AUTOPHAGIC PUNCTUM

Mitochondrial hitch-hiking of *Pink1* mRNA supports axonal mitophagy

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Abstract

Mitostasis, the process of mitochondrial maintenance by biogenesis and degradative mechanisms, is challenged by the extreme length of axons. PINK1 (PTEN induced putative kinase 1) is a mitochondrial protein that targets damaged mitochondria for mitophagy. In reconciling the short half-life of PINK1 with the need for mitophagy of damaged axonal mitochondria, we found that axonal mitophagy depends on local translation of the *Pink1* mRNA. Using live-cell imaging, we detected co-transport of the *Pink1* mRNA on mitochondria in neurons, which is crucial for mitophagy in distal parts of the cell. Here we discuss how the coupling of the transcript of a short-lived mitochondrial protein to the movement of its target organelles contributes to our understanding of mitostasis in neurons.

Keywords: Axonal biology**,** local translation**,** mitochondria, mitophagy, RNA transport

Microtubule-based transport supplies mitochondria to the axons and dendrites of neurons. The length of some axons requires mitochondrial proteins to be stable for days or even weeks if they are to survive the journey from the cell body to the extremities of the cell. While mitochondrial proteins generally have longer half-lives than most mammalian proteins, there are exceptions. PINK1, a Parkinson disease-related protein, has an estimated half-life of 30 min, and this short half-life is an essential part of the mechanism by which PINK1 senses mitochondrial damage. In healthy mitochondria, PINK1 is rapidly degraded; only in dysfunctional mitochondria is PINK1 stabilized on the outer mitochondrial membrane. This stabilization allows PINK1 and PRKN/parkin

to collaborate in driving the selective mitophagy of damaged mitochondria. Despite the short half-life of PINK1, this pathway functions in axons. How then can axonal and dendritic mitochondria have a supply of this short-lived, constantly turning-over protein ready to be stabilized and trigger mitophagy? This question prompted us to investigate the possibility of local translation of *Pink1* mRNA and the subsequent question of how *Pink1* mRNA is trafficked within a neuron.

In Harbauer et al. [1], we demonstrated that local translation of *Pink1* mRNA occurs in axons of cultured hippocampal neurons. Inhibiting local protein synthesis in axons but not cell bodies prevents the activation of the PINK1-PRKN pathway. Adult retinal ganglion cells transport tagged *Pink1* mRNA into their axons; thus, this mechanism is also active in mature neurons *in vivo*.

In situ hybridization for *Pink1* mRNA in axons and dendrites detects the transcript at the outside of mitochondria and live cell imaging of MS2/PP7-tagged *Pink1* mRNA reveals extensive co-transport of the transcript with mitochondria. Indeed, almost all the long-range movements of the tagged mRNA are accompanied by a mitochondrion, and inhibiting mitochondrial transport reduces *Pink1* mRNA transport. Thus, *Pink1* mRNA has its own version of organellar hitch-hiking. *Pink1* mRNA is not localized to mitochondria, however, in COS7 cells, HeLa cells, or fibroblasts. This difference implies a neuron-specific mechanism and hence a neuron-enriched RNA-tethering complex. We determined the tethering complex for the *Pink1* mRNA to consist of the mitochondrial outer membrane protein SYNJ2BP and a neuron-specific splice variant of SYNJ2 (synaptojanin 2), SYNJ2a. SYNJ2a is a 5' inositol-lipid-phosphatase but also contains a predicted RNA-recognition motif (RRM) domain that had not been studied. Expression of SYNJ2a in COS7 cells redirects *Pink1* mRNA to mitochondria. In addition, a construct in which SYNJ2a is artificially tethered to the mitochondrial outer membrane can bring the *Pink1* mRNA to mitochondria even after knockdown of SYNJ2BP. This rescue depends on the RRM domain as mutation of three conserved amino acids in the RNA-binding pocket abolishes its mitochondrial localization. UV crosslinking assays confirm the RNA-binding properties of the SYNJ2a RRM. There is specificity to the RNA-binding as well; *Actb* (actin, beta) transcripts are not recruited by SYNJ2a to mitochondria and the related protein SYNJ1, though it also contains a predicted RRM, does not recruit *Pink1* mRNA to mitochondria. RNA-seq revealed several hundred other transcripts that preferentially bound the wild-type SYNJ2a over the RNA-binding mutant, with a significant skew towards mitochondrial transcripts, some of which encode long-lived proteins. Thus, *Pink1* transcripts may not be the only mRNA using mitochondria for transport. It remains to be determined if those transcripts share features in their primary sequences or secondary structures that govern SYNJ2a binding.

For the *Pink1* transcript, we determined that a sequence within the coding region (1-675 bp) was sufficient to localize the transcript to mitochondria. This sequence encodes the N-terminal portion of PINK1 including the mitochondrial targeting sequence (MTS). Translation of the transcript is important for mitochondrial localization, but the MTS alone is not sufficient to confer mitochondrial localization to a reporter construct. This suggests a dual targeting mechanism, which involves first a co-translational targeting of the PINK1 MTS to mitochondria, probably via interaction of the nascent chain with the import complexes. This may facilitate formation of the *Pink1* mRNA-SYNJ2a-SYNJ2BP tethering complex as the interaction between SYNJ2a and SYNJ2BP is sensitive to translation inhibitors (Figure 1). However, many questions remain that will need to be clarified in future studies. How does translation favor the interaction between SYNJ2a and SYNJ2BP? Does binding of RNA by SYNJ2a allow simultaneous translation? Does translation occur all the time or is there a switch between translationally silent RNA transport and activation of translation at specific subcellular sites?

The importance of the transport mechanism for *Pink1* mRNA was demonstrated by determining whether mitochondrial depolarization can activate the PINK1-PRKN pathway in its absence, which we assayed by the presence of phospho-ubiquitin on depolarized mitochondria. This product of PINK1 activation is markedly reduced in neurites when SYNJ2BP is knocked down. Thus, the mitochondrial hitchhiking of the *Pink1* mRNA is of consequence for efficient mitophagy in axons and dendrites. Given the ability of SYNJ2a to bind additional transcripts, this hitchhiking mechanism may have more far-reaching implications for the maintenance of a healthy pool of mitochondria in the distant reaches of the neuron.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Reference

[1] Harbauer, A.B., Hees, J.T., Wanderoy, S., et al. Neuronal mitochondria transport Pink1 mRNA via synaptojanin 2 to support local mitophagy. Neuron 2022, Epub ahead of print. Available from: https://doi.org/10.1016/j.neuron.2022.01.035

Figure Legends

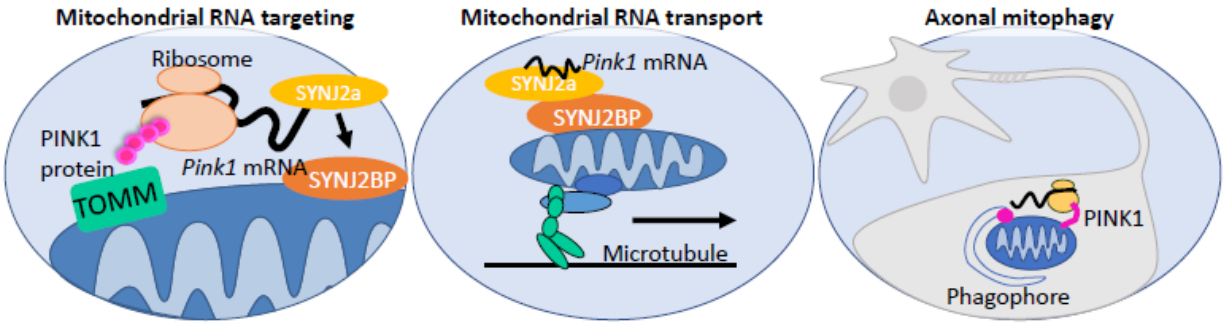

Figure 1. Model of mitochondrial RNA transport of *Pink1* and its role in axonal mitophagy. Translation of PINK1 targets the nascent chain-ribosome-mRNA complex to mitochondria. This allows the formation of the SYNJ2a-SYNJ2BP-*Pink1* mRNA tethering complex and subsequent co-transport of the *Pink1* mRNA along with mitochondrial trafficking. Local translation then supplies newly synthesized PINK1 protein to support axonal mitophagy.