

Review Article

Coordinating BNIP3/NIX-mediated mitophagy in space and time

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Mitochondria maintain organellar homeostasis through multiple quality control pathways, including the clearance of defective or unwanted mitochondria by selective autophagy. This removal of mitochondria, mitophagy, is controlled in large part by the outer mitochondrial membrane mitophagy receptors BNIP3 and NIX. While it has long been appreciated that BNIP3 and NIX mediate mitophagy by controlling the recruitment of autophagic machinery to the mitochondrial surface, the requirement for the carefully controlled spatiotemporal regulation of receptor-mediated mitophagy has only recently come to light. Several new factors that regulate the BNIP3/NIX-mediated mitophagy pathway have emerged, and various loss-of-function cell and animal models have revealed the dire consequences of their dysregulation. In this mini-review, we discuss new insights into the mechanisms and roles of the regulation of BNIP3 and NIX and highlight questions that have emerged from the identification of these new regulators.

Introduction

Mitochondria are multifaceted organelles present in virtually all eukaryotic cells. Beyond their well-known role in cellular energy production, mitochondria facilitate numerous processes including ion homeostasis, cofactor biogenesis, and the orchestration of cell death. These roles vary across cell types and physiological conditions, requiring mitochondrial populations to remodel and respond to distinct cellular and organismal demands. Additionally, mitochondria can become damaged over time, requiring the efficient removal and turnover of dysfunctional organelles. To address this, cells have appropriated the autophagic machinery to eliminate damaged or superfluous mitochondria in a process known as mitophagy. While cells engage multiple proteins and pathways to enable mitophagic clearance, including the extensively studied PINK1/Parkin pathway (reviewed in detail here: [1,2]), emerging evidence suggests that the receptor-mediated pathway mediated by BNIP3 and its paralog NIX (gene name *BNIP3L*) are critical in numerous physiological contexts and are extensively regulated.

Though initially characterized as cell death effectors due to their BH3-only domains [3–5], BNIP3 and NIX were linked to mitophagy upon the discovery that NIX is required to clear mitochondria from red blood cells during differentiation [6,7]. BNIP3 and NIX are targets of HIF1- α [8–10], and they are robustly up-regulated during hypoxia to mediate mitochondrial turnover. Due to this observation, BNIP3 and NIX have been viewed primarily as hypoxia-specific mitophagy factors due to their low expression levels in basal conditions. However, the recent identification of PINK1/Parkin-independent pathways that enable basal mitophagy *in vivo* [11,12] sparked renewed interest in pinpointing the factors responsible for this ‘housekeeping’ mitophagic program. Indeed, accumulating evidence suggests that BNIP3 and NIX likely influence basal mitophagy, which we define as steady-state mitophagic flux in cells and tissues in the absence of pharmacological (e.g., uncouplers), physiological (e.g., hypoxia), or genetic perturbations known to increase mitophagic flux. Multiple groups have detected low levels of endogenous BNIP3 and NIX protein levels in untreated, wild-type cells via immunoblotting, proteomic analysis, and immunofluorescence — consistent with their potential contribution to basal mitophagic flux (for example, [13–16]). Consistently, the most convincing

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link between BNIP3 and NIX and basal mitophagy is that genetic ablation of these two receptors in HeLa cells almost completely negates steady-state mitophagic flux detected using the fluorescent mitophagy reporter mito-mKeima [13,17]. These data suggest that in at least some cell types, BNIP3 and NIX influence mitophagy that is both induced by exogenous stimuli as well as in unstimulated conditions.

Recently, several groups have independently discovered new regulatory pathways influencing BNIP3- and NIX-mediated mitophagy, highlighting the severe cellular and physiological consequences of their unrestrained activity. Here, we discuss new players and emerging concepts in the regulation of BNIP3- and NIX-mediated mitophagy, including recent mechanistic insights as to how these receptors function and the pathways that mediate their stability, activity, and spatiotemporal regulation.

BNIP3/NIX as mitophagy receptors

BNIP3 and NIX are paralogous mitophagy receptors in mammals, sharing up to 65% sequence identity at the amino acid level (Figure 1A). Each receptor contains a single-pass, C-terminal transmembrane (TM) domain that anchors the protein in the outer mitochondrial membrane (OMM). This TM domain orients the bulk of BNIP3 and NIX into the cytosol, where each recruits the cellular autophagic machinery to mitochondria

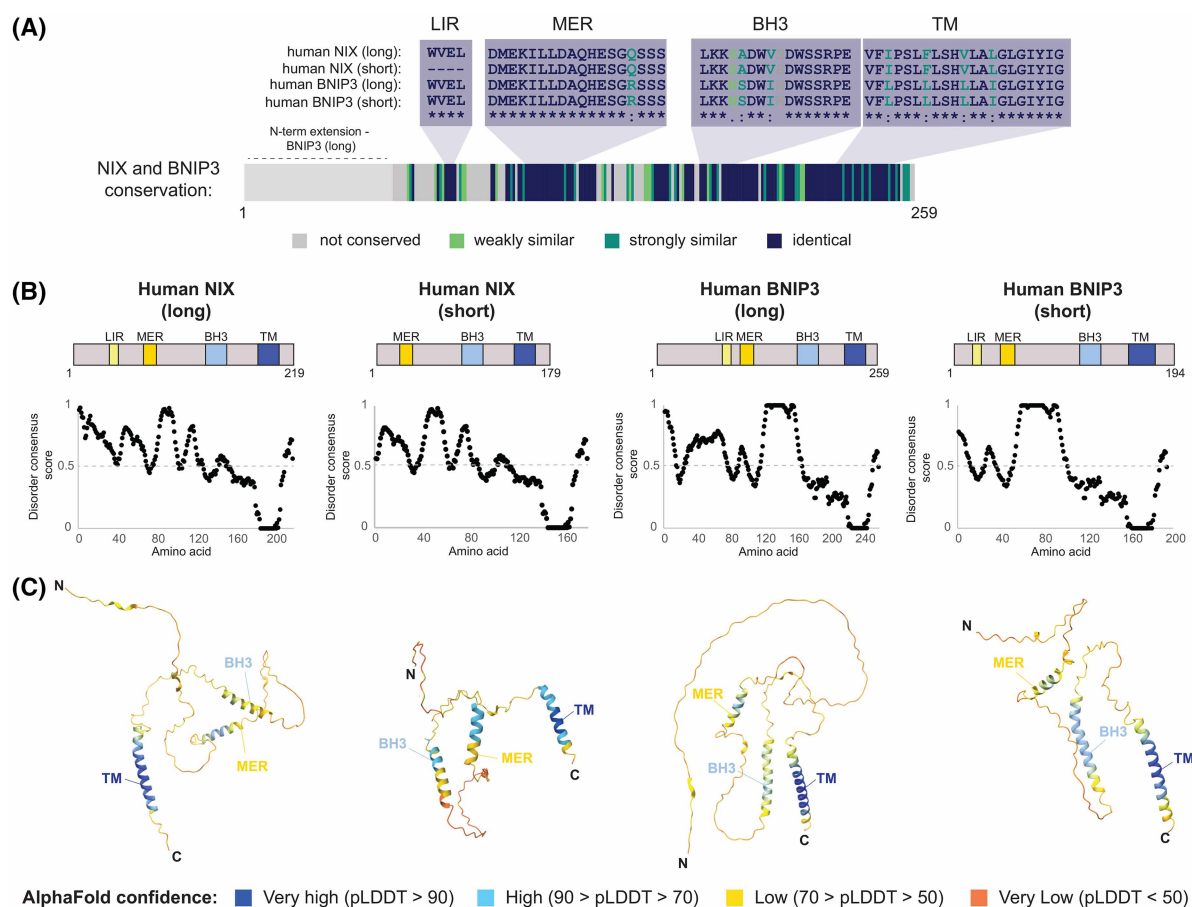


Figure 1. Conservation and domain architecture of human BNIP3 and NIX isoforms.

(A) Schematic showing conservation of residues across each of the long and short isoforms of human BNIP3 and NIX as indicated in the figure. Similarity was calculated using the Clustal Omega server and alignments are shown for four domains: the LC3 Interaction Region (LIR), the minimal essential region (MER), the BH3 only domain, and the transmembrane (TM) domain. **(B)** Top: schematics of human NIX (left) and human BNIP3 (right) isoforms, indicating the relative position of each domain. Bottom: Disorder consensus scores across amino acids of each isoform, as calculated by Metapredict [74]. Amino acids with a disorder consensus score of above 0.5 are considered to be in disordered regions. **(C)** AlphaFold predictions of human NIX (left) and human BNIP3 (right) isoforms. Select domains with predicted secondary structure are labeled.

through an LC3-interacting region (LIR) [18,19] and/or a minimal essential region (MER) [19–21]. In addition, BNIP3 and NIX share an atypical BH3-only domain [22] which may influence BNIP3 protein stability [23], though its functionality in mitophagy remains poorly understood.

Beyond these well-characterized domains, little is known about how BNIP3 and NIX function, as large portions of each receptor are unstructured (Figure 1B). Such substantial disorder, which for NIX has been experimentally verified through biophysical techniques such as circular dichroism and NMR [21], presents notable challenges for deciphering the molecular regulation of these receptors. Indeed, AlphaFold structural modeling reveals the only predicted folded regions within BNIP3 and NIX lie within the MER, BH3, and TM domains (Figure 1C).

To add to the complexity of these receptors, BNIP3 and NIX likely have multiple isoforms, with evidence that each encodes a long and short form [24,25] (Figure 1B). Recently annotated in Uniprot [25], a non-canonical long isoform of BNIP3 contains an N-terminal extension of 65 amino acids of unknown function. Ensembl also reports a non-canonical isoform for NIX [24], which notably is truncated at its N-terminus immediately after its LIR domain. In each case, it is unclear whether the non-canonical isoforms have distinct expression patterns or functions. In addition to potential differences in isoform expression and activity, BNIP3 and NIX harbor multiple post-translational modifications, such as phosphorylation, that could alter their ability to induce mitophagy [16,23,26–28]. Furthermore, the receptors can dimerize through a glycine zipper motif embedded within their TM domains, and evidence suggests that BNIP3 and NIX dimerization status promotes mitophagic induction [18,29,30]. In summary, BNIP3 and NIX have multiple avenues that allow the regulation of mitophagy in basal conditions, developmentally, and during hypoxia. However, until only recently, the identity and contribution of interacting regulators were largely a mystery.

The discovery of BNIP3/NIX regulators reveals the stark consequences of unchecked mitophagy

While BNIP3 and NIX were first implicated in mitophagy 15–20 years ago, the full consequences of their dysregulation were not known until recently. Within the last few years, a flurry of research has revealed three key negative regulators of BNIP3- and NIX-mediated mitophagy: the E3 ubiquitin ligase FBXL4, the phosphatase PPTC7, and the auxiliary MICOS interactor TMEM11.

In 2023, four groups independently identified FBXL4 as an essential suppressor of BNIP3 and NIX accumulation in mammalian cells and mice [31–34]. Each employed unique discovery-based approaches ranging from CRISPR screening to affinity purification-mass spectrometry to identify a link between FBXL4 and BNIP3/NIX. All studies found that knockout of *FBXL4* led to robust, post-transcriptional increases in BNIP3 and NIX protein levels and correspondingly displayed elevated mitophagy. Meanwhile, independent lines of research revealed a surprising role of the mitochondrial protein phosphatase PPTC7 in mitophagy [16,35]. The first clue that PPTC7 may influence BNIP3/NIX derived from the observation that tissues from *Pptc7* knockout mice had globally reduced mitochondrial protein content, with the notable exceptions of BNIP3 and NIX [36]. Subsequent work revealed that, like FBXL4 depletion, loss of *Pptc7* increased mitophagy through a transcription-independent increase in BNIP3 and NIX levels and activity [14,16,35]. Indeed, recent evidence suggests that FBXL4 and PPTC7 operate together [35,37,38], and further molecular insights into their relationship and functional roles are discussed below.

Simultaneous to the discovery that FBXL4 and PPTC7 modulate levels of BNIP3 and NIX, an affinity purification-mass spectrometry approach focused on the poorly characterized mitochondrial membrane protein TMEM11 revealed that it forms a protein complex with BNIP3 and NIX [13]. Like FBXL4 and PPTC7, TMEM11 depletion increases BNIP3 and NIX-dependent mitophagy, suggesting it plays a key role in their regulation. Furthermore, TMEM11, along with FBXL4 and PPTC7, was independently identified in a CRISPR screen as amongst the most significant negative modulators of basal mitophagy [31]. However, unlike FBXL4 and PPTC7, loss of TMEM11 does not appear to grossly increase BNIP3 and NIX levels [13], implying a role independent from FBXL4 and PPTC7 in mitophagic regulation.

The discovery of these BNIP3/NIX modulators has revealed the surprisingly destructive potential of unrestrained mitophagy. Multiple *FBXL4* mutations have been identified in human patients to cause mitochondrial DNA (mtDNA) depletion syndrome 13 (MTDPS13), a subtype of mitochondrial disease characterized by encephalopathy, myopathy, lactic acidosis, and general developmental delays [39–43]. Knockout mouse models of *Fbxl4* presented with some overlapping phenotypes: reduced birth weights, significantly lower mtDNA levels,

and metabolic dysfunction relative to their control littermates, with most *Fbxl4* KO animals perishing within the perinatal period [31,34,44]. Accompanying these defects, tissues from *Fbxl4* knockout mice as well as MTDPS13 patient fibroblasts harbored global down-regulation of mitochondrial protein levels [31,44], which originally were attributed to increased degradation through the lysosomal pathway [44], but were later linked to BNIP3/NIX-mediated mitophagy [31,34]. MTDPS13-associated variants in FBXL4 exhibit a decreased ability to mediate the turnover of BNIP3 and NIX, and thus a decreased ability to suppress mitophagy [31–34]. Initial characterization of *Pptc7* knockout mice revealed highly similar phenotypic defects to those observed in the *Fbxl4*-null animals: *Pptc7* KO animals were smaller, presented with global metabolic dysfunction, and none survived the perinatal period [36] — a finding reproduced in more recent work [35,38]. Collectively, these data suggest that excessive mitophagy is particularly detrimental within the perinatal period, and that an inability to turn over BNIP3 and NIX can result in mtDNA depletion-associated mitochondrial disease in humans.

Unlike *FBXL4* and *PPTC7*, knockout of *TMEM11* does not result in lethality in mice [45]. Knockout of the *TMEM11* homolog PMI (referred to hereafter as dTMEM11) has been studied in *Drosophila melanogaster*, where *dTMEM11* KO flies exhibited a widened mitochondrial diameter rather than an obvious loss of mitochondrial content [46,47]. While a role for dTMEM11 in mitophagy has yet to be examined, mitochondrial enlargement notably also occurs in human cells harboring *TMEM11* knockdown [13,47], and likely reflects an additional consequence of BNIP3/NIX mitophagic activation, as depletion of the receptors alleviates this phenotype [13]. On a physiological level, *dTMEM11* mutant flies have neuronal activity defects, are prone to paralysis after mechanical shock, and have a significantly shortened lifespan [46]. Whether the neuronal defects can be attributed to increased mitophagy remains to be determined, but it is likely that the more subtle physiological defects associated with loss of *TMEM11* in comparison with *FBXL4*/*PPTC7* are due to differences in the mechanisms by which each regulates BNIP3/NIX-mediated mitophagy.

Cellular mechanisms to limit BNIP3/NIX accumulation

Emerging evidence suggests that cells expend a substantial amount of effort to suppress BNIP3- and NIX-mediated mitophagy, as recent studies have found multiple pathways that regulate BNIP3 and NIX protein expression post-transcriptionally and in normoxic conditions [14,31–35,48].

The importance of ubiquitin-mediated turnover in BNIP3/NIX-mediated mitophagy emerged with the initial studies on *FBXL4*. As *FBXL4* functions as the F-box protein component of the Skp1-Cullin 1-F Box Protein (SCF) E3 Ubiquitin ligase complex [49], the most straightforward model for BNIP3 and NIX regulation posits that *FBXL4* promotes the ubiquitin-mediated turnover of these mitophagy receptors. Indeed, previous literature suggested that BNIP3 and NIX accumulate in the presence of proteasomal inhibitors such as MG-132 or MLN4924, a pan inhibitor of Cullin-RING E3 ligases [4,14,23,33,34]. Four recent studies found that BNIP3 and/or NIX become ubiquitinated in an *FBXL4*-dependent manner [31–34], which is dependent on the efficient recruitment of Skp1 and Cullin 1 to generate a functional SCF E3 ligase complex [31–34]. Interestingly, these groups also found that *FBXL4* mutations associated with MTDPS13 disrupted the assembly of functional *FBXL4*-SCF complexes, thus inhibiting BNIP3 and NIX turnover [31–34]. Collectively, these data suggest that *FBXL4*-mediated ubiquitination of BNIP3 and NIX is key for restraining receptor-mediated mitophagy.

Just as *FBXL4* was predicted to promote the ubiquitination of BNIP3/NIX, *PPTC7* was initially assumed to promote BNIP3/NIX turnover in a manner dependent on its phosphatase activity. Indeed, BNIP3 and NIX were found to be hyperphosphorylated across *Pptc7* knockout mouse tissues, and recombinant *PPTC7* efficiently dephosphorylated both receptors *in vitro* [16]. However, the necessity of *PPTC7* phosphatase activity was called into question after multiple groups reported that *PPTC7* acts as a scaffold that bridges *FBXL4* to BNIP3/NIX independent of its phosphatase activity [35,37,38]. Mechanistically, *PPTC7* has been shown through elegant structural modeling and biochemical approaches to bind to a disordered loop of BNIP3 and NIX just downstream of their atypical BH3 only domains [35,37]. This disordered loop of BNIP3/NIX binds to the *PPTC7* active site, and thus would be predicted to block phosphatase activity. Consistent with this model, a *PPTC7* mutant (D290N) with diminished phosphatase activity efficiently promotes BNIP3 and NIX turnover [37], suggesting full catalytic activity of *PPTC7* is not required to keep levels of BNIP3 and NIX in check. It remains unclear what, if any, role *PPTC7*-mediated dephosphorylation plays in the regulation of mitophagy, and rather it seems as though *PPTC7* has acquired a secondary, non-catalytic function at the OMM to influence mitophagy, despite BNIP3 and NIX hyperphosphorylation in *Pptc7* knockout mouse tissues [16,36].

The involvement of *PPTC7* as a regulator of BNIP3/NIX-mediated mitophagy came as a surprise, as this phosphatase, as well as its yeast ortholog *Ptc7p*, were previously shown to localize to the mitochondrial matrix

[50–52] and influence matrix-localized phosphorylation events [16,36,53,54]. However, several groups independently found that in addition to its matrix targeting, a portion of PPTC7 localizes to the OMM, rendering this phosphatase dual-localized [14,35,37,38]. PPTC7 has been reported to have a weak mitochondrial targeting sequence that may facilitate this dual localization [35] and thus has been postulated to be a ‘mitophagy sensor’. However, it is unclear what mediates the recruitment of PPTC7 to the OMM. A ‘tug-of-war’ model proposes that the weak MTS of PPTC7 competes with BNIP3/NIX interactions at the OMM [35], and, consistently, OMM-PPTC7 dynamically accumulates in pseudohypoxic conditions in which BNIP3 and NIX are stabilized [14,37]. However, evidence suggests that disruption of the PPTC7-BNIP3/NIX interaction does not block PPTC7 targeting to the OMM [14,35,37]. These data render the necessity of the BNIP3/NIX interaction unclear in mediating PPTC7 localization, and further work will be necessary to understand what regulates PPTC7 targeting and stability at the OMM versus its import into the matrix. Nonetheless, the dynamic localization of PPTC7 affords cells with an additional mechanism to modulate mitophagy activity.

In addition to ubiquitin-mediated degradation pathways, recent studies suggest other pathways may also regulate BNIP3/NIX levels. For example, excess BNIP3 and NIX can be mistargeted to the endoplasmic reticulum (ER) instead of to mitochondria, where the receptors can be subsequently delivered to lysosomes for degradation [48]. Thus, cells likely adapt and utilize multiple avenues to subvert the detrimental effects of unchecked BNIP3/NIX levels and activity. A recent study by the Ganley group demonstrated that NIX (and likely BNIP3) can be targeted to peroxisomes to mediate pexophagy [55], and one possibility is that the ER-lysosome pathway protects cells from issues that arise from the targeting of the receptors to multiple cellular compartments. However, it remains to be determined whether cells use the safeguard mechanisms afforded by ubiquitin-mediated and lysosomal pathways selectively or in concert with one another.

Spatial and temporal regulation of mitophagy

In addition to the regulation of BNIP3/NIX turnover, further pathways likely dictate the spatiotemporal control of mitophagy initiation sites to selectively remove damaged or unwanted portions of mitochondria. Notably, pseudo-hypoxic activation of BNIP3/NIX induces the recruitment of LC3 to discrete sites on intact mitochondrial tubules, even in the absence of mitochondrial fission machinery [56], suggesting that mitophagy is spatially controlled. In support of this idea, endogenous BNIP3 and NIX protein expression can be detected in unstimulated cells, where they are relatively evenly distributed throughout the mitochondrial network at steady-state [13] (Figure 2A). This low level of BNIP3 and NIX expression implies additional controls beyond protein turnover exist to prevent these mitophagy receptors from inappropriately recruiting LC3, and likewise, could activate the proteins to trigger mitophagy at specific sites within the mitochondrial network.

Interestingly, depletion of the BNIP3/NIX interactor TMEM11 leads to an increase in mitophagy without affecting total BNIP3/NIX levels [13], suggesting it may play a role in regulating the spatial activation of mitophagy. Accordingly, BNIP3/NIX and TMEM11 co-enrich at LC3-positive punctate structures during stimulation with pseudo-hypoxia treatments [13] (Figure 2B), suggesting TMEM11 acts locally to influence mitophagosome formation. Notably, though TMEM11 co-enriches with BNIP3/NIX at LC3-positive sites, depletion of TMEM11 leads to *increased* steady-state basal mitophagy and mitophagosome numbers, indicative of negative regulation. However, the functional role of TMEM11 enrichment at mitophagy sites has not yet been elucidated.

While recent work has defined TMEM11 as an OMM-localized interacting partner of BNIP3/NIX, TMEM11 was previously described as an auxiliary partner of the mitochondrial inner membrane cristae-organizing MICOS complex [57]. MICOS forms a supercomplex with the OMM sorting and assembly machinery (SAM), termed the mitochondrial intermembrane space bridging (MIB) complex [58], and TMEM11 likely associates with MICOS through OMM MIB components. These secondary interactions between TMEM11 and MICOS/MIB beg the question of whether the localization of mitophagy induction is related to these interactions. Indeed, MICOS destabilization via knockdown of MIC60 increases BNIP3/NIX-dependent basal mitophagy to a similar extent as TMEM11 depletion [13]. One model consistent with these data is that TMEM11 serves in mitochondrial surveillance, and that its interaction with MICOS/MIB could be a conduit through which TMEM11 and/or BNIP3/NIX sense localized dysfunction inside mitochondria to locally activate basal mitophagy. Interestingly, recent work has determined that individual cristae locally maintain their own membrane potential [59], further suggesting that organellar dysfunction could be limited to subregions within otherwise intact, tubular mitochondria. It will be interesting to explore in the future if and how the TMEM11 association

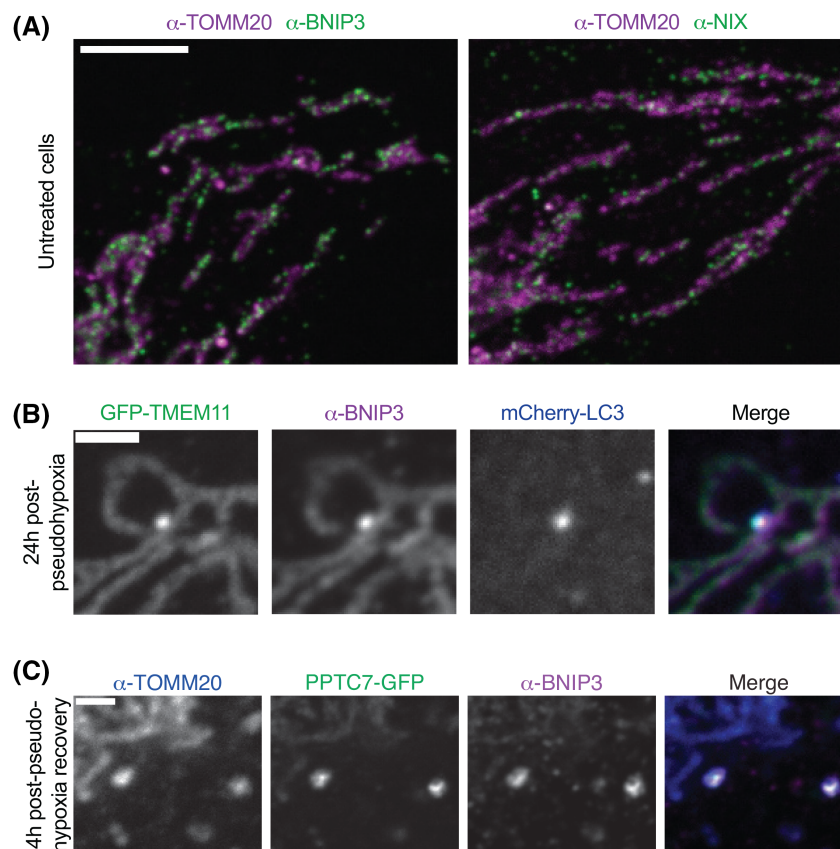


Figure 2. BNIP3 and NIX are spatially and temporally regulated to activate mitophagy.

(A) Low levels of BNIP3 and NIX are relatively evenly distributed throughout the mitochondrial network at steady-state. Confocal microscopy images are shown of immunolabeled BNIP3 (left) and NIX (right) localization within mitochondria (labeled with TOMM20) in untreated U2OS cells, an osteosarcoma cell line. (B) BNIP3 and TMEM11 co-enrich within the mitochondrial network at mitophagic sites co-labeled with LC3 upon treatment with pseudo-hypoxic insults. Images are shown of a U2OS cell transfected with GFP-TMEM11 and mCherry-LC3, treated for 24 hours with the pseudohypoxia mitophagy stimulant CoCl_2 , and fixed and immunolabeled for BNIP3. (C) PPTC7 dynamically co-enriches with BNIP3 during recovery from pseudo-hypoxic insult. Images are shown of a U2OS cell transfected with PPTC7-GFP, treated for 24 hours with the pseudohypoxia drug deferiprone, and allowed to recover 4 h prior to fixation and immunolabeling of BNIP3 and TOMM20. Scale bars (A) 5 μm , (B,C) 2 μm . Images in (A,B) were adapted from [13]. Images in (C) were adapted from [14].

with MICOS/MIB co-ordinates with the activation of BNIP3/NIX to trigger local mitophagy, and, if this is the case, how local dysfunction is sensed and communicated.

Similar to mechanisms underlying the spatial regulation of BNIP3/NIX, recent work suggests important temporally dynamic processes mediate their turnover after mitophagy initiation. While BNIP3 and NIX are transcriptionally up-regulated by hypoxia or pseudohypoxic insults such as iron chelation, this induction in BNIP3/NIX expression is reversible, particularly upon washout of iron chelators in cultured cells [14]. Remarkably, temporal analysis of the proximity labeling-based association between PPTC7 and BNIP3/NIX revealed increased interactions of the proteins during recovery from pseudohypoxic drug treatments [14]. This temporal link between PPTC7 and BNIP3/NIX was concomitantly observed to have a spatial relationship: while PPTC7 was observed to co-enrich at a small fraction of BNIP3-marked mitophagosome sites during pseudohypoxia treatment, their frequency substantially increased within hours of iron chelator washout [14] (Figure 2C). One possibility consistent with these data is that PPTC7 (and perhaps FBXL4) act in an additional manner beyond the repression of basal mitophagy to mediate the resolution of mitophagy, a situation which additionally necessitates BNIP3/NIX turnover. Interestingly, while TMEM11 co-enriches with a majority (~75%) of BNIP3 and NIX-marked mitophagosomes, many BNIP3/NIX sites do not appear co-enriched with TMEM11

[13]. Likewise, TMEM11 enriches at sites that are not co-enriched for BNIP3 or NIX. One intriguing possibility is that a stepwise temporal relationship exists between TMEM11, PPTC7, and FBXL4 at sites of mitophagy. Future studies to carefully study the spatiotemporal regulation of these proteins across mitophagy-activating and resolving conditions are warranted.

Unknowns and next steps for the field

With the recent surge in discoveries and subsequent identification of new regulators of BNIP3 and NIX-mediated mitophagy, the field has made rapid progress in understanding how BNIP3/NIX may be utilized and how cells control their activity. However, these new breakthroughs have raised as many questions as they have answered.

The molecular interactions between FBXL4 and PPTC7, their similar loss-of-function phenotypes, and the ubiquitin ligase and turnover role of FBXL4 have laid forth a relatively straightforward molecular model in their regulation of BNIP3/NIX. However, outstanding issues remain. In particular, the dynamic localization of PPTC7 to the OMM and the increased interaction and co-enrichment of PPTC7 with BNIP3/NIX after removal of mitophagy induction suggests that we still do not fully understand how the cell utilizes FBXL4 and PPTC7 to limit mitophagy. Because of the more subtle phenotypes associated with the loss of TMEM11 and its lack of well-characterized functional domains, its role in mediating BNIP3/NIX-mediated mitophagy has been more enigmatic. However, the molecular interactions and spatial link between TMEM11 and BNIP3, NIX, and LC3 strongly imply that TMEM11 directly regulates mitophagy. In the future, it will be important to determine the specific function of TMEM11 in mitophagy and its interplay with the FBXL4/PPTC7 regulatory pathway (Figure 3).

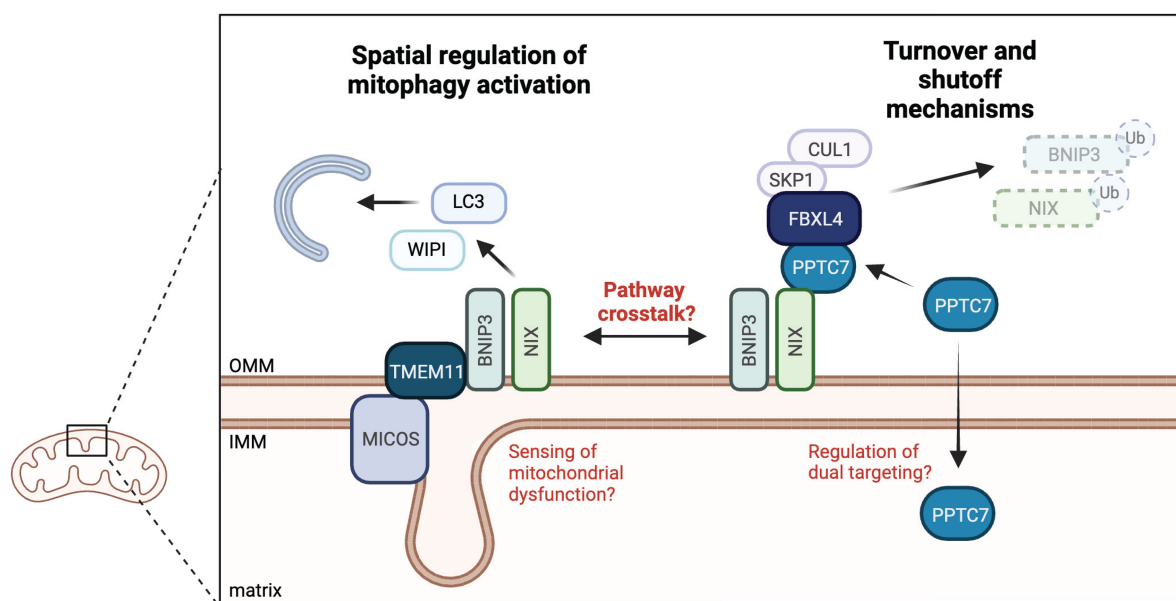


Figure 3. A working schematic of BNIP3 and NIX regulation.

BNIP3 and NIX are mitophagy receptors that recruit the autophagic machinery to the outer mitochondrial membrane (OMM) through the recruitment of LC3 or WIPI autophagy adapters (left). Recent evidence suggests that TMEM11 may spatially co-ordinate these mitophagic induction sites. TMEM11 is also known to interact with MICOS, a complex of the inner mitochondrial membrane (IMM), suggesting the functions of TMEM11 may allow sensing of mitochondrial dysfunction to trigger local mitophagy. The E3 ubiquitin ligase FBXL4 recruits subunits of the SCF1 complex, including SKP1 and CUL1, to BNIP3 and NIX to facilitate their ubiquitination and turnover. This ubiquitination is enabled by interactions with the mitochondrial phosphatase PPTC7, which is proposed to scaffold FBXL4 to its substrates BNIP3 and NIX (right). The mechanisms regulating FBXL4 activity, as well as PPTC7 dual targeting, have yet to be fully elucidated. Figure made with Biorender.com.

Additional handles likely exist to enable the regulatory control and activation of each receptor that we do not yet understand. One aspect of BNIP3 and NIX that has not yet been elucidated is how receptor dimerization promotes mitophagic activation. Additionally, both BNIP3 and NIX are heavily post-translationally modified, with several phosphorylation sites that are thought to regulate their function [16,23,27,28,60,61]. These sites are predominantly localized within the unstructured regions of the protein, and potentially contribute by modulating protein stability or turnover, access of the LIR and MER domains to autophagic machinery, and/or influencing interaction with other regulatory proteins. Going forward, unraveling these intricate details of BNIP3 and NIX regulation will be instrumental to reveal their context-specific utilization and functions.

While this review has focused on the collective activity of BNIP3 and NIX as mitophagy receptors, we have not focused on a key aspect of their biology: their differential expression, activity, and interaction profiles across tissues and physiological contexts. Notably, in many cell and tissue types, both BNIP3 and NIX are expressed. Despite this, the relative contribution of each protein to basal or hypoxia-induced mitophagy appears to be different, particularly in physiological contexts such as hepatic fasting [15], tissue- and cell-specific differentiation programs [62–64], and cardiovascular disease [65,66]. Furthermore, BNIP3 and NIX have distinct half-lives in 293T cells [14], suggesting differences exist in their turnover rates. Finally, a new study suggests BNIP3 and NIX have distinct affinities with downstream components of the autophagic machinery; while NIX binds to WIPI2, BNIP3 has a stronger affinity for WIPI3 [19]. These data suggest that, although they are paralogs, BNIP3 and NIX have important and nuanced regulation that will be critical to explore in more detail in the future.

Finally, an additional complication that must be navigated is the relationship between BNIP3/NIX-mediated mitophagy and other mitophagy pathways, including other potential LC3 receptors such as FUNDC1 [67], BCL2L13 [68], FKBP8 [69], PHB2 [70], and SAMM50 [71], as well as proteins facilitating ubiquitin-mediated mitophagy, such as PINK1/Parkin [72,73]. While certain cell types such as HeLa cells appear nearly exclusively reliant on BNIP3/NIX-dependent basal mitophagy [13,17], the compensatory effects of different mitophagy pathways may complicate our understanding of the context-dependent role of each.

Ultimately, the increasingly apparent roles of mitophagy pathways in mitochondrial homeostasis and the links of their defects with a broad range of disorders including mitochondrial disease, neurodegenerative diseases, heart disease, liver disease, and aging, make these timely and crucial questions to answer in the coming years.

Perspectives

- Mitophagy is a critical process for the maintenance of mitochondrial homeostasis during basal cell activity, in development processes, and under stress conditions.
- Several recent discoveries have unveiled new players and pathways by which cells regulate basal receptor-mediated mitophagy mediated by BNIP3 and NIX.
- Studies aimed toward understanding the interplay between different regulatory pathways and context-dependent utilization of mitophagy in different cell types and tissues present an important but exciting challenge in the coming years.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ER, endoplasmic reticulum; LIR, LC3-interacting region; MER, minimal essential region; mtDNA, mitochondrial DNA; OMM, outer mitochondrial membrane; SAM, sorting and assembly machinery; TM, transmembrane.

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