Antioxidants put Parkinson flies back in the PINK

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Parkinson's disease (PD) is the second most frequent neurodegenerative disease, affecting ≈1% of people above age 50. PD is clinically characterized by age-dependent uncontrollable tremor, postural imbalance, slowness of movement, and rigidity. The most salient pathological feature of PD is a progressive loss of dopaminergic neurons in the substantia nigra region of the midbrain (1, 2). The majority of PD cases are sporadic; however, ≈10–15% are familial, and mutations in at least six loci are known to cause PD. Several of these PD genes are associated with ubiquitin-mediated protein degradation and the abnormal accumulation of proteins such as α-synuclein in cytoplasmic aggregates known as Lewy bodies. There is also evidence that oxidative stress and defects in mitochondrial function, particularly in complex I, may contribute to PD (3). Exposure of humans or mice to the environmental toxins MPTP, paraquot, or rotenone results in acute and irreversible parkinsonism. These toxins impair mitochondrial function and consequently increase free radical production and oxidative stress. The age-dependent and progressive nature of the disease, as well as the fact that most PD cases are sporadic, suggest that environmental factors also play important roles in the pathogenesis of the disease. A favored hypothesis of PD pathogenesis is that genetic changes sensitize dopaminergic neurons to intrinsic or extrinsic insults, leading to the eventual loss of these neurons and to parkinsonism. Previous studies have shown that the fruitfly *Drosophila melanogaster* is a suitable model for analyzing the function of genes involved in PD (4, 5). In this issue of PNAS, Wang *et al.* (6) expand the analysis of PD in *Drosophila* to examine the role of PTEN-Induced Kinase 1 (PINK1), a mitochondrial protein. They find that reducing the activity of the single *Drosophila* homolog of PINK1 (dPINK1) induces a progressive loss of dopaminergic neurons and retinal neurons, which are phenotypes similar to the pathological changes in human PD. The authors also find that the human PINK1 gene can substitute for the function of dPINK1, indicating that this kinase functions as part of a highly conserved genetic pathway. One of the most important findings of this study, which is relevant to the development of potential therapies for PD, is that treatment with antioxidants such as vitamin E or expression of human SOD1 can protect dPINK1-deficient flies from neural degeneration.

The Parkinson Pathway

The Wang *et al.* (6) study complements recent parallel reports (7–9) examining the phenotypes of dPINK1 mutant flies to provide new insights into the integrated function of cytoplasmic and mitochondrial proteins in the etiology of PD. An important puzzle in the etiology of PD is that it seems to involve both cytoplasmic and mitochondrial functions, yet it is unclear how these functions might be linked. PINK1 is likely to function in conjunction with another mitochondrial protein known as DJ-1 because mutations in this gene also cause autosomal recessive forms of PD. Consistent with this notion, a recent study reports that digenic mutations of PINK1 and DJ-1 are associated with early-onset familial PD cases (10). In addition, expression of these two pathogenic proteins potentiates susceptibility of SH-SY5Y cells to oxidative stress, and PINK1 and DJ-1 can form a complex when coexpressed in mammalian cells (10). PINK1 and DJ-1 appear to function in the same pathway as Parkin, which encodes an E3 ubiquitin ligase that is thought to function primarily in the cytoplasm, presumably to target specific protein targets for degradation or modified function/localization (11). Parallel studies to those of Wang *et al.* (6) revealed that the loss of dPINK1 function resulted in a set of neuronal and flight muscle phenotypes very similar to those present in *dParkin* mutant flies (7–9). Although the protein targets regulated by Parkin that are involved in PD are not known for certain, one clear candidate is α-synuclein because elevated levels of this protein are observed in Parkin mutant cells and because at least one form of α-synuclein is a substrate for Parkin-mediated ubiquitination (12). In addition, as mentioned above, overexpression of mutant forms of α-synuclein can cause PD. Another candidate Parkin target is a G protein-coupled membrane protein known as Pael-R. Overexpression of Pael-R function can cause dopaminergic neural degeneration, and this effect is suppressible by Parkin (13). It is also noteworthy that Parkin mutant cells are hypersensitive to oxidative stress, which suggests some link to mitochondrial function. Epistasis experiments indicate that Parkin mediates an important effect of PINK1/DJ-1 because overexpression of Parkin can rescue PINK1 mutants (8, 9). Overexpression of DJ-1, however, cannot rescue dPINK1 mutants (7), in accord with DJ-1 and PINK1 acting as unit. Consistent with models in which PINK1/DJ-1 acts on Parkin, Parkin levels are reduced in PINK1 mutants. PINK1/DJ-1 therefore may influence the transcription and/or translation of Parkin, although it is unclear whether this effect would be direct or indirect. This study by Wang *et al.* (6) further indicates that antioxidants also act downstream of PINK1 and suggests that PINK1 may play a role in sensing oxidative damage rather than in responding to it. In line with this view, dPINK1 mutants are hypersensitive to agents causing oxidative damage such as rotenone or paraquot (8).

PTEN-Induced Kinase 1 may play a role in sensing oxidative damage.

The epistasis experiments described above could be interpreted in terms of a linear sequence of events beginning with a PINK1/DJ-1-dependent phosphorylation event in the mitochondria and eventuating in Parkin-mediated degradation or modification of specific cytoplasmic proteins such as α-synuclein or Pael-R. However, this view may be an oversimplification. One reason for suspecting that the two compartmentalized processes actually may be acting as part of a single regulatory loop is that dParkin mutants have mitochondrial defects similar to those observed in dPINK1 mutants. There is evidence that some Parkin protein also is present in mitochondria and that Parkin and DJ-1 complex when cells are under oxidative stress (14). In addition, the loss of dopaminergic neurons resulting from over-

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expression of the Parkin substrates α-synuclein or Pael-R is thought to trigger a cellular stress response that is most likely sensed by mitochondria. The ability of overexpressed Parkin or antioxidants to suppress PINK1 loss-of-function mutant phenotypes therefore may simply be due to these agents reducing cellular stress directly, thereby bypassing the need for PINK1-dependent detection of such stress (caused by either protein unfolding or oxidative damage). In line with such a view, overexpression of the mitochondrial protein BCL2, which mediates the effects of a wide variety of antiapoptotic signals, also can suppress the loss of dPINK1 function (9). Further experiments examining the epistatic relationships between PINK1, DJ-1, and Parkin and additional components of the mitochondrial stress/apoptosis pathway should help resolve the interrelationships between cytoplasmic and mitochondrial elements of the PD pathway.

PINK1 as a Discovery Tool

Another interesting possibility raised by the various studies of dPINK1 in flies is that genetic background may contribute significantly to the phenotype resulting from dPINK1 loss of function. For example, in contrast to the dramatic loss of dopaminergic neurons observed in the study by Wang *et al.* (6), Park *et al.* (9) observed that a null allele for dPINK1 caused only a mild loss of dopaminergic neurons, whereas Clark *et al.* (8) reported no loss at all of these neuronal cells. One explanation for this variation in phenotype is that genetic background may play an important role in the severity and/or penetrance of the dPINK1 phenotype. Perhaps the degree of baseline oxidative damage depends on genetic background, which would be consistent with the finding by Wang *et al.* (6) that antioxidants can protect against even the most severe dPINK1 loss-of-function phenotype. It also has been suggested that the environment and genetic background similarly play a key role in the age of onset and severity of PD in humans. Both environmental and genetic factors may contribute to sporadic PD cases. For example, in addition to damage linked to oxidative stress in the brains of sporadic PD patients, mutations in PINK1 have been found in some sporadic cases (15, 16). If background genetic factors determining the sensitivity to loss of PINK1 function are similar in flies and humans, flies may prove suitable for conducting second-site modifier screens to identify additional genes acting in the conserved PINK1/DJ-1/Parkin pathway. For example, screens for enhanced loss of dopaminergic cells in the fly might turn up new core elements of the PD pathway affecting a broad range of tissues as well as genes mediating the preferential susceptibility of DA cells in the mammalian brain to reduced activity of this pathway. The ability to target loss of dPINK1 function in a cell type-specific fashion in the RNAi system established by Wang *et al.* (6) also opens the door to conducting high-throughput screens for small molecules that can suppress specific neuronal degeneration phenotypes. Such molecules ultimately could lead to the development of drugs that would put PD patients back in the pink.

1. Lang, A. E. & Lozano, A. M. (1998) *N. Engl. J. Med.* **339,** 1130–1143.
2. Lang, A. E. & Lozano, A. M. (1998) *N. Engl. J. Med.* **339,** 1044–1053.
3. Beal, M. F. (2003) *Ann. N. Y. Acad. Sci.* **991,** 120–131.
4. Feany, M. B. & Bender, W. W. (2000) *Nature* **404,** 394–398.
5. Auluck, P. K. & Bonini, N. M. (2002) *Nat. Med.* **8,** 1185–1186.
6. Wang, D., Qian, L., Xiong, H., Liu, J., Neckameyer, W. S., Oldham, S., Xia, K., Wang, J., Bodmer, R. & Zhang, Z. (2006) *Proc. Natl. Acad. Sci. USA* **103,** 13520–13525.
7. Yang, Y., Gehrke, S., Imai, Y., Huang, Z., Ouyang, Y., Wang, J. W., Yang, L., Beal, M. F., Vogel, H. & Lu, B. (2006) *Proc. Natl. Acad. Sci. USA* **103,** 10793–10798.
8. Clark, I. E., Dodson, M. W., Jiang, C., Cao, J. H., Huh, J. R., Seol, J. H., Yoo, S. J., Hay, B. A. & Guo, M. (2006) *Nature* **441,** 1162–1166.
9. Park, J., Lee, S. B., Lee, S., Kim, Y., Song, S., Kim, S., Bae, E., Kim, J., Shong, M., Kim, J. M. & Chung, J. (2006) *Nature* **441,** 1157–1161.
10. Tang, B., Xiong, H., Sun, P., Zhang, Y., Wang, D., Hu, Z., Zhu, Z., Ma, H., Pan, Q., Xia, J. H., *et al.* (2006) *Hum. Mol. Genet.* **15,** 1816–1825.
11. Shimura, H., Hattori, N., Kubo, S., Mizuno, Y., Asakawa, S., Minoshima, S., Shimizu, N., Iwai, K., Chiba, T., Tanaka, K. & Suzuki, T. (2000) *Nat. Genet.* **25,** 302–305.
12. Shimura, H., Schlossmacher, M. G., Hattori, N., Frosch, M. P., Trockenbacher, A., Schneider, R., Mizuno, Y., Kosik, K. S. & Selkoe, D. J. (2001) *Science* **293,** 263–269.
13. Yang, Y., Nishimura, I., Imai, Y., Takahashi, R. & Lu, B. (2003) *Neuron* **37,** 911–924.
14. Moore, D. J., Zhang, L., Troncoso, J., Lee, M. K., Hattori, N., Mizuno, Y., Dawson, T. M. & Dawson, V. L. (2005) *Hum. Mol. Genet.* **14,** 71–84.
15. Tan, E. K., Yew, K., Chua, E., Puvan, K., Shen, H., Lee, E., Puong, K. Y., Zhao, Y., Pavanni, R., Wong, M. C., *et al.* (2006) *Movement Disorders* **21,** 789–793.
16. Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E., Dekker, M. C., Squitieri, F., Ibanez, P., Joosse, M., *et al.* (2003) *Science* **299,** 256–259.