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Mitochondria-ER cooperation: NLRX1 detects mitochondrial protein import stress and promotes mitophagy through the ER protein RRBP1

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ABSTRACT

Mitochondria rely on efficient protein import across their membranes for optimal function. We have shown that numerous mitochondrial stressors all converge on a common pathway disrupting this import efficiency. We identified a novel pathway involving NLRX1 and RRBP1 that responds to this import stress, resulting in LC3 lipidation, mitochondrial targeting and ultimate degradation. Furthermore, we demonstrated the relevance of this mitophagy axis in murine skeletal muscle following acute exercise. We propose that mitochondrial protein import stress is an underlying, common trigger for mitophagy, offering a novel avenue for therapeutic exploration and mechanistic insight.

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Main text

Mitochondria are essential components of eukaryotic cells, and like other pillars of homeostasis, mitochondria quality is closely monitored by multiple mechanisms. While mitochondria have the necessary cellular machinery to synthesize proteins within the organelle itself, most mitochondrial proteins are encoded in the nucleus and translated in the cytosol prior to import across one or both mitochondrial membranes to reach their destination. These circumstances highlight the importance of efficient import of mitochondrial proteins, without which core cellular processes like oxidative phosphorylation (OXPHOS) would be affected. While mitochondria may be engulfed through basal levels of constitutive bulk autophagy, dysfunctional mitochondria can also be selectively targeted for clearance. This rapidly expanding field of selective autophagic degradation of mitochondria (mitophagy) has defined a linear sequence of events following severe types of stress, such as depolarization of the inner mitochondrial membrane. Upon these cellular cues, current models position the PINK1-PRKN/parkin-facilitated accumulation of ubiquitin occurring upstream of mitophagophore expansion and LC3 lipidation. Importantly, the physiological relevance of PINK1-PRKN has been questioned lately, as mice lacking this pathway do not exhibit any overt phenotype or mitophagic impairment. Interestingly, depolarization and other independent mitophagy inducers such as iron depletion or misfolding of mitochondrial proteins can also disrupt mitochondrial protein import, but mechanistic understanding of if and how protein import stress could induce mitophagy remained unexplored.

In our recent work [1], we characterized the role of NLRX1 in sensing and responding to mitochondrial protein import stress (MPIS) by promoting mitophagy. Nod-like receptors (NLRs) are innate immune intracellular sensors of microbial or danger-associated signals. Upon signal detection, NLRs often promote inflammatory signaling pathways, cell death, and autophagy, or form high molecular weight signaling platforms known as inflammasomes. One NLR, NLRX1, is localized to the mitochondria, which strongly suggested it would sense some mitochondrial ligand or danger. Previous studies characterizing NLRX1 have revealed diverse functions such as regulating antiviral immunity, apoptosis, and ROS production. Still, the identification of a specific stress or danger was lacking. We hypothesized an underlying function is responsible for maintaining the mitochondrial network, which would implicate NLRX1 in these processes that are affected by mitochondrial function.

Through its *N*-terminal localization sequence, NLRX1 is constitutively targeted to the mitochondrial matrix. We showed that following MPIS, the import of NLRX1 is disrupted, leading to its retention and stabilization in the cytosol. This relocalization raised one possibility for the role of NLRX1 following MPIS, serving as a danger sensor like other NLRs. In support for this hypothesis, we showed that in this new location, NLRX1 interacts with an endoplasmic reticulum (ER) transmembrane protein RRBP1, and together they facilitate local LC3 lipidation and mitophagophore progression. Importantly, this NLRX1-driven LC3 lipidation arm of the mitophagy pathway is independent of the PINK1-PRKN system, which we showed instead is important for downstream mitochondrial clearance (Figure 1). This separation from canonical PINK1-PRKN signaling challenges the notion of a linear sequence of events for mitophagosome formation following mitochondrial stress.

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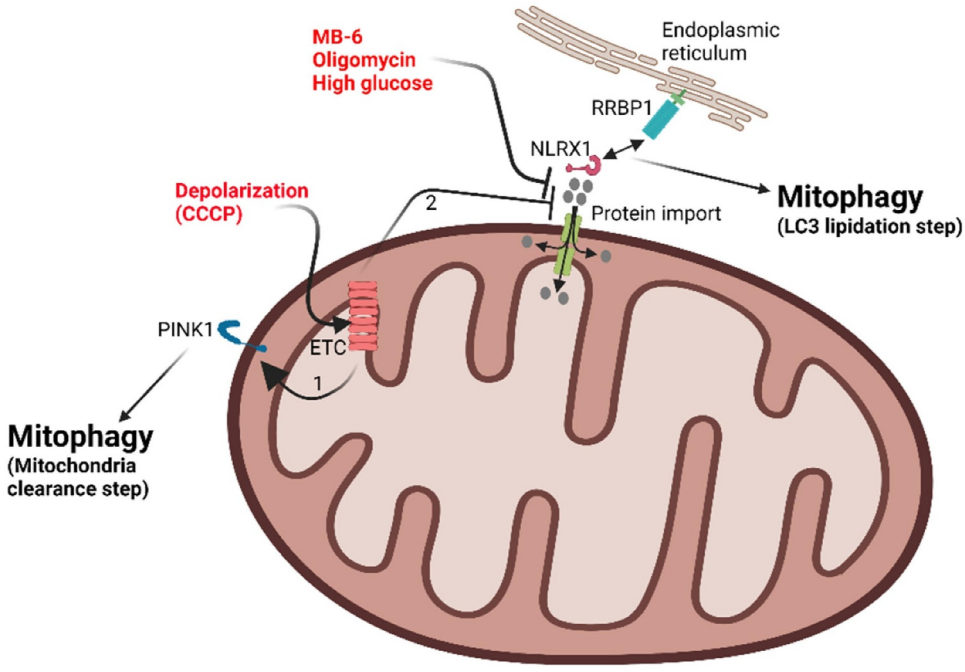

Figure 1. The NLRX1-RRBP1 axis mediates LC3 lipidation following mitochondrial protein import stress. Depolarization agents such as CCCP lead to PINK1 stability on the outer mitochondrial membrane (1) and prevent efficient import of other mitochondrial proteins, generating an import stress signal (2) which is sensed by NLRX1. The cytosolic stabilization of NLRX1 allows it to interact with its binding partner on the endoplasmic reticulum, RRBP1, and together they mediate LC3 recruitment and lipidation around the dysfunctional mitochondria. Import stress can also be triggered in the absence of PINK1 stabilization by additional treatments such as MB-6, oligomycin and high glucose. CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; MB-6, MitoBlock-6; ETC, electron transport chain. Created with BioRender.Com.

We have uncovered a new role for the ER in mitophagosome progression as the transmembrane protein RRBP1 is a crucial partner in NLRX1-driven LC3 lipidation. This builds off the current understanding that the ER serves as the primary source of membrane for the growing phagophore, because it would be efficient for the cell to use a transmembrane protein within the source of the growing mitophagosome to also promote LC3 lipidation and membrane expansion. RRBP1 is constitutively found in the perinuclear, rough ER, likely a result of its involvement in mRNA recruitment to ribosomes for protein translation. We showed that after various forms of translational stress, RRBP1 relocalizes peripherally, and is no longer restricted to the rough ER. In this secondary location, RRBP1 is in close vicinity to the mitochondrial network, a necessary step for facilitating mitophagy. Unexpectedly, we demonstrated that RRBP1 is important for recovering translation of mitochondrial proteins upon removal of the translational stress. This may be related to the ability of RRBP1 to recruit mRNAs to ribosome sites, and the accumulation of RRBP1 at mitochondria-ER contact sites may represent the cell's initial response to a decrease in the local translation of proteins at the mitochondrial outer membrane. We thus propose that the primary role for RRBP1 recruitment to mitochondria-ER contact sites during MPIS may be to assist local translation; if the MPIS persists, the progressive retention of NLRX1 to the cytosol and its association with RRBP1 would serve to redirect cellular response toward mitophagy. This way, NLRX1 would serve as a danger sensor and a gauge of MPIS. Finally, the possibility remains that RRBP1 could also facilitate other forms of selective autophagy, where some local signals, acting in a similar fashion as NLRX1 during mitophagy, would direct RRBP1 to mediate LC3 lipidation around the cargo of interest. This hypothesis remains to be tested experimentally.

Immediate next steps after our work involve exploring the NLRX1-RRBP1 axis of mitophagy in specific cell types of interest. Our work began to address this, by inducing mitophagy in mice through a forced exercise treadmill regimen and assessing different muscle tissue. We found that NLRX1 is essential for exercise-induced mitophagy, as assessed by LC3 lipidation in the heavy membrane fraction of cell lysates. This suggests that the NLRX1-RRBP1 pathway is translatable to an in vivo setting and demonstrates the importance of additional investigation and characterization of this pathway. Promoting efficient mitophagy can be one avenue to pursue when handling diseases involving muscle weakness or atrophy. Alternatively, resolving the issues in protein import into the mitochondria by increasing chaperones or removing aggregation-prone proteins may also prevent mitochondrial dysfunction without the need for complete degradation of the organelle in question.

PINK1 and PRKN are genetic risk factors for Parkinson disease, and a growing body of additional evidence inextricably links mitochondrial dysfunction to many neurodegenerative diseases. Future work into the role of the NLRX1-RRBP1 mitophagy pathway in neuronal cell lines, patient-derived induced pluripotent stem cells, and in vivo models of neurodegenerative disease will be exciting avenues to pursue. When considering the therapeutic strategies

our findings might inform, the most prominent is whether the efficiency of mitochondrial protein import could be modified in a selective, cell type-specific way without causing overt damage to the cells of interest or the organism. As with most therapeutics, a fine balance would need to be reached, where the cell can promote mitophagy in stressed mitochondria without impeding the function of the remaining healthy mitochondria.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Reference

[1] Killackey SA, Bi Y, Soares F, et al. Mitochondrial protein import stress regulates the LC3 lipidation step of mitophagy through NLRX1 and RRBP1. Mol Cell. 2022;82(15):2815–2831.e5. DOI:10.1016/j.molcel.2022.06.004