Regulation of Parkin-dependent mitophagy by Bcl-2-associated athanogene (BAG) family members

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Mitochondria are essential organelles that play a central role in cellular metabolism and physiology. Their broad range of functions include supplying energy, regulating signaling pathways, and maintaining control of cell proliferation and apoptosis. As defective mitochondria can perturb cellular homeostasis, quality control mechanisms have evolved to preserve mitochondrial fidelity in response to stress and aging (Palikaras et al., 2018). Persistent defects, however, trigger elimination of the entire organelle by an evolutionarily conserved set of cellular processes that specifically remove dysfunctional or surplus mitochondria. This selective degradation of the mitochondria through autophagy, termed mitophagy, is important in fine-tuning mitochondrial number, integrity and ultimately function. Mitophagy impairments can cause progressive accumulation of defective mitochondria, particularly in terminally differentiated post-mitotic cells, like neurons, which remain alive and functional for decades. The ensuing rise in oxidative stress and aggregation of proteins is likely to contribute to tissue damage linked to a broad spectrum of pathological conditions, such as neurodegenerative diseases, myopathies, inflammation, metabolic disorders and cancer (Palikaras et al., 2018). Despite major milestones in the mitophagy field within the last decade, important questions regarding the *in vivo* role of specific components, their interplay in different mitophagy pathways and their spatiotemporal regulation in distinct physiological and pathological contexts remain unanswered (Pickles et al., 2018). This short perspective will focus on recent advances towards elucidating the molecular mechanisms that govern mitophagy and downstream cell fate decisions, with specific attention to the potential contribution of the co-chaperone Bcl-2-associated athanogene (BAG) protein family focusing on BAG5 in Parkinson's disease (PD), ischemia and aging-associated inflammation.

Multiple aspects of mitochondrial physiology, including fission/fusion dynamics, biogenesis, transport and recruitment of autophagic machinery, converge on the canonical phosphatase and tensin homologue-induced putative kinase 1 (PINK1)–Parkin pathway (Palikaras et al., 2018). In healthy mitochondria, PINK1 is transported into the inner mitochondrial membrane, where it is processed and cleaved by several proteases before it is exported into the cytosol and degraded by the ubiquitin-proteasome system (Pickles et al., 2018). Following mitochondrial membrane potential dissipation, PINK1 is stabilized on the surface of damaged mitochondria where it phosphorylates both ubiquitin and the cytosolic E3-ubiquitin ligase Parkin at their respective Ser65 residues. PINK1-dependent phosphorylation alters Parkin conformation, promoting its translocation to the outer mitochondrial membrane and triggering its E3 ligase activity. Parkin in turn ubiquitinates several outer mitochondrial membrane components, generating poly-ubiquitin chains, which are substrates for PINK1, thereby amplifying mitophagy signals. This feedforward process results in the recruitment of autophagy receptors that bind ubiquitinated cargo and connect them to the autophagosome for engulfment of damaged mitochondria. PINK1 and Parkin also regulate other mitochondrial quality control mechanisms, for instance by initiating the piece-meal degradation of mitochondrial components through the generation of mitochondrial-derived vesicles, as well as coordinating the selective removal of abnormal protein aggregates from within the mitochondria (Pickles et al., 2018).

How these quality control pathways sense different levels of mitochondrial damage and mediate cell fate decisions accordingly are incompletely understood. Since loss-of-function mutations in PINK1 and Parkin are causally linked to PD, both enzymes are generally assumed to have cytoprotective activity through the sequestering of damaged mitochondria to the lysosomes. However, recent work indicates that Parkin can also sensitize cells toward apoptosis by enhancing the proteasomal degradation of the pro-survival Bcl-2 family protein, Mcl-1, in response to mitochondrial depolarization (Carroll et al., 2014). This sensitization is not observed using other pro-apoptotic stimuli that fail to activate Parkin, or in cells expressing catalytically dead Parkin. Taken together, there is an emerging hypothesis that Parkin functions as a damage-gated molecular switch that governs context-dependent cell fate decisions in response to variable stress stimuli. During physiological or mildly stressful conditions, Parkin remains in its cytoprotective state, but switches to a cytotoxic state when mitochondria are severely damaged. The physiological intracellular signaling pathways and factors which govern these two distinct outcomes (mitophagy or apoptosis) remain elusive.

The BAG family is a multifunctional group of co-chaperones that interact with diverse binding partners known to act in both cell survival and cell death pathways. Each of the six BAG family members (BAG1 to BAG6) share a common evolutionarily conserved sequence, the 'BAG-domain', through which they each interact with the molecular chaperone Hsp70 (Kalia et al., 2010). BAG5 represents an exception in the BAG family since it contains multiple BAG domains covering the whole protein. Several lines of evidence suggest that members of the BAG family play a role in regulating the PINK1-Parkin signalling pathway. For instance, BAG2 binds to and stabilizes PINK1 by blocking its ubiquitination and enhances PINK1-dependent, Parkin-mediated mitophagy (Qu et al., 2015). BAG6 promotes Parkin-dependent relocalization of mitochondrial clusters to the perinuclear region after mitochondrial aggregation (Hayashishita et al., 2019). BAG3 regulates Parkin levels and is recruited to depolarized mitochondria along with Parkin to facilitate mitophagy (Tahrir et al., 2017). BAG4 directly interacts with Parkin, impeding its translocation to damaged mitochondria (Hasson et al., 2013). We were the first to show that a BAG protein, specifically BAG5, forms a complex with Parkin and enhances its sequestration within protein aggregates (Kalia et al., 2004). Functionally, BAG5 is a negative regulator of Parkin's E3 ubiquitin ligase activity and mitigates Parkin-dependent preservation of proteasome function and inhibition of cell death (Kalia et al., 2004). Similar to BAG2, BAG5 directly interacts with and protects PINK1 from degradation (Wang et al., 2014), but evidence that it regulates PINK1-Parkin mitophagy was lacking.

We initially identified a potential role for BAG5 in PD pathogenesis by demonstrating that it is present within Lewy bodies and enhances dopaminergic neuronal death *in vivo* (Kalia et al., 2004). BAG5 also engages with several proteins that are commonly mutated in genetically inherited forms of PD including α-synuclein (Kalia et al., 2011), LRRK2 (Beilina et al., 2014), as well as PINK1 and Parkin as outlined above. Importantly, BAG5's activity interferes with the degradation of proteins by the ubiquitin-proteasome (Kalia et al., 2011) and the autophagy lysosomal pathways (Beilina et al., 2014). In our recently published report, we probed the PINK1-Parkin pathway as a potential point of convergence between BAG5's upstream, PD-relevant interactions and its downstream promotion of cell death (De Snoo et al., 2019). We found that BAG5 delays the translocation of Parkin from the cytosol to mitochondria following treatment with the mitophagy-inducing protonophore carbonyl cyanide *m*-chlorophenyl hydrazone. Consistent with its suppression of Parkin recruitment to damaged mitochondria, BAG5 also impairs PINK1- and Parkin-dependent mitophagy and movement of damaged mitochondria into the lysosomes following protonophore carbonyl cyanide *m*-chlorophenyl hydrazone treatment. These findings demonstrate a novel functional relationship between BAG5 and Parkin in response to mitochondrial membrane potential dissipation. To dissect what role this interaction plays in cell fate decisions, we next examined apoptosis and found that BAG5 enhances Parkin-mediated Mcl-1 degradation and cell death following severe mitochondrial insult. Together, these results reveal a new function for BAG5 as a sensor that may regulate the bi-modal activity of Parkin, promoting cell death by suppressing Parkin-dependent mitophagy and enhancing Parkin-mediated Mcl-1 degradation. The role of BAG5 in regulating *in vivo* PINK1- Parkin mitophagy in neurons and in the context of PD remains an area of active investigation.

Beyond neurodegeneration, two recent papers suggest that BAG5 may play a central role in Parkin recruitment and mitophagy in different types of cell stress. Tan et al. (2019) found that BAG5 attenuates Parkin translocation by localizing to mitochondria and forming a molecular complex with Hexokinase-II (HK-II) in cardiomyocytes under steady-state conditions. Dissociation of mitochondrial HK-II (mitoHK-II) during ischemia induces Parkin-mediated mitophagy independent of the canonical mitochondrial membrane potential depolarization/PINK1 pathway. Overexpression of BAG5 attenuates the effect of mitoHK-II dissociation on mitophagy suggesting that the BAG5-HKII complex acts as a sensor to dampen mitophagy under basal conditions but confers cardioprotection in response to ischemic stress. These findings suggest BAG5 functions in parallel with BAG3, which is critical for the maintenance of mitochondrial homeostasis under stress conditions in cardiomyocytes (Tahrir et al., 2017). In another report, BAG5 was found to modulate mitophagy by regulating the ubiquitination status of PINK1 in response to miR-155, a microRNA that is overexpressed in inflammatory and aged bone marrow tissue (Tsujimoto et al., 2020). In this pathway, BAG5

downregulation by miR-155 induces excessive degradation of PINK1 and impairs mitophagy in human bone marrow-derived mesenchymal stem cells. Although the effects of BAG5 suppression on Parkin were not studied, these findings suggest a novel pathway connecting BAG5 to upregulated miR-155 in aging- associated inflammation and downregulated PINK1 in pathological conditions with mitochondrial dysfunction.

While progress has been made in understanding how BAG family members, including BAG5, modulate mitophagy and downstream cell fate decisions, much remains unknown at this point (**Figure 1**). Most of the data discussed above was generated under experimental conditions using immortalized cell lines, combined with the overexpression of PINK1 or Parkin, which is likely to obscure turnover of mitochondria in physiological contexts. For instance, more studies will be required to elucidate what role BAG5 plays, if any, in basal mitophagy, particularly in tissues with high metabolic demand, including the nervous system, heart, liver, kidney and skeletal muscle. Another unanswered question is whether BAG5 has a potential role in regulating programmed mitophagy in different cell types during development. Since BAG5 is a negative regulator of E3 ubiquitin ligase activity by both Parkin and carboxyl terminus Hsp70-interacting protein (Kalia et al., 2011), it is intriguing to speculate that it may impair other E3 ubiquitin ligases which function similarly in mitophagy by localizing to the outer mitochondrial membrane and generating poly-ubiquitin chains to trigger autophagy adaptor recruitment. Lastly, future studies with primary mammalian cells or *in vivo* models are required to define interactors of BAG5 and the mechanisms by which these complexes may modulate PINK1 and Parkin mitophagy, particularly in the context of pathological and aging-related disorders like neurodegenerative diseases.

Taken together, emerging evidence implicates co-chaperones like the BAG proteins in the spatiotemporal regulation of PINK1-Parkin mitophagy. These findings suggest that chaperones may be an underappreciated link tying mitochondrial dysfunction and impairments in proteostasis. If so, it remains to be seen whether modulating the activity of molecular chaperones may one day offer a novel approach for disease-modifying intervention in pathological conditions characterized by oxidative stress and protein aggregation.

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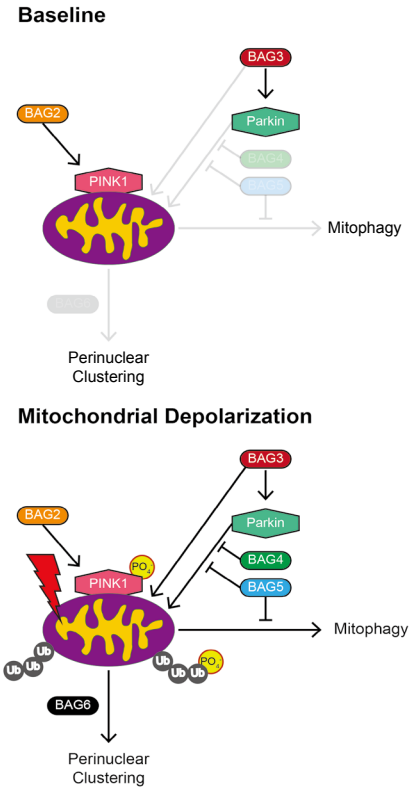

Figure 1 | BAG co-chaperones interact at various levels in the PINK1-Parkin mitophagy pathway. BAG2 binds to and stabilizes PINK1 by blocking its ubiquitination and enhances PINK1-dependent, Parkin-mediated mitophagy (Qu et al., 2015). BAG3 regulates Parkin levels and is recruited to depolarized mitochondria along with Parkin to facilitate mitophagy (Tahrir et al., 2017). BAG4 directly interacts with Parkin, impeding its translocation to damaged mitochondria (Hasson et al., 2013). BAG5 impairs Parkin E3 ligase activity (Kalia et al., 2004). BAG5 also delays Parkin recruitment and mitophagy following mitochondrial depolarization while enhancing Parkin-mediated proteasomal degradation of Mcl-1 (De Snoo et al., 2019). BAG6 promotes Parkin-dependent relocalization of mitochondrial clusters to the perinuclear region after mitochondrial aggregation (Hayashishita et al., 2019). BAG: Bcl-2-associated athanogene; PINK1: canonical phosphatase and tensin homologue-induced putative kinase 1.

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