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NEURODEGENERATIVE DISEASE

Forming fragments

> the lifespan of mitochondrial DLP1 complexes was shorter in *VPS35*^{WT}-overexpressing M17 cells than in controls

Mutations in the gene encoding the retromer complex component vacuolar protein sorting-associated protein 35 (VPS35) are associated with autosomal-dominant Parkinson disease (PD), but precisely how such mutations cause neurodegeneration is unclear. Now, Wang *et al.* show that overexpression of *VPS35* increases the turnover of mitochondrial dynamin-like protein 1 (DLP1) — a GTPase involved in mitochondrial fission — resulting in mitochondrial fragmentation, mitochondrial dysfunction and neuronal loss.

The authors overexpressed wild-type *VPS35* (*VPS35*^{WT}), or the mutant form *VPS35*^{D620N} or *VPS35*^{R524W} in cultured rat cortical neurons that also expressed a mitochondrial labelling protein. Compared with control cells transfected with vector only, cells overexpressing one of the three *VPS35* variants had shorter mitochondria (indicative of mitochondrial fragmentation) and had increased rates of cell death, and real-time imaging revealed an increase in the number of mitochondrial fission events. These effects were greatest in neurons expressing *VPS35*^{D620N}, a common mutation found in individuals with autosomal-dominant PD. Lentivirus-mediated transfer of *VPS35*^{WT}, *VPS35*^{R524W} or *VPS35*^{D620N} to the substantia nigra of wild-type mice also resulted in mitochondrial fragmentation and neuronal loss in that region.

Expression of *VPS35*^{WT}, *VPS35*^{R524W} or *VPS35*^{D620N} in human dopaminergic neuroblastoma (M17) cells resulted in mitochondrial dysfunction, as indicated by increased levels of reactive oxygen species and decreased mitochondrial membrane potential. Notably, the mitochondrial dysfunction seen in these cells was dependent on DLP1 function, as these effects were prevented by co-expression of a dominant-negative form of *DLP1* or a mitochondrial fission inhibitor targeting DLP1.

Furthermore, the density and size of DLP1 puncta in mitochondria were decreased in *VPS35*^{WT}-overexpressing M17 cells, and increased in M17 cells in which *VPS35* was knocked down. Measurement of the time required for recovery of DLP1-associated fluorescence in mitochondria after photobleaching of these cells indicated that the lifespan of mitochondrial DLP1 complexes was shorter in *VPS35*^{WT}-overexpressing M17 cells than in controls, and extended in M17 cells in which *VPS35* was knocked down. These findings suggest that *VPS35* is involved in regulating DLP1 complex turnover in mitochondria.

The authors also showed that DLP1 interacts directly with VPS35 and that this interaction is increased in M17 cells expressing *VPS35*^{D620N} compared with cells expressing *VPS35*^{WT}. Fibroblasts from an individual with PD who carried the D620N mutation also showed upregulation of this DLP1–VPS35 interaction compared with fibroblasts derived from a healthy individual.

Finally, the authors showed that DLP1 is a cargo of mitochondria-derived vesicles (MDVs), which carry proteins for lysosomal degradation. Localization of DLP1 to MDVs in M17 cells was increased following overexpression of *VPS35*^{WT} and decreased in cells lacking *VPS35*.

Together, these findings show that PD-causing mutations in *VPS35* contribute to neurodegeneration by increasing rates of mitochondrial fission, a process previously linked to other neurodegenerative disorders, and that this effect is mediated by increasing DLP1complex turnover in mitochondria.

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ORIGINAL ARTICLE Wang, W. *et al.* Parkinson's disease-associated mutant VPS35 causes mitochondrial dysfunction by recycling DLP1 complexes. *Nat. Med.* http://dx.doi.org/10.1038/nm.3983 (2015)

FURTHER READING Small, S. A. & Petsko, G. A. Retromer in Alzheimer disease, Parkinson disease and other neurological disorders. *Nat. Rev. Neurosci.* **16**, 126–132 (2015)