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In a flurry of PINK, mitochondrial bioenergetics takes a leading role in Parkinson's disease

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For many years research in Parkinson's disease (PD) has linked mitochondrial dysfunction with the characteristic loss of dopaminergic neurons of the substantia nigra, accumulation of cytoplasmic inclusions termed Lewy bodies, and motor dysfunction (Henchcliffe & Beal, 2008). The most compelling connection is that Parkinsonism can be observed in both humans and animals following exposure to inhibitors of complex I of the electron transport chain (Betarbet et al, 2002).

An understanding of how mitochondrial dysfunction arises in the tissue of a person afflicted with the disease has been elusive. The discovery of seemingly unrelated mutant genes responsible for familial forms of PD, including α synuclein (PARK1/PARK4), LRRK2 (PARK8), parkin (PARK2), DJ-1 (PARK6), ATP13A2 (PARK9), initially seemed to confound rather than solve the mystery, until the discovery of PD-associated mutations in a *bona fide* mitochondrial protein, PINK1 (PTEN-induced kinase 1 or PARK6) (Valente et al, 2004). Since then, a flurry of studies have detailed how this serine threonine kinase affects mitochondrial function and dynamics, and have put forward different hypotheses to explain the role(s) of mutant PINK1 in Parkinson's disease.

In this issue of *EMBO Molecular Medicine*, Morais et al make a significant contribution to our understanding of the PINK1-mediated mitochondrial protection. Using Drosophila and mouse models, the researchers assert that an early effect of PINK1 deficiency is the disruption of Complex I function. This results in decreased mitochondrial membrane potential and compromised transmission at neuromuscular junctions in Drosophila, that can be rescued by supplementing ATP in the synaptic terminal. Thus, the work lends insight into functional consequences of the mitochondrial defects in neuromuscular junctions that could account for defects in normal motor control in the disease. The authors also found that complex I activity and synaptic function could be replenished by expression of human wild type but not by PINK1 PD clinical mutants. Their findings highlight the pivotal role of PINK1 in maintaining respiration and mitochondrial ATP production and its relevance in PD.

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The consequences reported so far regarding the genetic manipulation of PINK1 on mitochondria are multifaceted, and discrepancies exist between model systems and between laboratories even when measuring similar endpoints (Table I). There is, however, a general agreement of a drop in mitochondrial membrane potential as a result of PINK1 deficiency, and evidence that mitochondria are hydrolyzing glycolytically produced ATP to generate the membrane potential (Morais et al, this issue; Gandhi et al, 2009). Morais et al propose that defects in the mitochondrial respiratory chain lie upstream of these alterations. Deficiencies in several respiratory complexes have been reported in PINK1 deficient cells (Gautier et al, 2008; Gegg et al, 2009; Hoepken et al, 2007; Piccoli et al, 2008); however, this study pinpoints the respiratory defect specifically at the level of complex I activity. This is an important distinction, as a generalized loss of respiratory competence may signal a different underlying mechanism than specific complex I inhibition. Another recent publication ascribes similar importance of PINK1 in the maintenance of mitochondrial bioenergetic function, but with a slightly different twist (Gandhi et al, 2009). Instead of complex I as the primary dysfunction resulting from PINK1 deficiency, these authors suggest a key effect on the Na^+/Ca^{2+} exchanger at the inner mitochondrial membrane that is responsible for Ca^{2+} efflux from mitochondria in electrically excitable cells. Uptake and efflux of Ca^{2+} from mitochondria are important

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Table I. Recent data on the effects of PINK1 knockout or knockdown on mitochondria and related functions

Publication	Model	Decreased mitochondrial respiration or complex activity	Rate of ATP synthesis or total ATP	Mitochondrial membrane potential	Altered mitochondrial Ca^{2+} handling	Increased ROS Generation	Altered mitochondrial morphology by EM	Proteasomal activity, autophagy/mitophagy	Association with machinery for mitochondrial movement
Morais et al, 2009	*Drosophila* NMJ, mouse fibroblasts & mitochondria from fibroblasts, liver, brain	Complex I-dependent respiration	Decreased	CsA-sensitive decrease (with oligomycin)			No changes observed		
Gandhi et al, 2009	Immortalized human neurons, mouse primary neurons	Intact cell respiration, reversed with pyruvate, Me-succinate		CsA-sensitive decrease (with oligomycin)	Inhibition of Na^{+}/Ca^{2+} exchanger	Yes			
Gegg et al, 2009	SH-SY5Y (human)	Lower trend (complex I, II, IV enzyme activity)	Decreased rate (complex I, II, IV), and decreased total ATP	Decreased		Decreased GSH, Increased Carbonylation			
Liu et al, 2009	HEK293, HeLa, SH-SY5Y (human)	Intact cell respiration on pyruvate	Decreased Rate on Complex I substrate					Decreased proteasome function	
Gautier et al, 2008	Mouse striatum	Complex I and II	No Change			Decreased Aconitase Activity	Increased number and size		
Piccoli et al, 2008	Primary human fibroblasts	Intact cell respiration, complex IV, and ATPase activity	Decreased total ATP			Yes			
Hoepken et al, 2007	Primary human fibroblasts and immortalized lymphoblasts	Complex I enzyme activity				Yes			
Wood-Kaczmar et al, 2008	Immortalized human neurons, mouse primary neurons			Decreased		Yes	Increased number	Increase in lysosomes	
Exner et al, 2007	HeLa (human)			Decreased			Fragmented cristae		
Dagda et al, 2009	SH-SY5Y (human)					Yes	Fragmented cristae and enlarged mitochondria	Increased autophagy/mitophagy	
Weihofen et al, 2009	HEK293-FT (human), COS7 (monkey)						Fragmented and aggregated mitochondria		Binding of PINK1 to Miro and Milton
Xiong et al, 2009	HEK293, SH-SY5Y (human)							Decreased proteasome function	
Clark et al, 2006; Park et al, 2006; Deng et al, 2008; Poole et al, 2008; Yang et al, 2008; Yun et al, 2008	*Drosophila* cells and tissue						Consistent with PINK1 loss inducing cristae fragmentation in spermatids, and with normal PINK1 promoting fission.		

aspects of normal neurotransmitter responses; however, excessive matrix Ca^{2+} is known to significantly compromise mitochondrial function by multiple mechanisms. Each of these patterns of inhibition would predict increased reactive oxygen species (ROS) production, as reported in multiple studies.

A clear link between the loss of PINK1 expression and alterations in mitochondrial morphology has been established (see Table I). The observations of both morphological and bioenergetic compromise raise the quintessential 'chicken and egg' question, as these two aspects of mitochondrial function are closely intertwined (see Twig et al, 2008). Genetic studies in Drosophila (Deng et al, 2008; Poole et al, 2008; Yang et al, 2009) suggest that PINK1 normally plays a role in promoting fission, although Morais et al have not observed defects in the morphology or number of mitochondria at the Drosophila neuromuscular junction (NMJ) of Pink1 mutants. The data available for mammalian cells and tissues are also not clear; Morais et al, did not observe changes in mitochondrial morphology of Pink1 mouse mutant neurons while others have described cristae and mitochondrial fragmentation as well as an increased size (Table I). If mitochondrial respiratory dysfunction or enhanced Ca^{2+} retention is indeed the primary consequence of PINK1 deficiency, one might expect mitochondrial fission rather than fusion to result, as mitochondrial depolarization and Ca^{2+} sequestration have generally been associated with fission events (Saotome et al, 2008; Twig et al, 2008). Clearly, the link between mitochondrial bioenergetics and morphology is complex, and much remains to be revealed on the topic.

> *» A direction in which the PINK flurry should now converge. «*

What can explain the divergent observations of effects of PINK1 deficiency? The simplest is that PINK1 may be a multifunctional protein with numerous binding partners and kinase targets that are differentially expressed in the many models that have been created. The reported effectors–substrates of PINK1 include the mitochondrial molecular chaperone TRAP1 (Pridgeon et al, 2007), the matrix serine protease HtrA2/Omi (Plun-Favreau et al, 2007), and the ubiquitin E3 ligase parkin (Kim et al, 2008). It is currently unclear how these fit into pathways controlling bioenergetic function, although a theoretical argument can easily be made that chaperone and protease function could have important effects on protein import and respiratory complex assembly. The identification of the targets of PINK1's kinase activity and/or binding partners will help to further unravel the role of PINK1 in the regulation of mitochondrial function and PD. Morais et al provide us with a direction in which the PINK flurry should now converge—we can narrow the search considerably by focusing on substrates involved in Complex I activity and/or regulation, and hope that this approach will soon bring us novel insights and additional PD therapeautic targets.

The author declares that she has no conflict of interest.

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