with functions in effector cell priming and direct pathogen clearance. Recent studies have linked TFEB to the regulation of the immune response through the endolysosomal pathway and by direct transcriptional activation of immune related genes. In this review, we discuss the current understanding of TFEB’s function and the molecular mechanisms behind TFEB activation. Finally, we discuss recent advances linking TFEB to the immune response that positions lysosomal signaling as a potential target for immune modulation.

INTRODUCTION

Since the discovery of transcription factor EB (TFEB) as the master regulator of autophagy and lysosome biogenesis [1,2], there has been a rapid expansion of cellular processes understood to be directly or indirectly regulated by TFEB (reviewed in [3]). Lysosomes, the primary cellular degradative compartment, were initially thought to be static housekeeping organelles [4]. Recent advances have changed that view, as it was dis-
covered that the lysosome pathway is dynamically up or down-regulated dependent on the cellular environment [1] (reviewed in [5]). TFEB transcriptionally regulates the lysosomal pathway and therefore affects both inputs and outputs of the lysosomal system. Extracellular cargo is delivered to lysosomes either via endocytosis and the endolysosome system [6], or through phagocytosis and subsequent phagosome-lysosome fusion [7]. Intracellular cargo, on the other hand, is delivered to lysosomes via macroautophagy (referred to autophagy herein). In further contrast to the view of lysosomes as static, it has now been appreciated that lysosomes provide output to the cell both by serving as signaling platforms and by facilitating cellular clearance through lysosomal exocytosis [8].

The innate and adaptive arms of the immune system act in concert to protect host organisms from foreign pathogens. The innate immune system, which is typically considered the first line of defense against infection, constitutes non-specific defenses and is mediated primarily by macrophages, neutrophils, and cell autonomous defense. The adaptive immune system, consisting primarily of B and T lymphocytes, is responsible for specific and long-lived defense against pathogens (reviewed in [9]). The adaptive and innate immune systems both utilize autophagy and the endolysosomal system in a variety of capacities to mediate immunity [10] (reviewed in [11,12]). As such, recent studies have linked TFEB to the immune response both through effects on the endolysosomal system and by direct transcriptional activation of immune related genes. In this review, we discuss the current understanding of TFEB function, as well as the molecular mechanisms behind TFEB activation. Finally, we discuss recent findings linking TFEB to the immune response, positioning lysosomal signaling as a potential target for immune modulation.

**Figure 1. MiT Family Member Structure.** The MiT Family members share common structural characteristics. AD: Transactivation Domain, bHLH: basic-helix-loop-helix domain, Gin: Glutamine Rich Region, LZ: Leucine Zipper Region, Pro: Proline Rich Region, Pro+Arg: Proline + Arginine Rich Region, Ser: Serine Rich Region.

**MICROPHTHALMIA FAMILY OF bHLH-zip TRANSCRIPTION FACTORS (MiT FAMILY)**

TFEB, along with microphthalmia-associated transcription factor (MITF), transcription factor E3 (TFE3), and transcription factor EC (TFEC) constitute the microphthalmia subfamily of basic helix-loop-helix-leucine zipper (bHLH-zip) transcription factors (MiT family) [13-16]. All MiT family members are conserved in vertebrates and share structural similarities; they contain an identical basic domain that binds specific DNA regions, and HLH and ZIP domains critical in protein-protein interactions (Figure 1). The HLH and ZIP domains mediate the formation of homo- and hetero- dimers, which is required for DNA binding and transcriptional activation of target genes. TFEB, TFE3, and MITF also contain a conserved transactivation domain needed for transcriptional activation [17,18], while the most divergent member of the family TFE3 lacks this domain and thus acts to repress transcription in complex with other subfamily members [16]. Outside of the conserved regions, these proteins differ significantly from one another and have important differences from other bHLH-zip transcription factors. Like most bHLH-zip transcription factors
Expression of MiT family members in different cell types and their relevant phenotypes:

| MiT Member | Expression | KO Dysfunction | Phenotypic Manifestation | Ref  |
|------------|------------|----------------|--------------------------|------|
| Mitf       | Melanocyte | Melanocyte Differentiation | Coat color defects       | [14] |
|            | Osteoclast | RPE Differentiation     | Small eyes               | [24] |
| Mast Cell  | Osteoclastogenesis |                   | Osteopetrosis           | [25] |
| Macrophage | Mast Cell Differentiation |                | Reduction in B cells     | [26] |
| NK Cell    | Notch Signaling |                          | Reduction in NK cells    | [27] |
| B Cell     |                        |                | Reduction in Macrophages  | [28] |
| Heart      |                        |                | Reduction in Mast cells   | [29] |
| TFE3       | Ubiquitous | Osteoclastogenesis | Osteopetrosis           | [25] |
|            | Mast cell function |            | Decreased mast cell degranulation | [30] |
|            | T cell (with TFEB) |                   | Hyper-IgM Syndrome       | [31] |
| TFEB       | Ubiquitous | Placental Vascularization | Embryonic Lethal – E9-10 | [33] |
|            | Osteoclast Function |            | Increased bone mass      | [34] |
|            | Dendritic Cell Function |        | Dec Phagosomal acidication | [35] |
|            | ESC Wnt Signaling |                | Endodermal differentiation defects | [36] |
| TFEC       | Myeloid Cells | None | None | [16,23,37] |

The expression patterns of MiT family members vary significantly; TFEB and TFE3 are broadly expressed and present across most cell types, while TFEC is the most restricted and is expressed only in myeloid cells [22,23]. MITF is predominantly expressed in melanocytes, osteoclasts, mast cells, macrophages, natural killer (NK) cells, B-lymphocytes, and the heart [17]. The MiT family members are important in cell differentiation and the development, as evidenced by the variety of defects seen in mice harboring mutations in these genes (summarized in Table 1). Mice with mutations in the MITF locus have defects in neural crest derived melanocyte and retinal pigment epithelium differentiation, osteoclastogenesis, mast cell differentiation, and notch signaling. These manifest phenotypically as changes in coat color, small eyes, osteopetrosis, and a reduction in NK cell, B cell, and macrophage numbers [14,24-29]. On gross examination, TFE3 knockout mice are viable and do not have any visible abnormalities. However, closer examination shows that TFE3 KO mice have qualitative changes in mast cell activation (e.g., decreased mast cell degranulation), and that TFEB operates in tandem with MITF to regulate osteoclast differentiation and in tandem with TFEB for proper T cell activation and function [25,30,31]. TF3E has also been implicated in development as TF3E restricts embryonic stem cell (ESC) differentiation, thus retaining ESC pluripotency and self-renewal capability [32]. TF3E KO mice are embryonic lethal at E9.5 to 10.5 due to placental vascularization defects [33]. Experiments in tissue specific TF3E KO mice and cell lines have shown that TF3E also plays an important role in osteoclast differentiation, dendritic cell function, and in endodermal lineage differentiation in ESCs [34-36]. Finally, the TF3F KO mice are viable with no major phenotype. The only differences seen between TF3F KO and WT cells are subtle changes in the macrophage compartment after response to T-helper 2 (Th2) cytokine stimulation [16,23,37].

The evidence of some functional overlap between MiT family members is expected, as lower organisms contain a single MiT ortholog capable of functioning similarly to several mammalian MiT members [38]. This ortholog is called Mitf in Drosophila melanogaster and HLH-30 in Caenorhabditis elegans [39,40]. Both Mitf and HLH-30 contain the conserved basic regions and HLH-zip domains seen in mammalian MiT members – suggesting similar mechanisms of DNA binding [41]. The D. melanogaster Mitf is equally related to human MITF and TFEB [41], rendering these organisms as a suitable model for the study of MiT family members. Apart from their role in development, recent advances have linked TFEB to the lysosome system, which will be discussed ahead.

**TFEB IN AUTOPHAGY AND LYSOSOME BIOGENESIS**
Lysosomes are the terminal degradative compartment of the endolysosomal system. They are critical for cellular homeostasis as they have a role in many essential cellular processes, including cellular recycling, endocytosis, autophagy, lysosomal exocytosis, cholesterol homeostasis, downregulation of surface receptors, bone remodeling, repair of the plasma membrane, antigen presentation, and inactivation of pathogenic organisms (reviewed in [42]). Importantly, calcium signaling is involved in many major lysosomal processes, including endosome-lysosome fusion, lysosome exocytosis, and membrane repair [43,44]. The first indication that lysosome biogenesis is transcriptionally regulated came from the observation that multiple lysosome-related genes were synchronously regulated in response to sucrose-induced lysosomal stress [45]. A later microarray analysis by the Ballabio group demonstrated more broadly that a variety of genes encoding for lysosomal proteins are co-regulated under different conditions across multiple cell lines [1]. Promoter analysis of the concomitantly expressed genes revealed a common 10-base E-box-like palindromic sequence (GTACGTCGAC) located typically within 500 base pairs of the transcription initiation site. This motif was named the Coordinated Lysosomal Expression and Regulation (CLEAR) element, and genome wide chromatin immunoprecipitation sequencing (ChIP-seq) of CLEAR elements showed enrichment in lysosomal genes and confirmed direct TFEB binding [46]. In line with this observation, overexpression of TFEB results in an increased number of lysosomes, an increase in lysosomal enzyme levels (including lysosomal hydrolases, v-ATPases, and lysosomal transmembrane proteins) and enhanced lysosome degradation capability – confirming TFEB as a bona-fide master regulator of lysosome function [1].

As the lysosome system has a variety of inputs and outputs, it would follow that these processes may be concurrently regulated with lysosome biogenesis. Ensuing work found that the CLEAR network and TFEB regulated a larger set of genes, including a subset of essential autophagy genes. This landmark paper by the Ballabio group is remarkable because it placed TFEB as the master regulator of two critical cellular degradation pathways [2]. Autophagy is an essential lysosome-dependent degradation pathway that plays a role in the breakdown of cellular organelles and protein complexes too large for proteasomal degradation. In this process, a double membrane vesicle termed the autophagosome envelops cytoplasmic constituents and eventually fuses with the lysosome. After fusion, the vesicle is termed an autophagolysosome and the enveloped components are degraded (reviewed in [47]). Autophagy plays an important role in cellular clearance, and TFEB activation/overexpression has been shown to mediate enhanced degradation of bulk autophagy substrates [2,48-51]. Moreover, TFEB has also been implicated in facilitating the clearance of damaged mitochondria through mitophagy and of lipid droplets through lipophagy [52,53]. Finally, TFEB has also been shown to play a role in regulating endocytosis [54] and lysosomal exocytosis [55], a process by which lysosomes fuse to the plasma membrane to promote cellular clearance by secretting its contents into the extracellular space. These findings indicate an important role for TFEB in modulation of the endolysosomal system and clearance of cellular debris, which has led to discoveries implicating TFEB in the pathogenesis of or as a viable therapeutic modality for a number of protein aggregation based diseases, including Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, Amyotrophic Lateral Sclerosis, Frontotemporal Dementia, and others [49,56-60].

While TFEB was the first member of the MiT family to be identified as a master regulator of lysosomal biogenesis, recent work has shown that TFE3 also binds CLEAR elements and induces lysosomal biogenesis and autophagy independent from TFEB [61]. The same study did not see an increase in expression of lysosomal genes following the overexpression of MITF-A isoform in ARPE-19 cells [61], but an analysis of 51 melanoma lines in another study showed correlation between MITF expression and lysosomal gene expression [62]. While the role of MITF in the control of lysosomal gene transcription is still unclear, additional studies must be done to account for the ability of MiT family members to heterodimerize and influence each other’s relative contribution to lysosomal gene expression.

**TFEB Activation as a Response to Cellular Stress**

**Molecular Mechanisms of TFEB Activation**

TFEB activation and cellular localization is controlled by post-translational modifications and protein-protein interactions. Thus far, multiple phosphorylation sites and upstream kinases/phosphatases have been identified (summarized in Figure 2). Of note, the serine/threonine kinase mammalian target of rapamycin (mTOR) phosphorylates Serine 211 (S211), and its inhibition by Torin-1 promotes TFEB nuclear translocation. Phosphorylation of this site mediates TFEB binding to 14-3-3 proteins and its subsequent cytoplasmic sequestration [63,64]. Conversely, the phosphatase calcineurin (specifically calcineurin catalytic subunit isoform beta; PPP3CB) promotes S211 dephosphorylation upon activation, enabling TFEB nuclear translocation [65]. Interestingly, the S211A TFEB mutant basal shows diffuse cytoplasmic and nuclear localization, but paradoxically retains regulation by Torin-1 [66]. A recent report demon-
important for TFEB nuclear translocation and activation [54]. However, a recent report definitively showed that S467 is phosphorylated by Akt (protein kinase B) and inhibits the nuclear translocation of TFEB [69]. Thus, it will be important to parse out the individual contributions of the phosphorylation sites on the serine-rich region to gain a more complete understanding of TFEB regulation. Finally, a recent study showed Serine 134 (S134) and serine 138 (S138) are phosphorylated by GSK3β, inhibiting TFEB nuclear translocation. Phosphorylation of S134 and S138 appear to be important in directing TFEB to the lysosomal surface, where it interacts with mTORC1. The S134A and S138A TFEB double mutant has decreased lysosomal localization, decreased S211A phosphorylation, and decreased interaction with 14-3-3 proteins, leading to its nuclear localization [68].

Figure 2. TFEB Activation Schematic. TFEB is phosphorylated at several sites, some are activating and some are inhibitory. TFEB localization to the lysosome is important for its inhibitory phosphorylation by the mTORC1 complex. Dashed lines indicate hypothesized mechanisms of action. P(red): Inhibitory phosphorylation, P(green): Activating Phosphorylation.

strated that this is due to multistep regulation of TFEB by the mTOR containing mammalian target of rapamycin complex 1 (mTORC1), which phosphorylates TFEB Serine 122 (S122) in addition to S211, contributing to mTORC1 mediated inhibition of TFEB nuclear translocation [66]. Serine 142 (S142) is another important site in the regulation of TFEB subcellular localization. S142 phosphorylation is mediated by mTORC1 and ERK2 [2,67], and it has been suggested that S142 dephosphorylation mediates TFEB nuclear translocation by reducing S211 phosphorylation, although the mechanism is unknown [68].

TFEB contains a conserved serine-rich C terminal region that is reportedly phosphorylated by protein kinase C isoform β (PKCβ) [34]. In a mouse osteoclast model, phosphorylation of the mouse Serine 461 (S461) and/or S462, S466, and S468 residues by PKCβ was important for TFEB stability after activation of PKCβ by RANKL. Another study on human TFEB mutated the corresponding C-terminal serine residues (S462, S463, S466, S467, and S469) to the phosphomimetic aspartic acid. The resulting 5x serine to aspartic acid mutant showed increased nuclear localization, suggesting that concomitant phosphorylation of these residues may be important for TFEB nuclear translocation and activation [54]. However, a recent report definitively showed that S467 is phosphorylated by Akt (protein kinase B) and inhibits the nuclear translocation of TFEB [69]. Thus, it will be important to parse out the individual contributions of the phosphorylation sites on the serine-rich region to gain a more complete understanding of TFEB regulation. Finally, a recent study showed Serine 134 (S134) and serine 138 (S138) are phosphorylated by GSK3β, inhibiting TFEB nuclear translocation. Phosphorylation of S134 and S138 appear to be important in directing TFEB to the lysosomal surface, where it interacts with mTORC1. The S134A and S138A TFEB double mutant has decreased lysosomal localization, decreased S211A phosphorylation, and decreased interaction with 14-3-3 proteins, leading to its nuclear localization [68].

Nutrition Deprivation and mTORC1-Dependent TFEB Activation

Autophagy is a cellular adaptation to nutrient deprivation, as it prevents the accumulation of damaged proteins and organelles in the cytosol while allowing the recycling of essential cellular building blocks. mTORC1 is recruited to the lysosome surface by the small Rag
(Ras-related GTP-binding) GTPases and Regulator under nutrient replete conditions. At the lysosome surface, the small GTPase Rheb activates mTOR, and active mTORC1 promotes anabolic processes and inhibits autophagy [70]. Under conditions of nutrient deprivation, mTORC1 dissociates from the lysosomal surface and is inhibited, promoting autophagy, and leading to the utilization of energy stores.

As autophagy is initiated during starvation, it is not surprising that TFEB responds to the level of nutrients within cells. In a fully fed state, TFEB is excluded from the nucleus and present primarily in the cytosol. This cytoplasmic sequestration is due to the delivery of TFEB to the lysosomal surface by Rags, where it is phosphorylated by mTOR at S211. However, under starvation conditions TFEB rapidly translocates to the nucleus due to dephosphorylation at S211. This dephosphorylation is believed to be mediated by two events: 1) a decrease in mTORC1 activity leading to less S211 phosphorylation and 2) the release of lysosomal calcium through mucolipin-1 (MCOLN1) and the subsequent activation of calcineurin's phosphatase activity that acts directly on S211 [71,72]. Of note, while one study reported decreased TFEB nuclear translocation in human mucolipidosis Type IV (MLIV; MCOLN1 loss of function mutant) human fibroblasts after starvation [65], another reported no reduction in TFEB nuclear translocation after starvation in human MLIV fibroblasts [71]. In support of the lysosomal calcium model, a recent study found that TFEB overexpression promotes lysosomal localization to the plasma membrane and increases lysosomal Ca2+ buffering capacity [72]. The lysosomal buffering capacity was also increased with starvation, constituting a positive feedback loop, and further indicating the importance of lysosomal calcium. Finally, another study identified sphingosine as a positive regulator of calcium release from acidic stores, resulting in TFEB translocation and autophagy [73]. Thus, it is clear that lysosomal calcium and calcineurin play an important role in S211 dephosphorylation, though the details of this pathway remain to be confirmed.

TFEB target gene transcription is also epigenetically activated during starvation, as the activation of AMP-activated protein kinase (AMPK) leads to increased levels of the TFEB transcriptional co-activator CARM1 (Co-activator Associated Arginine Methyltransferase 1) and subsequent increases in histone H3 Arg17 dimethylation [74]. Finally, the transcriptional repressor ZKSCAN3 (Zink Finger with CRAB and SCAN domains 3) acts in opposition to TFEB on autophagy and lysosome genes, and starvation promotes ZKSCAN3 translocation out of the nucleus into the cytosol suggesting coordinate regulation of TFEB and ZKSCAN3 [75].

There is mounting evidence that TFEB responds to a variety of cellular stresses in addition to starvation, including lysosomal stress, mitochondrial stress, endoplasmic reticulum (ER) stress, and reactive oxygen species (ROS) [1,52,64,67,71,76,77]. Soon after TFEB's discovery, it was recognized that TFEB responds to lysosomal stress, as treatment with lysosome alkalinizing agents such as chloroquine or Baflomycin-A1 induce TFEB nuclear translocation [64,67]. Another study also showed that aneuploidy, which causes lysosomal stress via accumulation of undegraded autophagic cargo as a result of altered protein composition, also activates TFEB [78]. The mechanism of TFEB activation after lysosomal stress is mTOR dependent, as mTORC1 is released from the lysosome membrane and inactivated in response to lysosome stress [70]. It will be interesting to see if there is additional overlap between the mechanisms of TFEB activation due to nutrient deprivation and lysosomal stress, including the involvement of lysosomal calcium and/or calcineurin.

TFEB can be regulated independently of mTORC1, and TFEB activation after mitochondrial stress is indeed mTOR independent. Mitophagy, the process by which damaged lysosomes are degraded via autophagy, occurs when the loss of mitochondrial membrane potential induces the PTEN-induced putative kinase 1 (PINK1) to recruit the E3 ligase Parkin to the outer mitochondrial membrane. After ubiquitination of mitochondrial proteins by Parkin, damaged mitochondrion are delivered to the autophagosome for eventual destruction [79]. Interestingly, induction of mitophagy by oligomycin and antimycin A result in potent TFEB (and TFE3) nuclear translocation dependent on PINK1, Parkin, Atg5, and Atg9a. The importance of this is corroborated by the observation that depletion of Mit family members causes impaired clearance of damaged mitochondrion [52]. TFEB also induces expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), the master regulator of mitochondrial degradation and biogenesis, and PGC-1α reciprocally regulates TFEB to induce mitophagy [53,59]. Recent work further implicates a role for TFEB in mitochondrial homeostasis as well as in the cellular response to exogenous and endogenous ROS [71]. Treatment with the oxidant chloramine T or with compounds known to generate endogenous ROS (such as rotenone, hydrogen peroxide, or cyanide m-chlorophenylhydrazone (CCCP)) induces TFEB activation following MCOLN1 mediated lysosomal calcium release and subsequent calcineurin activation. MCOLN1, and presumably TFEB activation, is critical in ROS induced mitophagy and the efficient clearance of ROS [71,80]. Together, this data indicates an important role for TFEB in mitochondrial homeostasis and in the response to oxidative stress.
Finally, ER stress also results in TFEB (and TFE3) nuclear translocation. When ER homeostasis is disrupted, accumulation of misfolding proteins in the ER lumen triggers the unfolded protein response (UPR) [81]. One of the key mediators of this response is the kinase double-stranded RNA activated protein kinase (PKR)-like ER kinase (PERK), and TFEB nuclear translocation in response to ER stress is PERK dependent [76]. PERK activation results in the global downregulation of translation (to reduce ER protein load) and upregulation of ATF4 (Activating Transcription Factor 4), which promotes cell survival by upregulating genes required for autophagy, redox homeostasis, and amino acid import. Importantly, active TFEB (along with TFE3) enhances the ATF4 mediated stress response, likely by inducing transcriptional upregulation of ATF4 itself [76]. While ATF4 is important in promoting cell survival initially, it can promote apoptosis in conditions of severe or prolonged ER stress. The role of TFEB in cell fate is likely complex and situation dependent, as TFEB appears to be protective against cell death in some studies, and has the opposite effect in others. After prolonged ER stress, the knockdown of TFEB and TFE3 attenuated cell death [76], while knockdown of TFEB enhanced cell death following severe lysosomal stress induced by rare earth oxide nanoparticles or GSK3β inhibition [77,82].

Protein Kinase C-mediated TFEB Activation

PKC has emerged as an important regulator of mTOR independent TFEB activation. PKC has many isoforms, and while PKCβ phosphorylates TFEB at the C-terminus, PKCα and PKCδ work in an inhibitory manner upstream of GSK3β to induce TFEB activation [68]. This finding is of some importance, as PKC lies downstream of many signaling pathways, including GPCR mediated Gαq signaling through diacylglycerol (DAG), the Toll-like receptors of innate immunity, and receptor tyrosine kinases [83,84]. Accordingly, phorbol-12-myristate-13-acetate (PMA, a direct PKC activator), antiotension II stimulation (through Gαq), and LPS (TLR4 ligand) all activated TFEB in a PKC dependent manner [68].

TFEB IN THE IMMUNE SYSTEM

TFEB-Related Processes are Essential Components of the Immune Response

The immune system is critical in protecting against foreign invasion, but tight regulation is essential as aberrant immune activation is detrimental to the host. Many TFEB-related cellular processes are important in host defense, and the autophagy/lysosome system has a variety of roles in immunity [11]. For one, autophagy is a responsive to innate immune receptors like TLRs and can directly eliminate intracellular pathogens [85]. It can also function to control the immune response, as autophagy limits inflammation by direct elimination of active inflammasomes [86]. Autophagy and the endolysosome system also play a critical role in antigen presentation, which is a key function of certain adaptive immune cells like B cells, dendritic cells (DCs), and CD4+ helper T cells [87]. Finally, the autophagic machinery is important both in conventional and non-conventional secretion of cytokines [11,88]. Additionally, the ER stress response is implicated in immunity as an improperly compensated response can aberrantly activate inflammation through direct NLRP3 inflammasome activation [89]. Finally, though the role of TFEB in cell fate determination is still unclear, cell fate decisions play an important role in immune development and self-tolerance, and can be protective in infection [90,91].

Pathogens also activate many of the signaling or cell stress pathways that result in TFEB activation. Pathogens activate autophagy (and likely TFEB) both directly and through nutrient deprivation due to competition by invading bacteria and viruses [11,92]. The *Pseudomonas aeruginosa* produced siderophores induce mitophagy, which is required for defense against *P. aeruginosa*, while the common fungal pathogen *Cryptococcus neoformans* induces mitochondrial depolarization and the mitochondrial stress response [93,94]. Certain bacterial virulence factors, such as pore-forming toxins (PFTs) and the cholera toxin, also directly activate the UPR [95,96]. Interestingly, Herpes simplex virus 1 selectively blocks PERK activation (which lies upstream of TFEB in the UPR response), indicating this pathway may have some antiviral effects [97]. While the understanding of the role of TFEB in the immune response is still in its infancy, it is clear some role for TFEB exists.

TFEB as a Direct Transcriptional Activator of Immune Genes

The first evidence alluding to a role for TFEB in the immune response came from studies on the TFEB ortholog HLH-30 in *C. elegans* [98,99]. The nematode gut mediates immunity in *C. elegans*, as this organism does not have specialized immune cells; thus *C. elegans* is considered a reasonable model for innate immunity [100]. Infection of worms with *Staphylococcus aureus* results in strong HLH-30 nuclear translocation, and worms lacking functional HLH-30 are more susceptible to death following infection despite similar bacterial burden [98]. Transcriptome analysis of WT and HLH-30 KO worms indicates that HLH-30 is responsible for upregulation of 77 percent of the genes typically increased following *S. aureus* infection, and gene-set enrichment analysis showed that the HLH-30 upregulated genes primarily belong to the cytoprotective, antimicrobial, and signaling catego-
CHiP-seq of TFE3 in RAW cells showed direct binding of transcription factors directly regulate immune genes, activation, and macrophage migration. To show that these generation of the proinflammatory response, macrophage and elucidates a role for these transcription factors in compensatory functions in the innate immune response, data indicates that TFEB and TFE3 have overlapping or seen in TFEB/TFE3 DKO BMDMs. Taken together, this in the transcriptional upregulation of ISGs in an interfer-on-independent but TFEB-dependent manner [104]. The authors show that TREX1 deficiency results in TFE3 and IRF7 are known to directly activate ISGs, though it may be prudent to see if ISGs are also directly transcriptionally regulated by TFEB/TFE3 based on recent advances in our understanding [3]. Moreover, as ISGs are important in mediating viral immunity [106], TFE3 may play an important role in the host response to viral infection. In fact, once the human immunodeficiency virus (HIV) has established a productive infection, the HIV protein NEF plays a role in sequestering TFE3 in the cytoplasm via mTOR activation to inhibit autophagy and promote viral replication [107].

**TFEB in the Innate Immune Response**

Studies from the Irazoqui group investigated the pathways upstream of TFEB after infection in *C. elegans* [108]. The authors found that TFEB nuclear translocation after infection is dependent on EGL-30 (Gq homologue), PLC-1 (phospholipase C (PLC) homologue), and DFK-1 (protein kinase D (PKD) homologue). Treatment with PMA, which produces DAG, induced translocation in the EGL-30 and PLC-1 but not the DKF-1 depleted worms, suggesting that in *C. elegans* an unknown G-protein coupled receptor (GPCR) couples to Gαq to activate PLC-1, which activates DFK-1 and TFEB through production of intracellular DAG. In the mammalian RAW cell model, authors confirmed the importance of PKD-1 and phosphatidylycholine (PC)-specific PLC. However, in mammalian cells it is also established that PKC lies upstream of PKD-1 [109], and the authors found PKCa but not PKCb, PKDγ, or PKCe to be necessary for TFEB activation after infection [108]. The involvement of PKCa in TFEB translocation is in agreement with another study that identified PKCa (and PKCb) as upstream of GSK3β and critical in TFEB activation [68], however these authors did not look at the involvement of GSK3β [108]. It will
be interesting to determine if this newly described PK-Cα-PKD-1 dependent pathway in macrophages is indeed upstream or parallel to the described GSK3β dependent phosphorylation of TFEB S134 and S138. Moreover, the involvement of PC-PLC argues against Gα involvement in murine macrophages, as PC-PLC lies downstream of macrophage CD14 signaling and not Gαq [110].

TFEB has a variety of effects on macrophage effector function and pathogen clearance. A study from our group identified Activator of G-protein Signaling 3 (AGS3), which is upregulated following LPS stimulation in macrophages, as another mediator of LPS induced TFEB activation [102]. BMDMs deficient in AGS3 had clearly decreased TFEB activation after LPS stimulation, while macrophages high in AGS3 expression had more nuclear TFEB, more lysosome mass, and increased lysosome proteolytic activity. Mechanistically, AGS3 mediated TFEB translocation is mTOR dependent, raising the possibility of mTOR dependent and mTOR independent effects on TFEB in macrophages after LPS stimulation [102,103]. Importantly, AGS3lo macrophages with more activated TFEB had a selective advantage against infection with *Burkholderia cenocepacia, Mycobacterium Tuberculosis,* and two strains of methicillin-resistant *S. aureus* (MRSA), while AGS3 deficient BMDMs were at a disadvantage [102]. Of particular significance, *B. cenocepacia* is known to escape endosomes to autophagosome that fail to merge with lysosomes, eventually allowing replication in the ER [111]. Our data shows that only ~10 percent of the infectious *B. cenocepacia* were able to escape the endolysosome system in AGS3lo cells, while 85 percent escaped in AGS3hi cells. Moreover, autophagy is known to be essential in controlling the replication of *M. tuberculosis* [10], and the heightened ability of AGS3hi macrophages to control the *M. tuberculosis* infection is likely due to upregulation of autophagy. Together, this data indicates that TFEB-mediated upregulation of the autophagy/lysosome system may be important in the clearance of dangerous intracellular pathogens [102]. In agreement with this, studies in the TFEB/TFE3 knockout RAW cells show that WT RAW cells have increased autophagosome formation and lysosome biogenesis after LPS stimulation while TFEB/TFE3 DKO RAW cells actually have a reduction in autophagosome and lysosome formation compared to the baseline [103].

Phagocytosis is a key mechanism by which macrophages take up large extracellular pathogens for eventual destruction (reviewed in [112]). Phagocytosis takes up opsonized particles by an Fcγ-receptor mediated mechanism and non-opsonized particles independent of the Fcγ-receptor. The early phagosome is an innocuous membrane that simply contains the uptaken particle, but phagosome maturation results a highly acidic vesicle containing ROS for pathogen killing; mature phagosomes eventually merge with the lysosome for destruction by mechanisms that utilize many components of the endolysosome system. Recently, it has been shown that TFEB is activated in an MCOLN-1 dependent manner after opsonic or non-opsonic phagocytosis [113]. Importantly, phagocytosis enhances lysosome-based proteolysis in a TFEB-dependent manner, and cells deficient in TFEB showed defects in their bactericidal ability after phagocytosis [113].

Finally, TFEB was shown to play an important role in cell autonomous defense to bacterial PFTs. TFEB-dependent autophagy promoted cell membrane repair in response to PFTs and promoted PFT engulfment and degradation, both of which were essential to organismal survival in a *C. elegans* [114]. Thus, TFEB plays a role in mediating innate immunity through direct transcriptional regulation of proinflammatory/cytoprotective genes and by upregulation of the autophagy/lysosome system following intracellular infection and phagocytosis.

**TFEB and Inflammasomes**

Inflammasomes are multimeric signaling complexes that are activated in response to pathogen-associated molecular patterns (PAMPs) or danger associated molecular patterns (DAMPs) to trigger pro-caspase-1 activation and the eventual cleavage and release of IL-1β and IL-18 [115,116]. Inflammasomes respond to a variety of stimuli including intracellular ATP, flagellin, cytosolic DNA, uric acid crystals, cholesterol crystals, and others to initiate the inflamatory cascade [117-120] (reviewed in [121]). Autophagy is a critical regulator of inflammasomes, as autophagy is able to directly engulf and destroy active inflammasomes to prevent autoinflammatory states [86]. A recent study in peritoneal macrophages from TFEB overexpressing mice (TFEB-Tg) showed decreased IL-1β secretion following NLRP3 inflammasome stimulation with cholesterol crystals [122]. This may be an expected result, as autophagy is known to dampen the inflammasome machinery. However, the defect in IL-1β secretion was retained in TFEB-Tg/ATG5 KO mice, indicating TFEB regulates the inflammasome independent of autophagy [122]. More work is needed to determine the molecular mechanisms through which TFEB regulates inflammasome activation and to see whether this regulation is NLRP3 specific.

**TFEB in the Adaptive Immune Response**

Much less is known about the role of TFEB in the adaptive than the innate immune response. In T cells, T cell receptor (TCR) stimulation increases TFEB level with no corresponding change in TFE3; no other MITF proteins are detectable after TCR stimulation [31]. A transgenic mouse model that inhibited the function of all
TFEB in the Immune Response

T cells
- T cell Dependent Immune Response: CD40L expression (CD4+ T cell)
- Activation: IL-2

Macrophages
- Proinflammatory: IL-1β, IL-6, TNF-α
- Migration: CCL2, CCL5
- Activation: CSF1

C. elegans:
- Transcription of 77% of genes involved in the nematode S. aureus immune response

Direct Transcriptional Activation of Immune Genes

Innate Immunity:
- Resistance PFT: membrane repair
- ISG Expression: STING/ST2K1 activation

Macrophages
- Pathogen Resistance: autophagy, lysosome mass, lysosome proteolytic activity
- Resistance to M. tuberculosis, B. cepacia, MRSA
- Phagosome Bactericidal Activity: phagosome acidification

Dendritic Cells
- Cross Presentation: MHC Class II presentation, cross presentation
- Lysosome Compartment, Autophagy

Figure 3. TFEB in the Immune Response. The effect of TFEB on the immune response is mediated by direct transcriptional activation of immune genes or by expansion of the autophagy/lysosome system.

MiT family members in T cells showed impaired germinal center formation and decreased levels of plasma cells despite proper CD4+ T cell localization following stimulation with sheep red blood cells (SRBCs) [31]. SRBCs are considered a T cell dependent antigen [123], and impaired germinal center response after SRBC stimulation is indicative of failure of the T-helper cell dependent B cell response. To test this directly, the authors stimulated the mice with the traditional T cell independent antigen trinitrophenol-Ficoll (TNP-Ficoll) and the traditional T cell dependent antigen trinitrophenol-keyhole limpet hemocyanin (TNP-KLH). The MiT deficient mice had a similar response to the control mice after TNP-Ficoll stimulation, but had decreased IgG titers in response to TNP-KLH stimulation indicating class-switch failure typical of Hyper-IgM syndrome [31]. The B cell/CD4+ helper T cell interaction is mediated in part by CD40 (present on B cells) and CD40L, which is transiently expressed on helper T cells following immunogenic stimulation [124]. The MiT deficient mice showed severely decreased CD40L expression, and promoter analysis/ChIP-seq identified multiple E-Box sequences that TFEB and TFE3 bind in vivo [31]. Interestingly, this phenotype was only present in mice deficient in both TFEB and TFE3, indicating functional or compensatory overlap in T cells. Additionally, a recent study linked mitochondrial respiration defects to TFEB activation and proinflammatory T cell status, though it is unclear whether the terminal phenotype is directly linked to TFEB activation [125].

DCs are professional antigen presenting cells that initiate the adaptive immune response by presenting intracellular antigens to CD8+ cytotoxic T cells and exogenous antigens to CD4+ helper T cells via MHC Class I and II respectively [126]. DCs are also capable of presenting exogenous antigens to CD8+ cytotoxic T cells via a specialized process called “cross-presentation,” which is critical for initiating the cytotoxic T cell response to tumor cells or pathogens that do not directly infect DCs. The lysosomal acidification machinery is important in cross presentation, as it also regulates proteolytic activity in endosomes/phagosomes and is essential in the generation of partially degraded antigens capable of escaping the endocytic/phagocytic system into the cytosol for delivery to MHC Class I [126]. However, a fine balance in lysosomal proteolysis is required, as too much is incompatible with cross presentation and favors MHC Class II presentation [127]. TFEB acts as a molecular switch in DCs regulating cross presentation via changes in lysosome function [35]. High TFEB levels promote MHC Class II presentation and reduce cross presentation, while low TFEB levels increase MHC Class I cross presentation. This was shown to have physiological significance, as DC maturation is associated with TFEB upregulation and a concurrent shift towards MHC Class II restricted...
antigen presentation [35]. As such, the use of TFEB activators or inhibitors may aid in the development of DC vaccines. Finally, although TFEB is highly expressed in B cells, very little is known about its function. More work is needed to fully understand the roles of TFEB in regulating cell-cell interactions in the adaptive immune system.

CONCLUSIONS AND PERSPECTIVE

TFEB has emerged as an important regulator of many biological processes at the cellular level. While much progress has been made in understanding the complex signaling mechanisms that regulate its activation, there is still much progress to be made in understanding its precise role in cell stress and the functional consequences of its activation in responding to stress and determining cell fate. Additionally, given emerging evidence that MiT family members have some functional overlap, it will be important to delineate which processes the MiT members have overlapping versus divergent functions and how interactions between family members affects terminal phenotype. Finally, given the evidence that TFEB plays an immunomodulatory role across many cell types (summarized in Figure 3), there is also an opportunity to better understand the pathogen-specific consequences of TFEB regulation in the hopes of identifying new therapeutic targets. Overall, there is no doubt that a better understanding of TFEB and the MiT family members can have a positive effect on human health and disease, and continued progress in the field will take us closer to this goal.

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