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Beyond Mitophagy: The Diversity and Complexity of Parkin Function

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Abstract

Parkin is an E3 ubiquitin ligase that mediates mitochondrial autophagy, or mitophagy, in multiple cell types. While discovered in the context of Parkinson's disease, Parkin is also an important regulator of mitophagy in the heart. In addition, while most current work is focused on the role of Parkin in mitophagy, accumulating evidence suggests that Parkin also impacts cellular physiology and function through additional processes. This Viewpoint discusses current controversies regarding the functional role of Parkin-mediated mitophagy and emerging evidence that Parkin regulates several additional pathways.

Keywords

Parkin; mitochondria; autophagy; heart

Ubiquitylation is a post-translational modification that involves covalently attaching ubiquitin to target proteins[1]. The process of ubquitylation requires a series of events including ubiquitin activation, conjugation, and ligation, catalyzed by E1, E2, and E3 enzymes respectively. Parkin is one of more than 500 E3 ubiquitin ligases encoded by the human genome that selectively bestows ubiquitin molecules onto particular proteins. One of Parkin's most widely appreciated roles is the ubiquitylation of proteins on the membrane of damaged mitochondria to designate them for clearance via mitophagy. Mitophagy is a selective form of macroautophagy that traffics damaged mitochondria to lysosomes for degradation, and serves as a critical mechanism in maintaining the health of the mitochondrial collective in a cell.

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CURRENT MODEL OF PARKIN IN PARKINSON'S DISEASE

Although it is clear that mutations that impair Parkin's E3 ligase function contribute to the attrition of dopaminergic neurons through cell death in Parkinson's disease[2], the underlying mechanisms are still not fully understood and there is no direct evidence that a defect in mitophagy is the underlying cause of Parkinson's disease. The assumption that faulty mitophagy is responsible for Parkinson's disease stems from observations that defects in mitochondrial morphology and function are key features of Parkinson's disease[2] and that loss of Parkin in *Drosophila* leads to mitochondrial dysfunction and muscle degeneration[3]. When combined with the seminal work from the Youle lab demonstrating that Parkin can mediate mitophagy[4], it is easy to see how this model—where defects in mitophagy due to loss of Parkin function cause accumulation of dysfunctional mitochondria and Parkinson's disease—was generated. However, Parkin deficiency in mice does not recapitulate the neuromuscular symptoms observed in the human disease[5], indicating that Parkin deficiency alone is insufficient for the development of the disease, at least in mice. Similarly, the presence of Parkin is not critical to maintaining a healthy population of mitochondria in the mouse heart, another mitochondria-rich tissue. Both global Parkin deficiency and cardiac-specific deletion of Parkin in the adult heart have no detectable effect on mitochondrial health and cardiac function in mice[6, 7]. Thus, either alternative mitochondrial quality control mechanisms exist to compensate for Parkin deficiency, or maintaining mitochondrial homeostasis under baseline conditions is not Parkin's primary function, and therefore not the cause of pathology in Parkinson's disease.

CHALLENGES WITH STUDYING PARKIN

The fact that numerous cell lines, such as mouse embryonic fibroblasts and HeLa cells, have undetectable levels of Parkin under normal conditions and still maintain networks of healthy mitochondria indicates that Parkin is not crucial for cellular homeostasis and survival. Additionally, cardiac Parkin protein levels are much lower than expected considering the high density of mitochondria in the adult heart[6], further supporting the notion that Parkin is not necessary under baseline conditions. However, Parkin is consistently upregulated in response to various conditions that affect mitochondrial health both in the presence and absence of cardiac dysfunction[6–8]. Dorn's group recently uncovered that Parkin is required for removal of cardiac mitochondria during the switch from glucose to fatty acid metabolism in early postnatal life[9], while our lab identified the necessity of Parkin for efficient removal of dysfunctional mitochondrial after myocardial infarction[7]. Thus, although not critical for baseline mitochondrial homeostasis, Parkin does appear to be necessary for adaption to stress or altered metabolic environment. Additional misconceptions in the field include grouping different types of mitophagy into the same category. Although the term mitophagy encompasses all autophagosome-mediated selective mitochondrial clearance, copious publications have shown over the past decade that different conditions (i.e. developmental, stress, maintenance) prompt distinct pathways to facilitate mitophagy.

Overall, these studies show that our current understanding of Parkin-mediated mitophagy needs to be expanded and reevaluated *in vivo* using relevant animal models. Moreover, while conditional Parkin knockout mice are useful models to uncover the function of Parkin in

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specific cells and tissues under various conditions, they have limited use in studying Parkin's role in the underlying pathogenesis of Parkinson's disease. Several of the disease-associated *Parkin* mutations still lead to the generation of a non-functional protein which could potentially alter cellular quality control mechanisms and other important processes in the cell. Therefore, we should focus on developing and studying knock-in mouse models with mutations in *PARK2* that more accurately mimic Parkinson's disease and then evaluate the impact on neuron and myocyte and respective tissue functions.

PARKIN BEYOND MITOPHAGY

Accumulating evidence suggests that Parkin also impacts cellular physiology and function through additional, non-mitophagy-related processes. Not surprisingly, these novel functions of Parkin also require the E3 ubiquitin ligase activity. Ubiquitylation, as its name suggests, has a hand in regulating countless cellular processes[1]. As an E3 ubiquitin ligase, Parkin function has the potential to impact numerous pathways/processes beyond mitochondrial quality control (Figure 1B). Among others, cells utilize ubiquitylation to degrade proteins through autophagy or the proteasome, change a protein's subcellular location, or alter protein-protein interactions. These diverse effects can have an immense impact on cellular functions including transcription, translation, DNA repair, organelle trafficking, cell survival/death, cell proliferation, and inflammation, to name just a few. While the precise mechanisms by which specific ubiquitylation events on a given protein bring about these effects are incompletely understood, important factors include which residues on the target protein undergo ubiquitylation, whether mono- or polyubiquitylation is involved, and the nature of the ubiquitin-ubiquitin linkages in the case of polyubiquitylation. For instance, polyubiquitylation often leads to protein degradation, while some instances of monoubiquitylation stabilize proteins or change their subcellular locations[1]. The functional diversity of ubiquitylation helps us to comprehend the extensive range of possible Parkin's substrates. Indeed, an unbiased proteomic analysis identified a broad swath of Parkin substrates - even when performed within the relatively circumscribed context of experimentally induced mitochondrial damage[10]. Members of the Parkin ubiquitylome identified in this study included mitochondrial proteins (as expected), as well as numerous cytosolic proteins. The functions of the proteins identified mapped to several distinct pathways including autophagy, proteasome function, mitochondrial function, mitochondrial dynamics, cell death, and metabolism.

It is also important to consider that Parkin affects mitochondrial biology and cellular metabolism independent of its role in mitophagy. Mitochondrial biogenesis must be coupled with mitochondrial degradation to ensure sufficient cellular energy production. Thus, it is not surprising that, in addition to mediating mitophagy, Parkin also activates mitochondrial biogenesis. Parkin mediates lysine 48-linked polyubiquitylation to target PARIS for degradation in the proteasome[11]. Because PARIS transcriptionally represses mitochondrial biogenesis regulator peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), Parkin-mediated degradation of PARIS increases mitochondrial biogenesis. Additionally, research by Kim *et al.* indicates that Parkin regulates lipid metabolism: Parkin-deficient mice on high-fat diet (HFD) withstand weight gain, steatohepatitis, and insulin

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resistance[12]. These effects are attributable, in part, to Parkin-mediated monoubiquitylation and stabilization of the plasma membrane CD36 lipid transporter.

Emerging evidence suggests that Parkin may also function as a tumor suppressor. Parkin deficiency has been observed in various human cancers and *Parkin*$^{-/-}$ mice have enhanced hepatocyte proliferation and develop macroscopic tumors with characteristics of hepatocellular carcinoma[13]. Of note, Parkin can regulate mitosis by mediating proteasomal degradation of cell cycle regulators such as Cyclin E, suggesting that Parkin deficiency may result in increased levels of these regulators and cell proliferation[13]. However, additional studies are clearly needed to dissect the function of Parkin in cancers.

Parkin has also been implicated in the regulation of cell death. Parkin inhibits the mitochondrial apoptosis pathway by ubquitylating Bax, prohibiting its translocation from the cytosol to mitochondria in response to apoptotic stimuli[10, 14]. However, the story is potentially much more complex. Parkin may conversely sensitize cells to apoptosis specifically induced by mitochondrial membrane depolarization, but not by classic mitochondrial and death receptor apoptosis activators, via ubiquitylation and degradation of the anti-apoptotic Bcl-2 protein, Mcl-1[15]. At a minimum, these findings suggest that the effects of Parkin on cell survival/death are context dependent and that further work is needed to understand the relevant molecular determinants. As depolarization of the inner mitochondrial membrane also stimulates PINK1-Parkin-dependent mitophagy, these observations raise questions as to how mitophagy and cell death events functionally integrate at mitochondria.

IMPLICATIONS ON FUTURE PARKIN RESEARCH

Given the major effort that has been devoted to the study of Parkin, why have the many non-mitophagy functions of Parkin been overlooked? The most likely reason is that experimental approaches used to study Parkin function – specifically the induction of mitochondrial dysfunction using “poisons” such as CCCP, Antimycin A, and oligomycin – have biased discovery toward mitophagy. Use of unbiased approaches similar to the proteomic analysis of the Parkin ubiquitylome will promote a broader understanding of the pathways impacted by Parkin. In addition, experimental approaches to facilitate a comprehensive investigation of Parkin biology necessitate refocusing studies on physiological paradigms that mimic *in vivo* Parkin activation. This includes the use of physiological stressors – as opposed to the aforementioned drugs – and cell systems that endogenously express Parkin to avoid overexpression artifacts in cells that appear to thrive, even in the absence of this protein.

In conclusion, the diversity and complexity of Parkin’s functions undoubtedly extend beyond our current models. While mitochondria may function as a convergence point for many pathways/processes regulated by Parkin (mitophagy, metabolism, dynamics, transport, biogenesis, apoptosis) (Figure 1B), we must be persistent in thinking more broadly. Only by achieving a deeper understanding of its pleiotropic effects will it be possible to intelligently exploit this protein to therapeutic advantage for neurological, cardiac, and other diseases.

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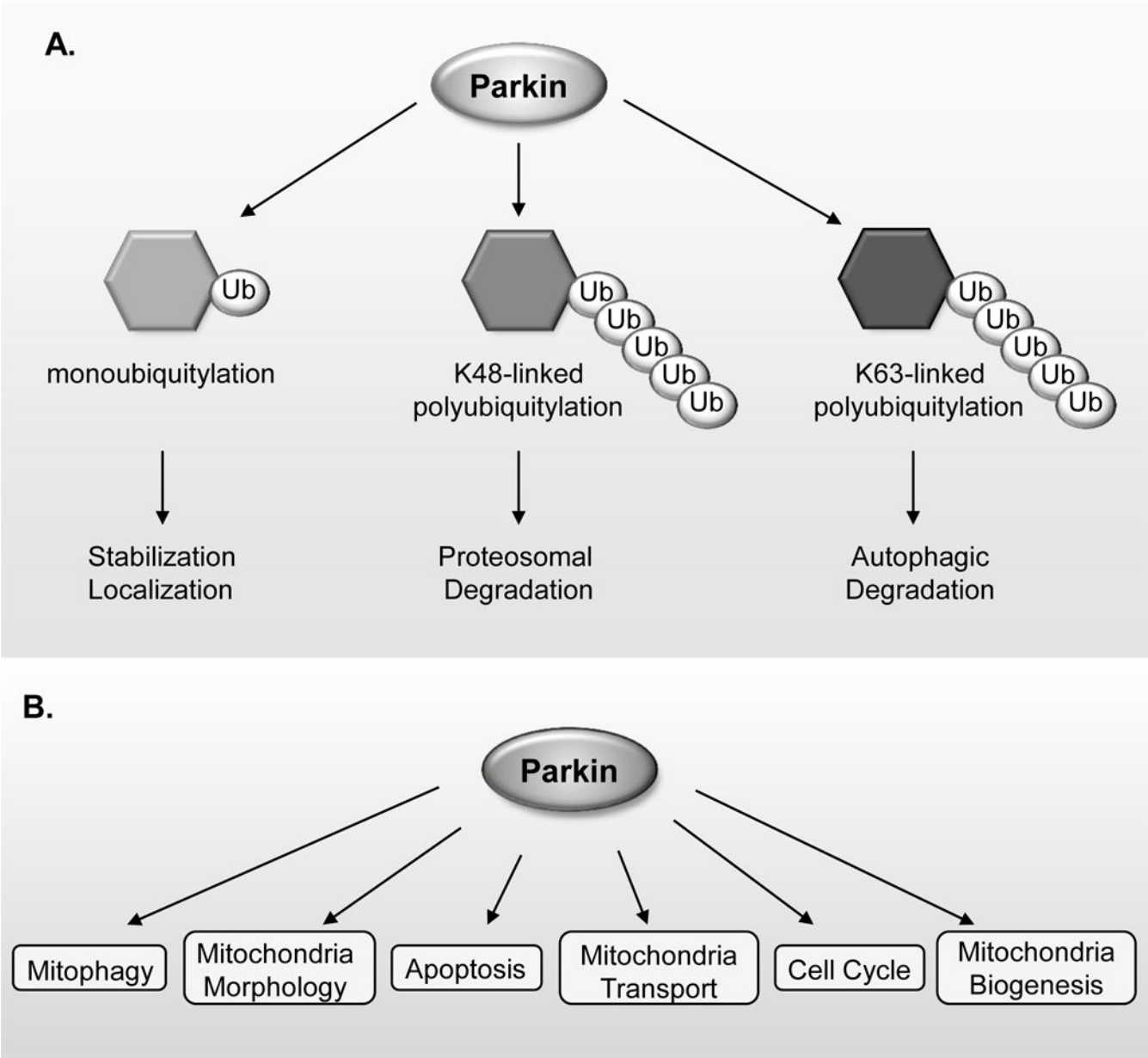

Figure 1.

Complexity of Parkin's cellular functions. **A.** Different ubiquitin linkages catalyzed by Parkin. **B.** Parkin regulates many diverse cellular processes in the cell.