Parkin mitochondria in the autophagosome

Heidi M. McBride

University of Ottawa Heart Institute, Ottawa, Ontario K1Y 4W7, Canada

Narendra et al. (see p. 795 of this issue) have made an exciting new discovery that links the fields of mitochondrial quality control and the genetics of Parkinson's disease (PD). Through an elegant series of high-resolution imaging experiments, they are the first to provide evidence that the PARK2 gene product Parkin is selectively recruited to damaged or uncoupled mitochondria. This recruitment leads to the clearance of the organelles through the autophagosome, demonstrating a primary function for Parkin in the regulation of mitochondrial turnover. This work significantly increases our understanding of PD and provides a new framework for the development of therapeutic interventions.

Mitochondrial dysfunction has long been associated with the onset of neurodegenerative states, including the selective loss of dopaminergic neurons in Parkinson's disease (PD; Schapira, 2008). However, it has been difficult to understand whether the degeneration of the mitochondria in these neurons is a cause or effect of the disease. One of the difficulties in the study of mitochondria in neurodegeneration has been our limited understanding of how damaged mitochondrial proteins and lipids are degraded in steady state. There are currently at least three distinct mechanisms known for mitochondrial protein turnover: the proteolysis of proteins within the matrix or intermembrane space (Arnold and Langer, 2002), autophagic degradation of entire organelles (Mijaljica et al., 2007), and proteasome-dependent outer mitochondrial membrane–associated degradation (OMMAD; Neutzner et al., 2007). With the exception of the mitochondrial proteases, which have been studied for some time, the molecular mechanisms and regulation of mitochondrial turnover via autophagy and the proteasome are less well characterized. There have been recent hints that mitochondrial protein turnover is selective; e.g., the ubiquitination and proteasome-dependent degradation of the anti-apoptotic Bcl2 family member Mcl-1 by the E3 ligase MULE occurs upon the induction of cell death (Warr et al., 2005; Zhong et al., 2005). Similarly, in yeast, the fusion GTPase Fzo1p was found to be selectively removed from the mitochondrial outer membrane through a proteasome-dependent mechanism (Neutzner et al., 2007). In addition, it was shown that whole mitochondria lacking electrochemical potential for extended periods will be selectively cleared through steady-state autophagy, or mitophagy (Twig et al., 2008). These data underscore the importance of a tightly regulated process to control the selective destruction of mitochondrial proteins caused by accumulated damage, but also during specific cellular processes. Furthermore, it is increasingly becoming evident that the clearance of cellular debris through autophagy is critical for human health, and defects in this process are becoming more tightly linked with neurodegenerative states (Mizushima et al., 2008).

Narendra et al. (see p. 795 of this issue) have now found a new function for the PARK2 gene product, Parkin, in the regulation of selective mitophagy. Parkin is a primarily cytosolic ubiquitin E3 ligase that contains a ubiquitin like domain (UBL), two RING finger domains, and a conserved region between the RING domains (Schapira, 2008). The PARK2 gene has been shown to be mutated in nearly 50% of autosomal recessive and 10–15% of sporadic early onset PD. There have been conflicting reports suggesting functions for Parkin in the cytosol, in the ER, on mitochondrial targets, and at the plasma membrane. Evidence for a primary function at the mitochondria was strengthened by the identification of a genetic link with the mitochondrial membrane–anchored kinase, and Parkinson's related protein, PTEN-induced kinase 1 (Pink1). In *Drosophila melanogaster*, the loss of Pink1 was rescued upon overexpression of Parkin, whereas loss of Parkin was not rescued by the overexpression of Pink1 (Clark et al., 2006; Park et al., 2006; Yang et al., 2006; Exner et al., 2007). In addition, the mitochondria in *D. melanogaster* cells lacking Parkin or Pink1 are highly fused, and the defects in the fly are rescued upon overexpression of the fission GTPase Drp1, or the loss-of-fusion factors Mfn/Marf or Opa1 (Deng et al., 2008; Poole et al., 2008; Yang et al., 2008). These data have led to an emerging model where mitochondrial dysfunction may play a central role in the onset of PD, and suggest possible links between the fission/fusion machinery and PD genes. Narendra et al. (2008) have now determined that Parkin is strikingly and specifically recruited to dysfunctional mitochondria. Like other studies, these authors found that at steady state, Parkin is primarily cytosolic; however, careful confocal imaging allowed them to visualize a handful of Parkin foci colocalizing with a few, fragmented mitochondria. Interestingly, treatment of YFP-Parkin–overexpressing cells with the mitochondrial uncoupler carbonyl cyanide

Correspondence to Heidi M. McBride: hmcbride@ottawaheart.ca

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 183 No. 5 757–759
www.jcb.org/cgi/doi/10.1083/jcb.200810184

m-chlorophenylhydrazone (CCCP) for 24 or 48 h led first to extensive Parkin recruitment, followed by the complete loss of mitochondria from the cell, an event that was not observed in the cells lacking Parkin. This loss of mitochondria was dependent on the presence of the autophagy-related gene Atg5, which demonstrates that the degradation of the organelles occurred in autophagosomes. Mitochondrial fragmentation has already been shown to occur upon CCCP treatment, and inhibition of these fission events using the dominant interfering mutant of DRP1 did not affect Parkin recruitment. This finding indicates that Parkin recruitment is independent of mitochondrial fragmentation. It is tempting to consider that Parkin may play an important role in selecting damaged mitochondrial proteins that would be pinched away from the healthy organelle by DRP1 (Fig. 1). The authors suggest that the increase in fusion observed in the Parkin null flies may help to buffer the accumulating damage. In this case, the residual fission may allow the transport of some mitochondrial fragments to the autophagosome. This could partially explain why the further loss of DRP1 and mitochondrial fission is lethal. Similarly, the rescue of the Parkin-null flies by the inhibition of mitochondrial fusion may be caused by the preservation of individually damaged organelles in a form that is amenable for mitophagic degradation. That the loss of Parkin can be rescued upon induction of fragmentation suggests that the protein is not essential for the targeting of the damaged fragments to the autophagosome; rather, it may function upstream in the selection of proteins for DRP1-mediated fission.

It is almost certain that the overexpression of Parkin and use of CCCP exaggerate the phenotype; however, the results clearly implicate the mitochondrial recruitment of Parkin in the targeted degradation of damaged organelles. To ensure that the phenomenon of Parkin recruitment was not unique to global CCCP treatment, the authors showed that overexpressed Parkin was also recruited to mitochondria upon an increase in complex 1–dependent reactive oxygen species (ROS) using the herbicide paraquat, a toxin similar to MPTP used to induce a PD phenotype in some animal and cultured models (Terzioglu and Galter, 2008). Similarly, genetic backgrounds that induce partial mitochondrial dysfunction also led to Parkin recruitment, further indicating that this is a general response to mitochondrial stress. Because the authors did not determine whether Parkin's ubiquitination activity was required, or what the mitochondrial targets might be, the potential role of ubiquitination in targeting the damaged mitochondrial fragments to the LC3-positive autophagosomes remains unknown. In addition, this study did not determine the relative contribution of endogenous levels of Parkin to mitochondrial turnover in neurons, which will be clinically important. Nevertheless, these results are the first to demonstrate a specific role for the ubiquitin E3 ligase on mitochondrial quality control, and provide further evidence that the etiology of PD may indeed be caused by breeches in mitochondrial integrity.

The identification of Parkin as the first protein to regulate the selective removal of mitochondria provides an important molecular tool to dissect this pathway and search for novel therapeutics. Parkin overexpression has already been shown to provide some protection against toxin-induced animal models of PD (Ulusoy and Kirik, 2008). If the expressed Parkin could be activated for efficient mitochondrial recruitment, then the efficacy of these studies may have been higher. What factors may help to activate Parkin? The results from the Narendra et al. (2008) study clearly indicate that Parkin is selectively recruited to damaged mitochondria, but it is not obvious how this ligase can distinguish healthy from damaged mitochondria. The signal to recruit may be

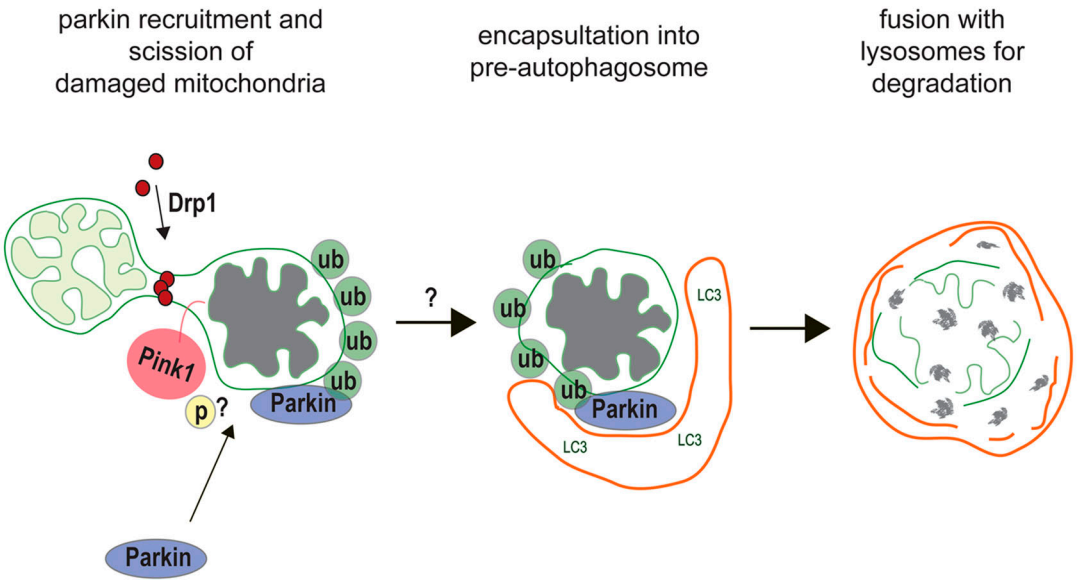

Figure 1. **Parkin recruitment leads to selective mitophagy.** Cytosolic parkin is selectively recruited to uncoupled or dysfunctional mitochondria. The outer membrane protein kinase Pink1 functions upstream of Parkin and may play a role in sensing mitochondrial damage and Parkin recruitment. The ubiquitination activity of Parkin was not directly examined in this study, but it will be important to determine whether it mono- or poly-ubiquinates its substrates. The recognition of Parkin-associated mitochondrial fragments by LC3-positive autophagosome requires Atg5. Fusion with lysosomes leads to the total degradation and clearance of the damaged mitochondria. p, phosphorylation; ub, ubiquitin.

the initiation of protein aggregation within the outer membrane, possible second messengers like ROS or NO, or through the activation of an unidentified mitochondrial receptor protein.

The most obvious candidate for a Parkin recruitment factor would be the mitochondrial outer membrane kinase Pink1 (Schapira, 2008). Interestingly, two of the proposed Pink1 substrates are also involved in quality control: the chaperone Trap1/Hsp75 (Pridgeon et al., 2007) and the serine protease HtrA2/Omi (Plun-Favreau et al., 2007). Upon cellular stress induced through the p38γ–Map kinase pathway, Pink1 was shown to be required for the phosphorylation and activation of HtrA2/Omi (Plun-Favreau et al., 2007). This activation presumably led to the degradation of unfolded or oxidized intermembrane space proteins, although the substrates were not defined. Similarly, peroxide-induced stress led to a Pink1-dependent phosphorylation of Trap1/Hsp75, whose chaperone activity appears to assist in the refolding of damaged proteins and reduction of mitochondrial ROS (Pridgeon et al., 2007). Pink1 was initially thought to reside within the intermembrane space, with a functionally relevant cytosolic pool (Silvestri et al., 2005; Haque et al., 2008). However, recent studies have provided compelling evidence for a single, outer membrane location for the enzyme with the kinase domain facing the cytosol (Zhou et al., 2008). Because HtrA2/Omi and Trap1/Hsp75 reside within the mitochondria, the likelihood of direct protein interactions between them seems less likely. Whether or not these interactions are direct, it indicates that Pink1 functions as a sensor of mitochondrial or cellular stress. A third effect of activated Pink1 may be in the activation of Parkin, either directly or indirectly, triggering the initiation of mitophagy.

Other PD-related genes may play roles in mitochondrial quality control, although the mechanisms are admittedly less obvious. For example, DJ-1 is encoded by the PARK7 gene and has been shown to function as a redox sensor granting protection to cells against ROS-induced toxicity (Schapira, 2008). This protection is evident in multiple models of cell death, including stroke (Aleyasin et al., 2007). Interestingly, DJ-1 was recently reported to translocate to the mitochondria within 3 h under conditions of oxidative stress (Junn et al., 2008). By 24 h, DJ-1 was relocalized into the nucleus, where it is proposed to bind multiple RNAs and regulate p53's transcriptional activity. Whether or not DJ-1 recruitment is regulated in the same manner as Parkin remains to be determined.

By providing a fundamentally new function for Parkin in mitochondrial quality control, this study opens up many new avenues of investigation. Whether and how the PD mutations in Parkin interfere with the maintenance of functional mitochondria will be the subject of intense future investigation.

Submitted: 30 October 2008
Accepted: 4 November 2008

References

Aleyasin, H., M.W. Rousseaux, M. Phillips, R.H. Kim, R.J. Bland, S. Callaghan, R.S. Slack, M.J. During, T.W. Mak, and D.S. Park. 2007. The Parkinson's disease gene DJ-1 is also a key regulator of stroke-induced damage. *Proc. Natl. Acad. Sci. USA.* 104:18748–18753.

Arnold, I., and T. Langer. 2002. Membrane protein degradation by AAA proteases in mitochondria. *Biochim. Biophys. Acta.* 1592:89–96.

Clark, I.E., M.W. Dodson, C. Jiang, J.H. Cao, J.R. Huh, J.H. Seol, S.J. Yoo, B.A. Hay, and M. Guo. 2006. *Drosophila* pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature.* 441:1162–1166.

Deng, H., M.W. Dodson, H. Huang, and M. Guo. 2008. The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in *Drosophila*. *Proc. Natl. Acad. Sci. USA.* 105:14503–14508.

Exner, N., B. Treske, D. Paquet, K. Holmstrom, C. Schiesling, S. Gispert, I. Carballo-Carbajal, D. Berg, H.H. Hoepken, T. Gasser, et al. 2007. Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. *J. Neurosci.* 27:12413–12418.

Haque, M.E., K.J. Thomas, C. D'Souza, S. Callaghan, T. Kitada, R.S. Slack, P. Fraser, M.R. Cookson, A. Tandon, and D.S. Park. 2008. Cytoplasmic Pink1 activity protects neurons from dopaminergic neurotoxin MPTP. *Proc. Natl. Acad. Sci. USA.* 105:1716–1721.

Junn, E., W.H. Jang, X. Zhao, B.S. Jeong, and M.M. Mouradian. 2008. Mitochondrial localization of DJ-1 leads to enhanced neuroprotection. *J. Neurosci. Res.* In press.

Mijaljica, D., M. Prescott, and R.J. Devenish. 2007. Different fates of mitochondria: alternative ways for degradation? *Autophagy.* 3:4–9.

Mizushima, N., B. Levine, A.M. Cuervo, and D.J. Klionsky. 2008. Autophagy fights disease through cellular self-digestion. *Nature.* 451:1069–1075.

Narendra, D., A. Tanaka, D.-F. Suen, and R.J. Youle. 2008. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J. Cell Biol.* 183:795–803.

Neutzner, A., R.J. Youle, and M. Karbowski. 2007. Outer mitochondrial membrane protein degradation by the proteasome. *Novartis Found. Symp.* 287:4–14 (discussion 14–20).

Park, J., S.B. Lee, S. Lee, Y. Kim, S. Song, S. Kim, E. Bae, J. Kim, M. Shong, J.M. Kim, and J. Chung. 2006. Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by parkin. *Nature.* 441:1157–1161.

Plun-Favreau, H., K. Klupsch, N. Moisoi, S. Gandhi, S. Kjaer, D. Frith, K. Harvey, E. Deas, R.J. Harvey, N. McDonald, et al. 2007. The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. *Nat. Cell Biol.* 9:1243–1252.

Poole, A.C., R.E. Thomas, L.A. Andrews, H.M. McBride, A.J. Whitworth, and L.J. Pallanck. 2008. The PINK1/Parkin pathway regulates mitochondrial morphology. *Proc. Natl. Acad. Sci. USA.* 105:1638–1643.

Pridgeon, J.W., J.A. Olzmann, L.S. Chin, and L. Li. 2007. PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. *PLoS Biol.* 5:e172.

Schapira, A.H. 2008. Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *Lancet Neurol.* 7:97–109.

Silvestri, L., V. Caputo, E. Bellacchio, L. Atorino, B. Dallapiccola, E.M. Valente, and G. Casari. 2005. Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Hum. Mol. Genet.* 14:3477–3492.

Terzioglu, M., and D. Galter. 2008. Parkinson's disease: genetic versus toxin-induced rodent models. *FEBS J.* 275:1384–1391.

Twig, G., A. Elorza, A.J. Molina, H. Mohamed, J.D. Wikstrom, G. Walzer, L. Stiles, S.E. Haigh, S. Katz, G. Las, et al. 2008. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J.* 27:433–446.

Ulusoy, A., and D. Kirik. 2008. Can overexpression of parkin provide a novel strategy for neuroprotection in Parkinson's disease? *Exp. Neurol.* 212:258–260.

Warr, M.R., S. Acoca, Z. Liu, M. Germain, M. Watson, M. Blanchette, S.S. Wing, and G.C. Shore. 2005. BH3-ligand regulates access of MCL-1 to its E3 ligase. *FEBS Lett.* 579:5603–5608.

Yang, Y., S. Gehrke, Y. Imai, Z. Huang, Y. Ouyang, J.W. Wang, L. Yang, M.F. Beal, H. Vogel, and B. Lu. 2006. Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of *Drosophila* Pink1 is rescued by Parkin. *Proc. Natl. Acad. Sci. USA.* 103:10793–10798.

Yang, Y., Y. Ouyang, L. Yang, M.F. Beal, A. McQuibban, H. Vogel, and B. Lu. 2008. Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. *Proc. Natl. Acad. Sci. USA.* 105:7070–7075.

Zhong, Q., W. Gao, F. Du, and X. Wang. 2005. Mule/ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquitination of Mcl-1 and regulates apoptosis. *Cell.* 121:1085–1095.

Zhou, C., Y. Huang, Y. Shao, J. May, D. Prou, C. Perier, W. Dauer, E.A. Schon, and S. Przedborski. 2008. The kinase domain of mitochondrial PINK1 faces the cytoplasm. *Proc. Natl. Acad. Sci. USA.* 105:12022–12027.