Mitophagy coordination with retrograde transport ensures the integrity of synaptic mitochondria

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ABBREVIATION: AD, Alzheimer disease; Aβ, amyloid-β; APP, amyloid beta precursor protein; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; LE, late endosome; $\Delta\psi_m$, mitochondrial membrane potential; RHEB, Ras homolog enriched in brain; RNAi, RNA interference; shRNA, small hairpin RNA; Tg, transgenic

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ABSTRACT

Mitochondria sustain various essential functions at synaptic terminals. Synaptic mitochondria deficits have been implicated in early Alzheimer disease (AD) pathophysiology. Mitophagy, a selective autophagy for removal of damaged mitochondria, plays a key role in mitochondrial quality control in neurons. However, fundamental questions remain unanswered as to whether mitophagy regulates synaptic mitochondrial integrity and whether early deficits in synaptic mitochondria associated with AD are attributed to mitophagy failure. We recently revealed that the integrity of synaptic mitochondria is maintained by a coordination of RHEB-mediated mitophagy with dynein- and SNAPIN-driven retrograde transport. We demonstrate that increased mitophagy initiation coupled with defective retrograde transport triggers mitophagy stress at AD synapses. Excitingly, SNAPIN-enhanced retrograde transport reduces synaptic mitophagy stress and ameliorates mitochondrial deficits, thereby counteracting synaptic damage in AD mouse brains. Therefore, our study provides new mechanistic insights into how mitophagy facilitates synaptic mitochondrial maintenance and how mitophagy failure exacerbates AD-linked mitochondrial defects and synaptic degeneration.

Mitochondria are the principal producers of cellular energy, supplying most of the ATP by oxidative phosphorylation/OXPHOS, which is crucial for neuronal function and survival. Mitochondria serve as local energy sources and play a pivotal role in sustaining synaptic activities. Given the high capacity to sequester and buffer intracellular Ca^{2+} levels, mitochondria are also involved in maintaining and regulating synaptic transmission. Thus, mitochondria at synaptic terminals are highly active and are more vulnerable to various insults and cumulative changes induced by deleterious factors. Mitophagy, a cargo-specific autophagy in which damaged mitochondria are sequestered within autophagosomes for their subsequent degradation within lysosomes, constitutes a major quality control system to maintain mitochondrial homeostasis in neurons. The critical understanding of the physiological roles of mitophagy and its link to pathological conditions remains very limited. In particular, the mechanism underlying mitochondrial maintenance at synaptic terminals is still poorly understood [1].

Mitochondrial dysfunction underlies cognitive impairment in neuronal aging and is one of the most notable hallmarks of age-related neurodegenerative diseases, including Alzheimer disease (AD). A number of recent studies demonstrated mitophagic abnormalities in AD brains. Importantly, mitophagy stimulation was shown to abolish AD pathology and reverse memory impairment in AD models. Synaptic stress is an early pathological feature in AD brains, and mitochondrial deficits have been proposed as a key player in AD-associated synaptic dysfunction. This raises yet another fundamental question: are such defects attributed to mitophagy failure at AD synapses?

RHEB (Ras homolog enriched in brain) was previously reported to target mitochondria for autophagy in non-neuronal cells upon increased mitochondrial respiration. We determined whether RHEB-mediated mitophagy plays a role in the quality control of mitochondria in neurons. Under basal conditions, RHEB is mainly present in the cytoplasm of axons and displays very limited association with mitochondria. Strikingly, upon treatment with carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), a mitochondrial membrane potential ($\Delta\psi_m$) uncoupler, RHEB is robustly recruited to depolarized mitochondria and RHEB-targeted mitochondria exhibit high motility and move exclusively in a retrograde direction along axons. Consistently, mitochondria associated with RHEB are engulfed by phagophores to form mitophagosomes, which display dominant retrograde movement in axons. It is known that mitophagic clearance primarily occurs in the soma of neurons, where degradative lysosomes are mainly located. Thus, our observations support the view that damaged mitochondria in distal axons recruit RHEB to initiate mitophagy, whereas retrograde transport enables axonal removal of mitophagosomes for lysosomal degradation in soma (**Figure 1**).

We and others have demonstrated that mitochondrial damage activates PRKN/Parkin-mediated mitophagy predominantly in the soma of neurons. In this study, we found that *Rheb* RNA interference (RNAi) does not affect PRKN translocation onto mitochondria upon $\Delta\psi_m$ dissipation, suggesting that

RHEB does not play a role in PRKN-mediated mitophagy in the soma. However, we have shown that BNIP3L/Nix, an outer mitochondrial membrane protein, facilitates RHEB association with damaged mitochondria and thereby controls mitochondrial targeting for autophagy in axons. Mitophagosome biogenesis is significantly impaired in axons expressing *Rheb* small hairpin RNA (shRNA) or *Bnip3l* shRNA, but not *Prkn* shRNA. Furthermore, disruption of RHEB-mediated mitophagy leads to aberrant retention of oxidatively stressed mitochondria within axons. Collectively, these results indicate that RHEB and BNIP3L are important for mitophagy in axons, in which stressed axonal mitochondria are targeted for autophagy.

We next assessed how RHEB-mediated mitophagy is regulated in response to chronic mitochondrial stress under pathophysiological conditions. By performing immunohistochemistry, biochemistry, and transmission electron microscopy analysis in AD-related mutant human APP (amyloid beta precursor protein; HsAPP) transgenic (Tg) mouse brains, we provided multiple lines of evidence showing synaptic mitophagy stress as reflected by robust accumulation of mitophagosomes, particularly at dystrophic presynaptic terminals surrounding amyloid plaques. This finding indicates that such a defect is relevant to the development of synaptic pathology in AD. RHEB association with mitochondria is markedly enhanced at AD synapses enriched with soluble amyloid β (Aβ) oligomers, suggesting increased initiation of RHEB-mediated mitophagy. We further examined whether mitophagy initiation can be efficiently induced upon dissipating $\Delta\psi_m$ in cortical neurons derived from mutant HsAPP Tg mouse brains. $\Delta\psi_m$ dissipation significantly augments RHEB recruitment to depolarized mitochondria and thus intensifies mitophagy stress within AD axons. Moreover, we determined whether mitophagy stress at AD synapses could also be the result of impaired retrograde transport. Indeed, retrograde movement of mitophagosomes along AD axons is remarkably impeded, leading to defects in the removal of mitophagosomes from synaptic terminals. Additionally, we excluded the possibility of a direct impact of AD-linked lysosomal deficits on synaptic mitophagy stress. Therefore, our findings indicate that increased mitophagy initiation coupled with defective retrograde transport results in mitophagy stress and thus exacerbates mitochondrial deficits at AD synapses (**Figure 1**).

Our previous studies established that the dynein-SNAPIN motor-adaptor complex mediates retrograde transport of autophagic vacuoles in the form of amphisomes—through fusion with late endosomes (LEs)—from distal axons toward the soma for lysosomal degradation. In this study, we have further demonstrated that newly generated mitophagosomes in axons rapidly fuse with the dynein-SNAPIN transport complex-loaded LEs to form mito-amphisomes so that these mitophagosomes gain retrograde transport motility. Such a mechanism facilitates mitophagic clearance within lysosomes in the soma and thus reduces mitophagosomal accumulation at synapses

(**Figure 1**). *Snapin* deficiency in neurons mimics mitophagy stress at AD synapses, inducing synaptic damage. Excitingly, increasing SNAPIN levels in AD neurons decreases mitophagic retention and attenuates mitochondrial defects in axons by enhancing retrograde transport of mitophagosomes. Mutant HsAPP Tg mouse brains transduced with AAV-SNAPIN exhibit reduced mitophagy stress and alleviated synapse loss. These *in vitro* and *in vivo* data collectively suggest that SNAPIN-enhanced retrograde transport ameliorates synaptic mitochondrial deficits by promoting removal of damaged mitochondria engulfed within mitophagosomes, thereby counteracting synaptic deterioration associated with AD.

In summary, our study provides new mechanistic insights into how mitophagy ensures mitochondrial integrity at synapses crucial for synaptic maintenance, and how mitophagy failure augments synaptic mitochondrial deficits and exacerbates synaptic defects in AD neurons. Our study establishes a foundation for future investigations into mitophagy enhancement through boosting retrograde transport of mitophagosomes to mitigate mitochondrial pathology and synaptic degeneration in AD.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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Figure legend

Figure 1. Maintenance of synaptic mitochondrial integrity through RHEB-mediated mitophagy and dynein- and SNAPIN-driven retrograde transport. In healthy neurons, mitochondrial damage in axons and at synaptic terminals activates RHEB-mediated mitophagy through which RHEB is recruited to damaged mitochondria and mediates their engulfment by phagophores and sequestration within mitophagosomes. Nascent mitophagosomes rapidly fuse with late endosomes (LEs) to form mito-amphisomes so that the dynein-SNAPIN transport machinery can be loaded onto mitophagosomes. As a result, mitophagosomes gain retrograde transport motility toward the soma for lysosomal degradation, thus reducing mitophagy stress in distal axons. Such a mechanism is critical for synaptic mitochondrial maintenance. In Alzheimer disease (AD) neurons, RHEB-mediated mitophagy is robustly initiated at synaptic terminals enriched with soluble oligomers of amyloid β (Aβ), the main

constituent of amyloid plaques in AD brains. However, impaired dynein- and SNAPIN-mediated retrograde transport fails to remove mitophagosomes from AD synapses, leading to mitophagy stress. Such defects augment synaptic mitochondrial deficits and exacerbates AD-associated synaptic damage.

Reference

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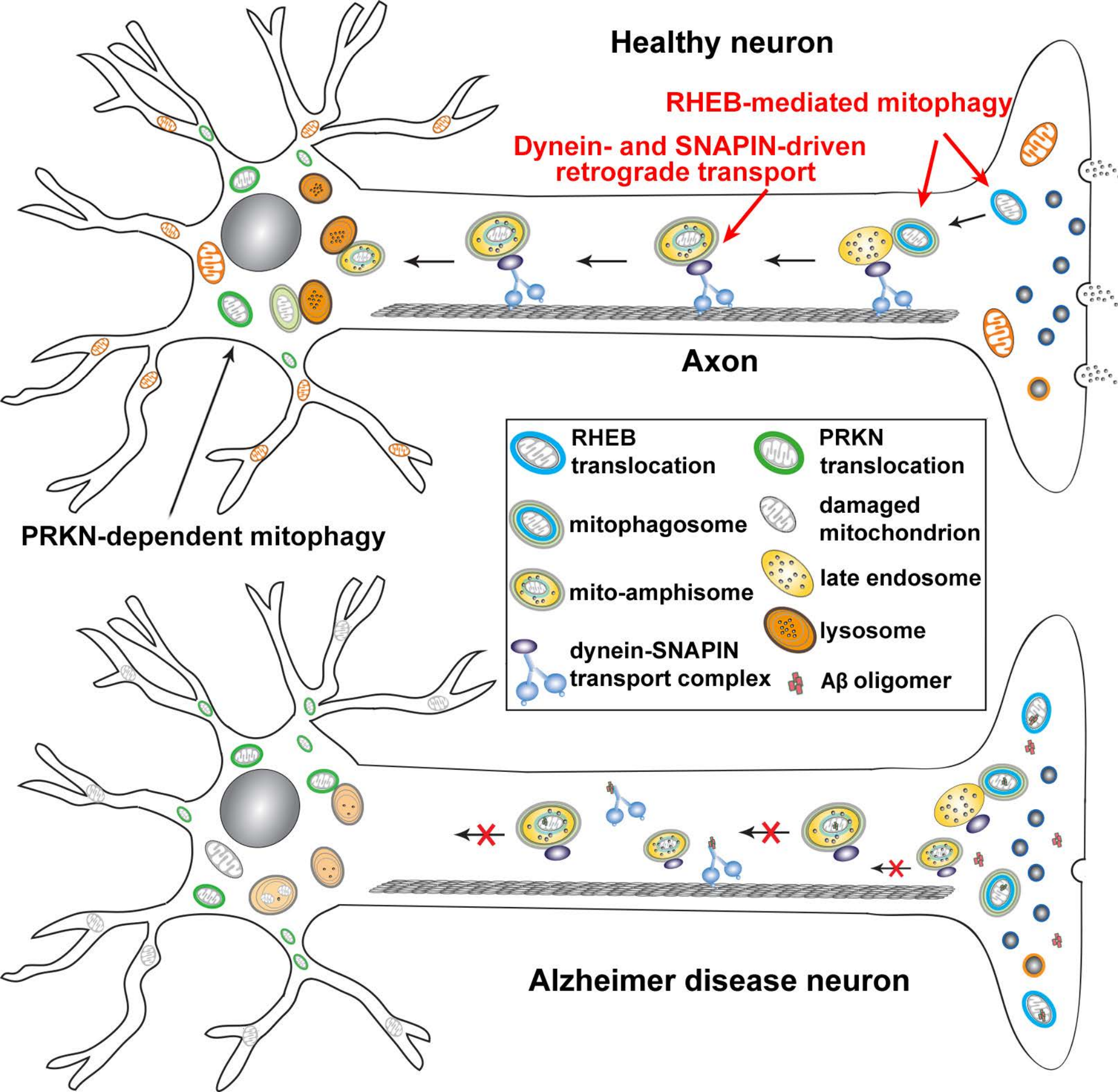
Healthy neuron
RHEB-mediated mitophagy
Dynein- and SNAPIN-driven
retrograde transport
Axon
PRKN-dependent mitophagy
RHEB
translocation
PRKN
translocation
mitophagosome
damaged
mitochondrion
mito-amphisome
late endosome
lysosome
dynein-SNAPIN
transport complex
Aβ oligomer
Alzheimer disease neuron