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SPOTLIGHT

Fine-tune TMEM11 to unleash basal mitophagy

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When mitochondrial damage threatens to disrupt cell and tissue homeostasis, selective autophagy (mitophagy) provides an important route to neutralize dysfunctional organelles. Whilst we understand much about stress-induced mitophagy, steady-state and spatial mechanisms remain elusive. In this issue, Gok et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202204021) reveal an unexpected role for TMEM11 in mitophagy regulation.

Transmembrane protein 11 (mammalian TMEM11, PMI in *Drosophila*) is a bona fide physiological regulator of mitochondrial network architecture. Genetic depletion of TMEM1/PMI in vivo induces robust mitochondrial dysfunction, with systemic effects on neural integrity and lifespan, complementing in vitro phenotypic observations in human cells (1, 2). Early yeast studies suggested TMEM11 interacts with the outer mitochondrial membrane (OMM) proteins BNIP3 and BNIP3L (also referred to as NIX), hinting at a possible role in mitophagy (3). BNIP3/BNIP3L are "eat-me" signals or selective autophagy receptors (SARs) that engage with the autophagy machinery to promote the elimination of damaged mitochondria. The TMEM11-BNIP3/NIX connection was challenging to reconcile, given contrasting data that localized TMEM11 at the inner mitochondrial membrane (IMM; 2), a spatially distinct neighborhood of the mitochondrial network.

So, what is the mechanistic function of mammalian TMEM11? The Friedman group used CRISPRi to explore this question, verifying that TMEM11 depletion disrupts mitochondrial ultrastructure and network architecture (4). Gok et al. then examined previously reported proteomic links and confirmed a small proportion of TMEM11 undergoes Mitochondrial Contact Site and Cristae Organizing System (MICOS)–dependent assembly within a larger MICOS/Mitochondrial Bridging Complex (MIB)–sized complex; however, the reciprocal depletion of TMEM11 did not modify MICOS stability or assembly. With the knowledge that TMEM11 assembled into smaller complexes, the authors deciphered its mitochondrial distribution. Super-resolution imaging of GFP-TMEM11 revealed a uniform distribution pattern distinct from HSP60 and MIC60. Ultrastructural characterization of APEX2-GFP-TMEM11 cells unearthed a surprise: APEX2 foci were present at the cytosol-facing mitochondrial surface, a characteristic feature of OMM proteins. This topographical discrepancy provoked a reassessment: Where exactly is endogenous TMEM11 localized? The biochemical profiling of intact mitochondrial preparations revealed that TMEM11 was digested upon protease treatment, providing further evidence for an OMM localization.

A mitophagic twist in the tale emerged when proteomics profiling revealed BNIP3/BNIP3L as major hits, consistent with previous studies (3). TMEM11-GFP immunoprecipitation experiments and reciprocal BNIP3/BNIP3L pulldown paradigms verified these interactions. After authenticating the TMEM11-BNIP3/BNIP3L association, the authors performed yeast two-hybrid studies with various mutants to dissect their interaction at greater mechanistic resolution. BNIP3 and TMEM11 interact using (or at a site proximal to) their respective transmembrane domains. Strikingly, BNIP3/BNIP3L depletion partially reversed the mitochondrial morphology defects in TMEM11-deficient cells. These results reveal a functional interplay that sustains mitochondrial homeostasis under steady-state conditions.

Because BNIP3/BNIP3L are well known to modulate mitochondrial turnover, the authors measured mitophagy levels upon TMEM11 depletion in two distinct cell lines. Remarkably, basal mitophagy levels increased upon TMEM11 depletion, while basal macroautophagy (non-selective autophagy) was unaltered. To further examine the requirement for TMEM11 in BNIP3-dependent mitophagy, the authors employed a hypoxia mimetic to stimulate increased levels of mitochondrial turnover. TMEM11 depletion enhanced $CoCl_2$-mediated mitophagy, a phenotype reversible by BNIP/BNIP3L silencing. Depleting the MICOS-interacting subunit MIC60 had similar BNIP-reversible effects, demonstrating that these interactions influence mitophagy induction. Further work revealed that TMEM11 resides with BNIP3/BNIP3L at discrete, LC3-enriched phagophore-like domains, and TMEM11 depletion increased BNIP3/BNIP3L abundance on the OMM. These data suggest TMEM11 associates with BNIP3/BNIP3L to restrict the formation of mitophagy initiation sites under normal, steady-state conditions (Fig. 1).

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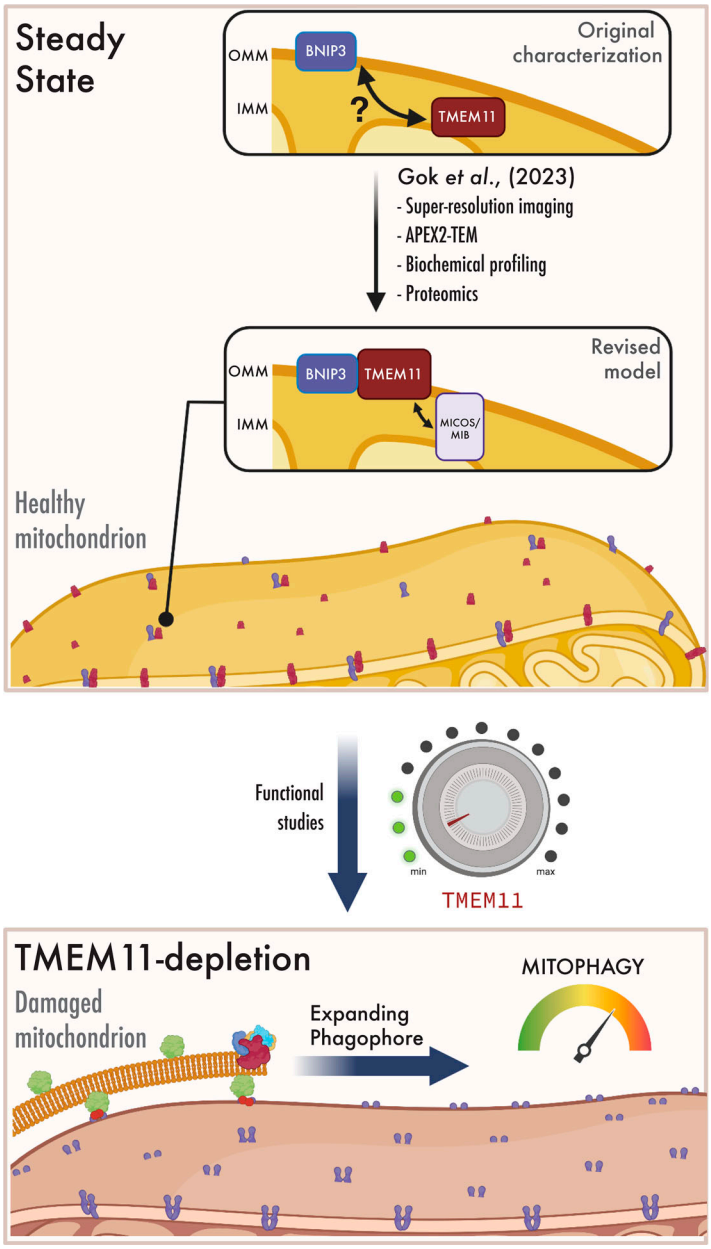

Figure 1. **Mitochondrial TMEM11 engages BNIP3/NIX to restrain basal mitophagy.** Initial studies of TMEM11 localized it to the IMM, and yeast two-hybrid screens identified the mitophagy-associated proteins BNIP3/BNIP3L as putative interactors. The molecular logistics of this association were unclear because of the distinct outer membrane localization of BNIP3/BNIP3L. Gok and colleagues (4) explored the functional roles of TMEM11 and performed a series of converging methods to pinpoint its localization at the OMM. Subsequent proteomics and biochemical profiling revealed the transmembrane domain of TMEM11 interacts with BNIP3/BNIP3L at its respective transmembrane region—which is known to be crucial for mitophagy progression. Strikingly, the authors report that TMEM11 depletion enhances mitophagy levels and leads to abundant BNIP3/BNIP3L microdomains on the OMM. These results identify a new mechanism that could explain differential levels of steady-state (basal) mitophagy. Created with BioRender.com.

Overall, this work is both evocative and timely. Most of our mitophagy knowledge comes from studies of tractable paradigms that induce mitophagy through severe mitochondrial dysfunction. Yet, we now understand that cells modulate mitochondrial recycling in a context-dependent manner, with factors such as metabolic state, hypoxia, and iron balance all playing key roles. Like non-selective macroautophagy, studies of mitophagy reporter mice, flies, and fish (5–7) have also revealed that mitochondrial turnover readily occurs within our tissues at resting steady state (basal mitophagy). While our knowledge of damage-induced mitophagy is extensive, our understanding of the signals that control basal mitophagy is comparably limited. Friedman and colleagues now provide essential clues into an adaptive mechanism that fine-tunes mitophagy levels, whereby TMEM11 appears to restrict steady-state BNIP3/3L activity. Several important mechanistic questions remain. Does TMEM11 alter BNIP3/NIX dimerization, which occurs via its transmembrane domains and appears essential for mitophagy (8)? Might TMEM11 inhibition modify the magnitude of mitophagy by influencing BNIP3/BNIP3L interactions with the autophagy machinery? Furthermore, how does TMEM11 depletion alter BNIP3/BNIP3L phosphorylation status, predicted to drive its activity and engagement with ATG8s? Understanding the influence of post-translational modifications that promote or prevent TMEM11–NIX crosstalk will prove informative.

BNIP/BNIP3L has a well-characterized role during developmental (programmed) mitophagy. Genetic ablation of BNIP3L leads to defective maturation of mouse hematopoietic and retinal tissues (9,10). It will be exciting to ascertain the tissue-specific contributions of TMEM11 to BNIP3/3L-dependent functions in vivo, notably as TMEM11-deficient mice (https://www.mousephenotype.org) manifest an array of phenotypes in neural, hematopoietic, and vascular tissues (11). Understanding the tissue-specific developmental regulation of TMEM11 could provide essential insights into the modulation of programmed mitophagy.

What stimulates the TMEM11–NIX interaction? Metabolic state changes are critical for mitophagy efficiency (12) and may impact the temporal dynamics of TMEM11–NIX engagement. It will be exciting to determine a possible role for TMEM11 in metabolic sensing, either within mitochondria through MICOS/MIB or at the perimitochondrial environment via NIX. The role of TMEM11 in maintaining mitochondrial integrity, along with its newly defined OMM localization, raises questions regarding its impact on organelle crosstalk. Understanding how TMEM11 governs the formation and abundance of mitophagy initiation sites on mitochondria may prove pivotal in cracking the code that controls

basal mitophagy, at least in specific tissue types.

Although TMEM11 appears to put the "proverbial brakes" on mitophagy, this story may extend beyond mitochondria. Recent data demonstrates BNIP3L/NIX functions as a poly-organellar SAR: NIX can localize to peroxisomes and elevated NIX levels drive their selective turnover (pexophagy; 13,14). It will be interesting to investigate peroxisomal homeostasis in the absence of TMEM11, particularly as the mitochondrial network also generates a pool of pre-peroxisomal vesicles (15). Despite inducing defects in mitochondrial morphology, TMEM11 depletion did not affect respiratory function. If the role of TMEM11 is restricted to mitophagy, fine-tuning its activity may represent a promising therapeutic strategy for different disease states, where modulating mitochondrial turnover could prove clinically advantageous.

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