Neurons are highly polarized cells with an extended axon, thus facing the special challenge of maintaining mitochondrial integrity in both health and disease. PARK2-mediated mitophagy is the key mechanism of mitochondrial quality control. It was reported that acute mitochondrial depolarization in cultured neurons with a high-dose (40 \mu M) of antimycin A1 (AA), a respiratory complex III inhibitor, quickly induces PARK2-mediated mitophagy in distal axons within ~50 mins. Such acute mitochondrial depolarization, however, prevents investigation into the early stages of mitochondrial quality control. Recent in vivo studies with genetic Pink1 and park/Parkin mutations in the Drosophila nervous system support the model that mitochondrial turnover is mainly restricted to the soma. These studies raise 2 fundamental questions: whether mitophagy is an early mechanism for neuronal mitochondrial quality control and whether axonal mitophagy could be induced by mild and chronic mitochondrial stress, a condition more relevant to the chronic pathological stress leading to axonal accumulation of damaged mitochondria. Therefore, the early removal of defective mitochondria from axons constitutes a critical step of mitochondrial quality control. We recently investigated the axonal mitochondrial response to mild stress in wild-type neurons and chronic mitochondrial defects in amyotrophic lateral sclerosis (ALS)- and Alzheimer disease (AD)-linked neurons. We demonstrated that remobilizing stressed mitochondria is critical for maintaining axonal mitochondrial integrity. The selective release of the mitochondrial anchoring protein SNPH (syntaphilin) from stressed mitochondria enhances their retrograde transport toward the soma before PARK2/Parkin-mediated mitophagy is activated. This SNPH-mediated response is robustly activated during the early disease stages of ALS-linked motor neurons and AD-related cortical neurons. Our study thus reveals a new mechanism for the maintenance of axonal mitochondrial integrity through SNPH-mediated coordination of mitochondrial stress and motility that is independent of mitophagy.

To test this model, we recapitulated chronic mitochondrial dysfunction by applying a mild stress with 5 nM AA, a 1000-times lower dosage than reported in the literature. This mild AA treatment induces a slow and reversible mitochondrial stress. Surprisingly, axonal mitochondria respond to this mild stress by the selective regulation of their biased directional transport, favoring the removal of stressed mitochondria out of axons. This regulation is triggered by the bulk release of SNPH in the form of cargo vesicles from stressed mitochondria. Such a dynamic response is captured at ultrastructural levels and by time-lapse super-resolution imaging. Upon release, these SNPH cargo vesicles undergo retrograde transport en route to the endosome-lysosome pathway for degradation. Importantly, the generation of SNPH cargo vesicles is also observed in spinal ventral root motor neuron axons during the early asymptomatic stages of familial ALS-linked human SOD1(G93A) expressing mutant mice. Progressive mitochondrial damage depletes SNPH after disease onset. Consistently, the SNPH-mediated response is also activated in the axons of AD-related cortical neurons from mutant human APP-expressing transgenic mice. The density of SNPH cargo vesicles in AD axons is robustly increased. Stressed mitochondria display reduced anterograde transport, favoring the removal of stressed mitochondria out of axons. This SNPH-mediated response is robustly activated during the early disease stages of ALS-linked motor neurons and AD-related cortical neurons.

Removing dysfunctional mitochondria from axons independent of mitophagy under pathophysiological conditions

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\textbf{ABSTRACT}

Chronic mitochondrial dysfunction has been implicated in major neurodegenerative diseases. Long-term cumulative pathological stress leads to axonal accumulation of damaged mitochondria. Therefore, the early removal of defective mitochondria from axons constitutes a critical step of mitochondrial quality control. We recently investigated the axonal mitochondrial response to mild stress in wild-type neurons and chronic mitochondrial defects in amyotrophic lateral sclerosis (ALS)- and Alzheimer disease (AD)-linked neurons. We demonstrated that remobilizing stressed mitochondria is critical for maintaining axonal mitochondrial integrity. The selective release of the mitochondrial anchoring protein SNPH (syntaphilin) from stressed mitochondria enhances their retrograde transport toward the soma before PARK2/Parkin-mediated mitophagy is activated. This SNPH-mediated response is robustly activated during the early disease stages of ALS-linked motor neurons and AD-related cortical neurons. Our study thus reveals a new mechanism for the maintenance of axonal mitochondrial integrity through SNPH-mediated coordination of mitochondrial stress and motility that is independent of mitophagy.

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and enhanced retrograde transport. Intriguingly, SNPH is largely abolished in mutant human APP-expressing transgenic mouse brains and postmortem brain specimens from human AD patients. Given the fact that the accumulation of damaged mitochondria in distal axons is the pathological hallmark of ALS and AD, defective motor machineries likely impair the removal of damaged mitochondria even though SNPH is depleted in the late disease stages.

The generation of SNPH cargo vesicles from stressed axonal mitochondria is initiated as early as 6 h after the start of mild stress conditions while PARK2-mediated mitophagy is not readily detected until 30 h under the same stress conditions. In addition, park2 knockout neurons display no impaired generation of SNPH cargo vesicles. Thus, the SNPH-mediated stress response is critical to maintaining axonal mitochondrial integrity before PARK2-mediated mitophagy is activated. When mitochondria undergo acute and global damage, this early mechanism is likely bypassed by activating mitophagy. Indeed, acute mitochondrial depolarization suddenly arrests mitochondrial transport probably by RHOT1/Miro1 degradation and mitochondrial fragmentation. If the acute depolarization model operates in vivo, one would expect the accumulation of damaged mitochondria in distal axons following geneticmutation of the PARK2 pathway. This model was recently examined by an in vivo study. Mutation of Pink1 or park/Parkin in Drosophila does not result in the accumulation of damaged mitochondria in axons or neuromuscular junctions. Thus, under chronic mitochondrial stress or pathological conditions, the early removal of those anchored dysfunctional mitochondria from axons constitutes an important pathway to maintain axonal mitochondrial integrity.

The generation of mitochondria-derived vesicles in non-neuronal cells can efficiently remove damaged or aggregated mitochondrial proteins to avoid the complete degradation of whole organelles. The selective incorporation of different protein cargoes depends on the nature of the mitochondrial stress. Our study reveals a new class of axonal mitochondria-derived cargoes that selectively release bulk SNPH in axonal compartments, where mitochondrial trafficking and anchoring are unique from non-neuronal cell types. To our knowledge, this is the first observation showing these mitochondria-derived vesicles in axons under physiological and pathological stress conditions. Axonal SNPH cargoes and non-neuronal mitochondrial-derived vesicles (TOMM20^+ matrix^-) share some common features: (1) the formation of both vesicles is LC3-, PARK2-, and DNM1L/Drp1-independent; (2) both vesicles are formed from the lateral segregation of the mitochondrial outer membrane; and (3) both vesicles are delivered to lysosomes for degradation. However, SNPH cargo vesicles have several unique features. First, SNPH cargo vesicles are specifically derived from axonal mitochondria under mild stress conditions long before PARK2-mediated mitophagy is activated. Second, SNPH cargo vesicles lose CYCS/cytochrome c and membrane potentials, and do not carry TOMM20. Third, the selective removal of those anchored dysfunctional mitochondria from axons constitutes an important pathway to maintain axonal mitochondrial integrity.
bulk removal of SNPH remobilizes stressed axonal mitochondria toward the soma.

Our study highlights that the generation of SNPH cargo vesicles ensures a quick response of axonal mitochondria to chronic and pathological stresses: dysfunctional mitochondria that are anchored in distal axons of mature neurons can be remobilized and transported back to the soma (Fig. 1). This is likely the first line of mitochondrial defense that sits in the early stage of the complete hierarchy of the mitochondrial quality control system in axons. Thus, our study reveals a new neuronal pathway that benefits the development of strategies to attenuate axonal mitochondrial pathology in the early stages of several major neurodegenerative diseases.

**Abbreviations**

| Abbreviation | Meaning                        |
|--------------|--------------------------------|
| AA           | antimycin A1                   |
| AD           | Alzheimer disease              |
| ALS          | amyotrophic lateral sclerosis   |
| SNPH         | syntaphilin                    |

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**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.