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Gut mitochondrial defects drive neurodegeneration

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Abstract

Neurodegenerative diseases, including Parkinson's disease, are linked to the accumulation of defective mitochondria in the brain and to microbial dysbiosis in the gut. However, the interplay between these factors is incompletely understood. Fedele et al. reveal how gut mitochondrial dysfunction activates intestinal inflammation to drive neurodegeneration in a Parkinson's disease model.

Nearly 2,500 years ago, Hippocrates is said to have stated that "all disease begins in the gut." In many cases, he may have been correct. Until recently, modern medicine has largely addressed gut health distinctly from most other pathologies, especially neurodegenerative diseases. This perspective has changed, largely thanks to the investment in the Human Microbiome Project by the US government and private sector, with studies concluding that gut health and the gut microbiota have a crucial role in the development of neurological disorders, including Parkinson's disease (PD)[1]. In a study published in *Nature Aging*, Fedele et al.[2] reveal a link between intestinal inflammation and neurodegeneration in a PD model that is initiated via mitochondrial dysfunction (Fig. 1).

PD is an age-related neurodegenerative disease with symptoms ranging from mild tremors, speech disturbances and impaired writing ability to more severe manifestations such as slowed movements (bradykinesia), rigid muscles, impaired posture and balance and loss of automatic movements. Over 10 million people worldwide have PD. As mitochondria accumulate damage with age, quality control mechanisms are essential to ensure homeostasis and maintain healthy cells and tissues. Dysfunctional mitochondria can be cleared and recycled via a specialized form of autophagy known as mitophagy. The gene *PINK1* regulates mitophagy through recruitment of parkin to the outer membrane of depolarized mitochondria to tag it for degradation[3]. Mutations in the *PINK1* gene have been linked to a form of familial PD and are associated with altered expression of metabolic and immune response genes[3]. Although hereditary mutations can affect cell physiology throughout the entire body, it seems reasonable to assume that impaired mitochondria in brain tissue has a prominent role in PD-related neurodegeneration. However, there

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are previously identified clues that suggest that alterations in the intestine could precede neurodegeneration in PD. For example, studies in patients with PD have shown that α-synuclein aggregates (which can contribute to PD) accumulate in peripheral nerves, including those that innervate the intestine[4]. These α-synuclein aggregates can propagate to the central nervous system, where they accumulate and cause neurotoxicity[4]. The primary question that the authors set out to address in this study[2] was whether mitochondrial dysfunction in brain tissue alone is responsible for PD-related neurodegeneration in the Pink1 *Drosophila* model of PD.

Previous work has shown that impaired mitophagy can induce inflammation, contributing to neurodegeneration[5], but the tissue-specific mechanisms involved are not well-known. Using an in silico approach, Fedele et al.[2] detected the upregulation of 45 immune-response genes in *Pink1* mutants, with *Relish* (which encodes a *Drosophila* NF-κB transcription factor) being required for the activation of most of these immune genes. To better understand the implications of immune-response activation in the phenotypes caused by a disruption in *Pink1* function, Fedele et al.[2] analyzed locomotor ability and the number of dopaminergic neurons (which are known to be reduced in number in PD models) in flies mutant for both *Pink1* and *Relish*. Importantly, these double-mutant flies recovered in movement activity level and dopaminergic neuron count, which indicates that suppression of Relish signaling could be neuroprotective in *Pink1*-dependent PD. Activation of the *Drosophila* immune response via Relish signaling results in the expression of a variety of immune genes, including those encoding antimicrobial peptides[6]. Fedele et al.[2] analyzed the expression of the antimicrobial peptide Attacin-A in *Pink1*-mutant flies and observed it to be highly upregulated in *Drosophila* intestinal cells. Previous reports have found that defective mitochondria and systemic immune response activation are tightly linked to intestinal barrier dysfunction[7,8]. Accordingly, the authors[2] used a noninvasive method known as the 'Smurf assay'[7] in which nonpermeable food dye is fed to animals to determine intestinal barrier integrity. Compared to healthy control flies, Fedele et al.[2] found blue dye spread throughout the body of a higher fraction of *Pink1*-mutant flies, indicating a loss of intestinal barrier integrity. They also show an increase in apoptotic cells and intestinal stem cells in the gut of the mutants relative to controls. Furthermore, Fedele and colleagues demonstrate that intestinal cell damage in *Pink1* mutants leads to the recruitment of a ring of fat body cells involved in wound healing and cell repair that is absent in age-matched wild-type controls. These intestinal defects in *Pink1*-mutant flies, including intestinal barrier dysfunction, can be rescued by genetically depleting the immune-response activator *Relish*.

How do dysfunctional mitochondria induce an innate immune response in *Pink1*-mutant flies? It is increasingly recognized that damaged or stressed mitochondria can release mitochondrial DNA (mtDNA) into the cytosol and that cytosolic mtDNA can activate the innate immune system and contribute to inflammatory pathology[9,10]. Indeed, recent work has shown that in cellular and zebrafish models of PD, mtDNA accumulates in the cytosol and activates the immune response[11]. To understand the relation of defective mitochondria and immune response activation in the gut, Fedele et al.[2] analyze the levels of cytosolic mtDNA in gut cells of *Pink1*-mutant flies, revealing increased levels of extramitochondrial DNA compared to controls. The Relish- and NF-κB-binding protein EYA has been shown to induce an innate immune response against cytosolic DNA in both flies and mammals[12,13].

Epistatic analysis found that Eya is responsible for activating the immune response in *Pink1* mutants. Fedele et al.[2] also demonstrate that loss of *Eya* can rescue neurotoxicity in *Pink1* mutants. These findings validate the hypothesis that the cytosolic mtDNA regulates immune response activation and neurotoxicity in *Pink1* mutants (Fig. 1).

Recent epidemiological studies in patients with PD have revealed that one of the nonmotor symptoms of PD is excessive daytime sleepiness[14]. In *Drosophila*, mutants for either of two PD-related genes (*Pink1* and *Parkin*) present altered circadian rhythms[15]. Fedele et al.[2] demonstrate that *Pink1* mutants have upregulated levels of the clock-controlled protein Takeout (To) and the sleep regulator *Drosophila* insulin-like peptide 2 (Dilp2). Appropriately named, Takeout is primarily involved in the regulation of feeding behavior and is highly expressed in the crop, fat body and antennae. In adult flies, the function of Dilp2 is to regulate blood sugar levels. Because both genes, *To* and *Dilp2* (also known as *Ilp2*), are involved in metabolic and energy homeostasis, the authors analyzed the mobilization of triglycerides. Insulin signaling regulates lipid storage via regulation of the transcription factor Foxo. However, the immune response can also modulate metabolic homeostasis through the regulation of *Drosophila* Foxo by Relish. Fedele et al.[2] show that triglyceride levels increase in *Pink1*-mutant flies, and that loss of *Relish* lowers triglyceride levels in *Pink1*-mutant flies and reduces the levels of Takeout, but not Dilp2. These results link the activation of the immune response with changes in metabolic stores and the expression of factors that affect feeding behavior.

The gut–brain axis is emerging as a major area of development in understanding neurological disease, with bidirectional communication between the central and enteric nervous systems having both local and systemic effects. Studies in mice have demonstrated that injection of α-synuclein in the pylorus and duodenum can initiate the accumulation of α-synuclein plaques in the brain via the vagus nerve[4]. Fedele et al.[2] studied the implications of immune-response activation and cell death in the gut for the onset and progression of PD. First, they inactivated the immune response by knocking down *Relish* in a subset of intestinal cells. Remarkably, this gut-specific manipulation rescued the neurotoxic effects caused by *Pink1* mutations. Perhaps the most exciting and surprising finding was that dampening inflammation, specifically in the gut, led to improved mitochondrial function in the brain (Fig. 1b). It will be fascinating to further investigate the underlying mechanisms involved. Fedele et al.[2] next investigated whether this intestinal cell death could be involved in neurotoxicity. To test this hypothesis, the authors overexpressed the antiapoptotic protein Buffy in intestinal cells and analyzed the neuronal effects in *Pink1*-mutant flies. Interestingly, inhibiting apoptosis in intestinal cells was sufficient to suppress neurotoxicity in *Pink1*-mutants. These findings demonstrate that immune-response activation in the gut, and resulting apoptosis of intestinal cells, is necessary for neurotoxicity in this model of PD. It is interesting to speculate, therefore, that intestinal barrier dysfunction may have a key role in this phenomenon.

This study in *Drosophila melanogaster* by Fedele et al.[2] highlights the importance of the mitochondrial dysfunction–immunity axis in the onset and progression of PD. Although recent studies investigating PD have emphasized the importance of the peripheral nervous system and the accumulation and propagation of α-synuclein in peripheral nerves (such as

the vagus nerve) in affecting the central nervous system[4], Fedele and colleagues highlight the relevance of the immune response in distal tissues and changes in mitochondrial homeostasis to induce immune activation. Specifically, this neurotoxicity is dependent on the gut by way of inflammation and cell death resulting from release of mitochondrial DNA by dysfunctional mitochondria. As mitochondrial dysfunction and inflammation are both hallmarks of aging, it will be interesting to investigate whether similar mechanisms are involved in tissue and organismal aging. Most importantly, the findings outlined in this exciting paper suggest that targeting gut inflammation may be a promising treatment for PD, opening new opportunities to treat this disorder.

Acknowledgements

References

1. Fung TC, Olson CA & Hsiao EY Nat. Neurosci 20, 145–155 (2017). [PubMed: 28092661]

2. Fedele G. et al. Nat. Aging 10.1038/s43587-022-00194-z.(2022).

3. Pickrell AM & Youle RJ Neuron 85, 257–273 (2015). [PubMed: 25611507]

4. Kim S. et al. Neuron 103, 627–641 (2019). [PubMed: 31255487]

5. Sliter DA et al. Nature 561, 258–262 (2018). [PubMed: 30135585]

6. Buchon N, Silverman N & Cherry S Nat. Rev. Immunol 14, 796–810 (2014). [PubMed: 25421701]

7. Rera M, Clark RI & Walker DW Proc. Natl Acad. Sci. USA 109, 21528–21533 (2012). [PubMed: 23236133]

8. Clark RI et al. Cell Rep. 12, 1656–1667 (2015). [PubMed: 26321641]

9. Pérez-Treviño P, Velásquez M & García N Biochim. Biophys. Acta Mol Basis Dis 1866, 165761 (2020). [PubMed: 32169503]

10. West AP & Shadel GS Nat. Rev. Immunol 17, 363–375 (2017). [PubMed: 28393922]

11. Matsui H. et al. Nat. Commun 12, 3101 (2021). [PubMed: 34035300]

12. Liu X. et al. PLoS ONE 7, e42725 (2012). [PubMed: 22916150]

13. Okabe Y, Sano T & Nagata S Nature 460, 520–524 (2009). [PubMed: 19561593]

14. Shen Y, Huang JY, Li J & Liu CF Chin. Med. J 131, 974–981 (2018). [PubMed: 29664059]

15. Julienne H, Buhl E, Leslie DS & Hodge JJL Neurobiol. Dis 104, 15–23 (2017). [PubMed: 28435104]
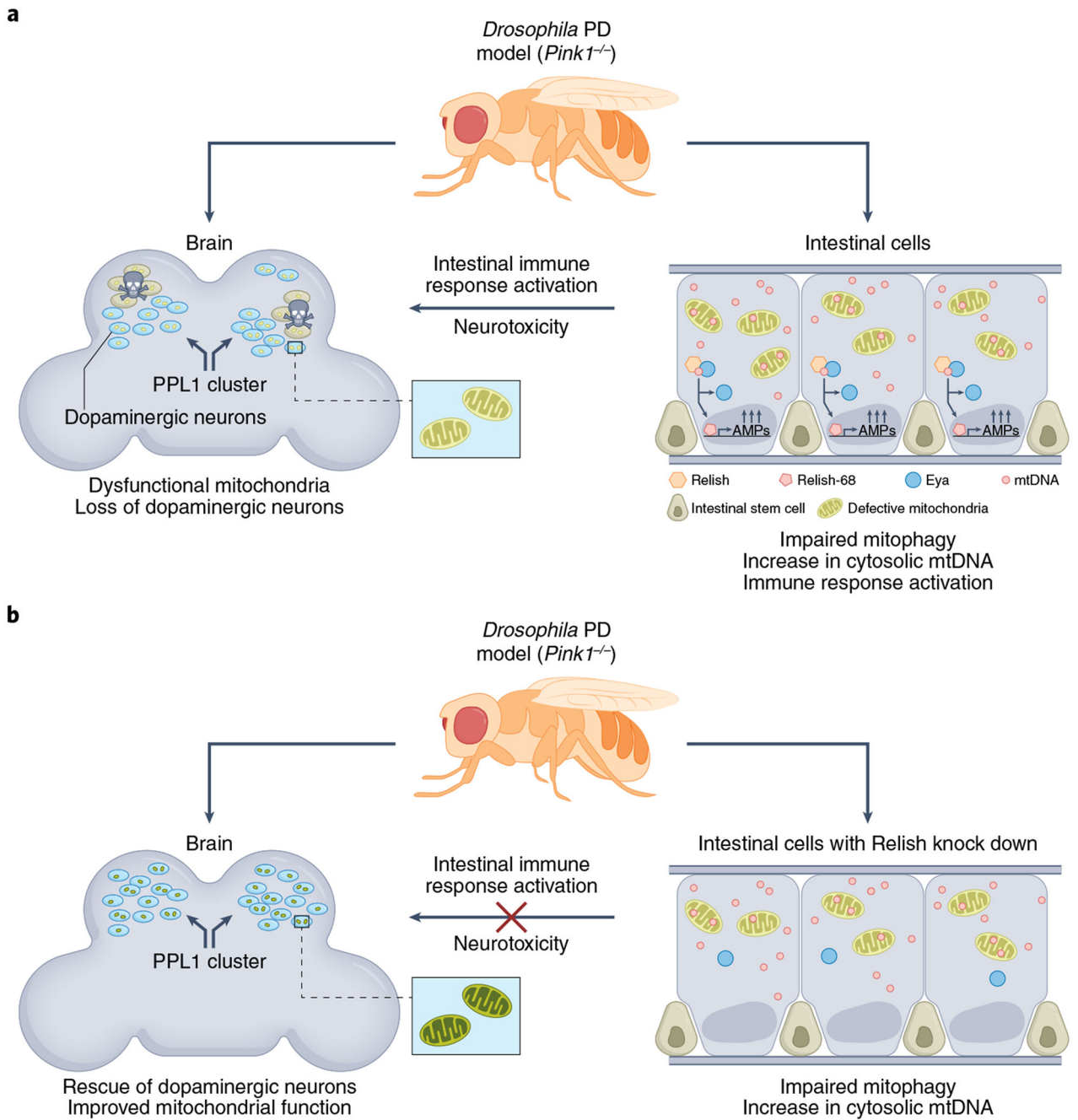
Fig. 1 |. Activation of the immune response in intestinal cells, due to mitochondrial dysfunction, is required for neurotoxicity in a *Drosophila* model of PD.

a, *Pink1*-mutant flies (a model of PD) present high levels of cytosolic mtDNA in intestinal cells due to defective mitochondria. The transcription factor Relish translocates from the cytosol to the nucleus as Relish-68, the activated N-terminal cleaved domain of Relish. Extramitochondrial DNA activates the immune response in intestinal cells via Relish, and via changes to interactions between Relish and Eya. Expression of immune genes encoding antimicrobial peptides (AMPs) in gut cells promotes neurotoxicity in brains and loss of cells

in a specific cluster of dopaminergic neurons known as the PPL1 cluster. **b**, Knockdown of Relish in intestinal cells in this PD model inhibits the activation of the immune response in the gut, even in the presence of gut mitochondrial dysfunction. This results in rescuing the number of dopaminergic neurons in the PPL1 cluster and mitochondrial activity in the brain.