A mitochondrial-derived vesicle HOPS to endolysosomes using Syntaxin-17

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Damaged mitochondrial content is packaged in mitochondrial-derived vesicles (MDVs), which are targeted for degradation through an unclear mechanism. McLelland et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201603105) show that the SNARE Syntaxin-17 mediates MDV fusion with endolysosomes, promoting the delivery of mitochondrial cargo to lysosomes for degradation.

Parkinson's disease (PD) is a devastating neurological disorder that affects 1–2% of the population. Initial symptoms include movement defects caused by the impaired functioning of the pars compacta region of substantia nigra. Dopamine-producing neurons in this region are progressively lost, which leads to the development of rigidity, tremor, slow movement, and postural instability in PD patients. Unfortunately, there is no cure for PD, as current treatments only provide symptomatic relief (Pickrell and Youle, 2015). Genetic studies of rare familial cases have identified a set of inheritable mutations linked to PD, including those found in genes called *PARK2* (encoding parkin) and *PINK1*. An early *Drosophila melanogaster* study described *parkin* mutant flies with locomotion defects caused by degeneration of their indirect flight muscles, likely as a consequence of abnormal mitochondria that accumulate in these cells (Greene et al., 2003). Strikingly, the similar phenotypes of flies lacking PINK1 could be rescued by overexpressing human parkin, suggesting that parkin acts downstream of PINK1 in a mitochondrial quality control pathway (Greene et al., 2003).

Mitophagy, the selective autophagic elimination of damaged, nonfunctional mitochondria, emerged as the route through which parkin and PINK1 exert their functions in various cultured cells, which was recently confirmed in distal neuronal axons (Ashrafi et al., 2014). During mitophagy, PINK1 kinase is stabilized on the surface of damaged mitochondria that fail to maintain mitochondrial membrane potential, which leads to the recruitment and activation of the ubiquitin ligase parkin. Parkin ubiquitinates several mitochondrial proteins, which serve as molecular signals for the capture of damaged mitochondria into autophagosomes. Finally, these double-membrane vesicles transport their cargo to lysosomes for degradation, the last step in the macroautophagic pathway (Pickrell and Youle, 2015; Fig. 1). Mitophagy can be triggered experimentally by carbonyl cyanide m-chlorophenyl hydrazine (CCCP) or valinomycin treatments, which dissipate the inner membrane potential of mitochondria. CCCP and valinomycin, along with pesticides and herbicides such as rotenone and paraquat, which inhibit the respiratory chain (RC) involved in mitochondrial oxidative phosphorylation, are commonly used to model PD in vitro and in vivo (Pickrell and Youle, 2015), but these models remain relatively artificial. Evidence for the central role of parkin and PINK1 in mitophagy under physiological conditions in vivo was first provided by a study of wild-type, autophagy-impaired, and *parkin* or *Pink1* mutant *Drosophila* (Vincow et al., 2013). Loss of parkin or PINK1 resulted in a decrease in mitochondrial protein turnover, albeit a less severe phenotype than that observed in autophagy-impaired flies for all mitochondrial proteins except RC proteins. This observation suggested a model in which parkin and PINK1 mediate the selective turnover of RC components in vivo, in addition to their roles in mitophagy. The existence of this pathway was recently confirmed by treating cultured cells with the RC complex III inhibitor antimycin A in the presence of galactose instead of glucose. The induced oxidative damage within mitochondria led to enhanced formation of mitochondrial-derived vesicles (MDVs) that incorporate selective cargo (Soubannier et al., 2012). These double-membrane MDVs deliver oxidized mitochondrial proteins to lysosomes, and their generation from mitochondria requires the actions of parkin and PINK1 at the budding site (Fig. 1; McLelland et al., 2014). In this issue, McLelland et al. expand on these findings to decipher the molecular mechanisms of MDV-mediated selective mitochondrial protein turnover.

Mitochondria form a network inside cells with constant fusion and fission that is typically considered independent of SNARE proteins, which mediate most membrane fusion events. Under the hypothesis that MDVs require SNARE-dependent targeting to reach the endolysosomal compartment, McLelland et al. (2016) focused on Syntaxin-17, a unique SNARE with two transmembrane domains on its C terminus, which was previously shown to partially localize to mitochondria (Itakura et al., 2012; Hamasaki et al., 2013; Arasaki et al., 2015). In vitro MDV reconstitution assays and electron microscopy and live-cell confocal microscopy analyses demonstrated that forming and mature MDVs that transport the mitochondrial proteins VDAC1, PDH E2, and SDHA are enriched for Syntaxin-17. This process was also shown to depend on parkin and PINK1, as knockdown of either impaired Syntaxin-17 cluster formation on the outer mitochondrial membrane during MDV budding.

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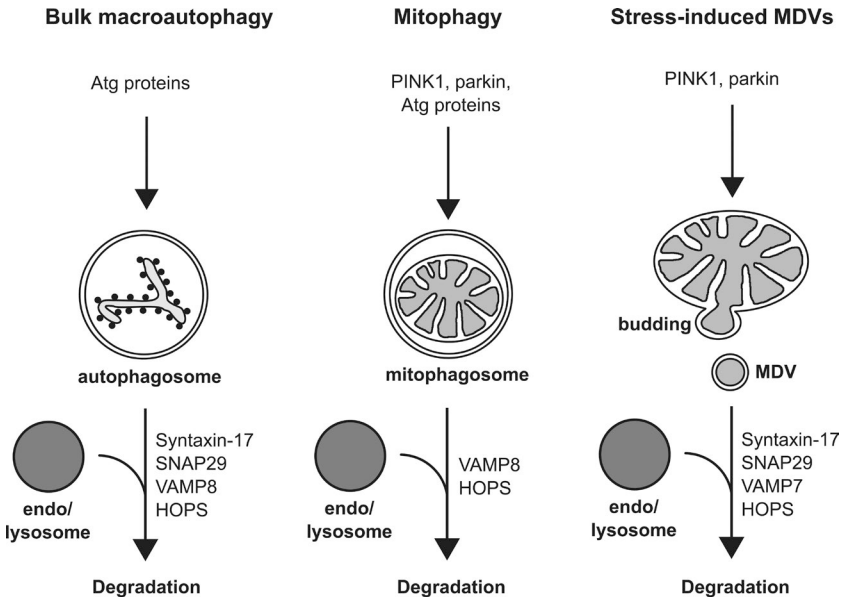

Figure 1. **A model of bulk macroautophagy, mitophagy, and MDV-mediated selective mitochondrial protein turnover in mammalian cells.** During bulk macroautophagy, Atg proteins mediate the formation of double-membrane autophagosomes, which subsequently acquire Syntaxin-17 and HOPS to enable their fusion with lysosomes and late endosomes. Mitophagy requires the actions of parkin, PINK1, and Atg proteins to promote formation of mitochondria-containing autophagosomes, which fuse with endolysosomes in a HOPS-dependent but Syntaxin-17–independent fashion. Parkin and PINK1 also mediate the stress-induced budding of MDVs from mitochondria, which fuse with endolysosomes in a Syntaxin-17– and HOPS-dependent manner.

Previous work established that Syntaxin-17 and SNAP29 mediate autophagosome fusion with late endosomes and lysosomes, and that Syntaxin-17 and SNAP29 form a complex with VAMP8 in mammals or VAMP7 in *Drosophila* (Itakura et al., 2012; Takáts et al., 2013). These studies prompted McLelland et al. (2016) to investigate whether Syntaxin-17 uses the same partners in targeting MDVs to lysosomes. Using colocalization studies and immunoprecipitation, McLelland et al. (2016) validate the interactions between Syntaxin-17 and SNAP29, VAMP7, and VAMP8. Silencing these candidates revealed that Syntaxin-17, SNAP29, and VAMP7 are critical for MDV delivery to lysosomes. Subsequently, the researchers used the biochemical properties of SNARE proteins to establish that a ternary complex of these SNAREs is indeed important for MDV clearance. Syntaxin-17 and SNAP29 are Q SNAREs, whereas VAMP7 is an R SNARE. Q (Glu) and R (Arg) refer to the amino acids present in the zero ionic layer of the assembled complex. In line with the importance of these positions, mutation of these residues in either Syntaxin-17 or VAMP7 rendered the proteins unable to bind each other, even though their subcellular localization remained unaffected. Interestingly, the simultaneous point mutation of both Syntaxin-17 (Q to R) and VAMP7 (R to Q) restored SNARE complex formation and function, indicating that these amino acids are interchangeable, and only the ratio of Q:R in the zero ionic layer was found to be important.

Further, McLelland et al. (2016) investigated the interaction of the Syntaxin-17–containing SNARE complex with the multisubunit tethering complex homotypic fusion and vacuole protein sorting (HOPS), which mediates the lysosomal targeting of autophagosomes. McLelland et al. (2016) found that HOPS is also required for the fusion of stress-induced MDVs with late endosomes and lysosomes, showing that its role is conserved for both types of double-membrane vesicles (Jiang et al., 2014; Takáts et al., 2014; McLelland et al., 2016). Unexpectedly, the researchers found that Syntaxin-17, SNAP29, and VAMP7 are largely dispensable for the canonical mitophagy pathway, unlike VAMP8 and HOPS. These differences may be explained by the unique characteristics of the larger-than-normal autophagosomes, which have been observed to form during mitophagy and which may contain specific endosomal components such as endocytic SNARE proteins (Politi et al., 2014; Yamano et al., 2014). This issue warrants further studies.

Syntaxin-17 is an ancient mitochondrial SNARE enriched at mitochondria–ER contact sites, an important structure that regulates mitochondrial metabolism through the transfer of lipids and other signals between the two organelles (Hamasaki et al., 2013; Arasaki et al., 2015). McLelland et al. (2016) now provide evidence that Syntaxin-17 is involved in another pathway that integrates mitochondria into the endomembrane system; stress-induced MDVs likely ensure a selective autophagic degradation pathway for damaged, oxidized mitochondrial components. The existence of such a vesicular transport route means that mitophagy may only be necessary for the elimination of extensively damaged mitochondria. How these observations relate to the development of PD remains to be established, but the simplified model of impaired canonical mitophagy being the only cause of pathology in patients harboring *PARK2* or *PINK1* mutations is challenged by these findings.

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