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Research Highlight

Fragmentation for selection: how the deleterious mtDNA is removed in the female germline

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Mitochondria are the energy factories and metabolic centers of cells, and their malfunction can lead to multiple human diseases. As highly dynamic organelles, mitochondria undergo frequent fission and fusion processes [1]. The fusion process enables the exchange of inner materials between individual mitochondrion. The materials from healthy mitochondria could potentially compensate for the defects found in the defective mitochondria. Fission processes not only increase the mitochondrial number but also separate the damaged parts of mitochondria from the healthy ones, thus, facilitating the elimination of the defective mitochondria through a process known as mitophagy. Excessive fusion leads to extra connections and branching of the mitochondria, whereas extra fission results in fragmentation of mitochondria. The fusion and fission processes are differently tuned in various tissues to adapt to different physiological requirements [2].

As semiautonomous organelles, mitochondria possess multiple copies of circular DNA, known as mitochondrial DNA (mtDNA), in the matrix. mtDNA encodes 13 subunits of the respiration complex and a few tRNAs and rRNAs in animal cells. In metazoans, mtDNA is maternally inherited [3]. The free radicals produced during oxidative phosphorylation are powerful mutagens to the mitochondrial genome. Therefore, mtDNA has relatively higher mutation rate than nuclear DNA. In addition, the repair capability of mtDNA is limited. As a result, certain levels of variations in mtDNA exist in the individual animal, which is known as heteroplasmy [4]. During mtDNA transmission, the deleterious mutations in mtDNA are selectively removed in the female germline to prevent the accumulation of harmful mutations in the successive generations [5]. However, the molecular mechanisms underlying this special selection process are poorly understood.

In a recent paper published in *Nature* [6], Thomas Hurd, Ruth Lehman, and their colleagues found that mitochondrial fragmentation is the driving force behind the removal of mutated mtDNA in the germline. To study the mechanisms of mtDNA selection in the germline, they required a model system that could generate heteroplasmic germ cells that contain, both, wild-type and mutant mtDNA. They also needed an assay to distinguish different types of mtDNA. Fruit fly has been proved to be an ideal model [7]. Fly germ line is segregated from the somatic linage cells at the very early stage due to the formation of germ plasm. Transplantation of the donor germ plasm to a recipient embryo that carries a different type of mtDNA enables the formation of germ cells with two types of mtDNA. Both, intra- and inter-specific plasm transplantation, could produce heteroplasmic germ cells [8]. The authors also took advantage of a *Drosophila melanogaster* strain whose mtDNA carries a temperature sensitive point mutation in *cytochrome c oxidase subunit I* (Col^{ts}) [7,9]. At the restricted temperature, cytochrome oxidase activity is greatly reduced. To generate heteroplasmic germ cells, the germ plasm from wild-type *Drosophila yakuba* was transplanted into the *Drosophila melanogaster* embryo carrying Col^{ts} mutation. By altering the temperature, the selection of mtDNA could be turned "on" or "off", which could be analyzed using fluorescent *in situ* hybridization (FISH) with the specific probes for the unique regions of the D-loops of mtDNA of either *Drosophila melanogaster* or *Drosophila yakuba*. With these neat experimental settings, the authors found that at the restricted temperature, the proportion of wild-type mtDNA relative to the mutant mtDNA increased dramatically in the germline but not in soma; this was observed only in the female germline but not in the male germ line. In *Drosophila*, the germline stem cells divide to self-renew and to produce germline cysts containing cells that undergo four rounds of division with incomplete cytokinesis. Further experiments have found that the mtDNA selection occurs in the germline cysts but not in the germline stem cells. Previous study has suggested that the selection could happen at the cellular level due to the cell death occurring in the cells with excessive mutant mtDNAs. However, the inhibition of cell death did not block the selection. Rescuing the mitochondrial defects with concurrent maintenance of the mutant mtDNA suppressed the selection, suggesting that the selection happens on the level of organelle and not on the level of mutant mtDNA itself.

Subsequently the authors examined the morphology and dynamics of the mitochondria in, both, germline stem cells and germline cysts. The stem cell mitochondria were observed to be tubular and branched, whereas the cyst mitochondria were found to be fragmented; this is likely due to the reduced expression of

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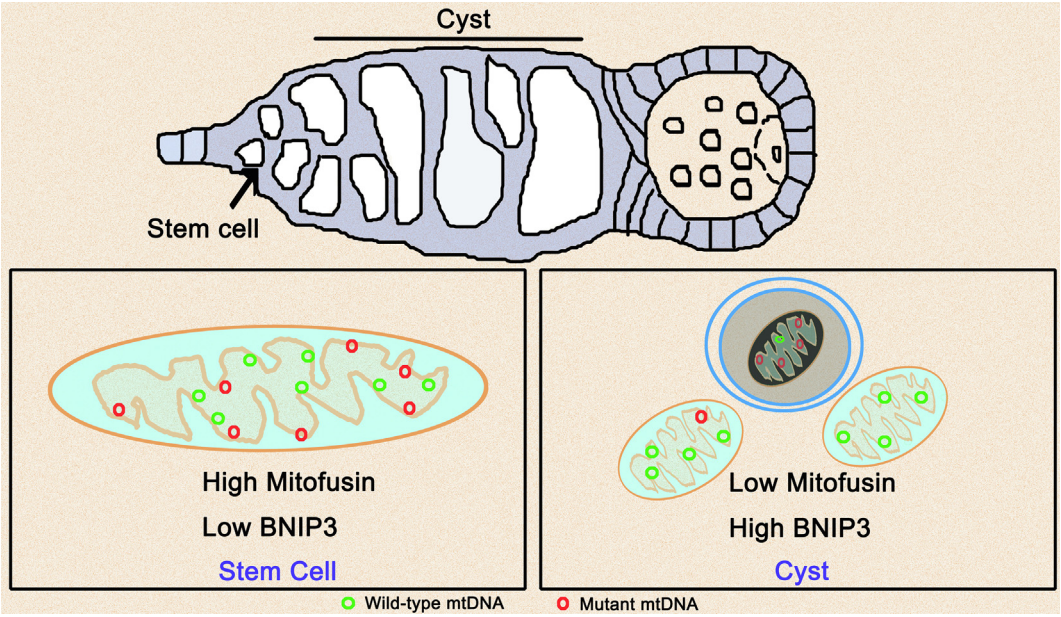

Fig. 1. A scheme to show the organization of the germarium of *Drosophila*. The stem cells divide asymmetrically to self-renew and produce cysts that undergo four rounds of division with incomplete cytokinesis. In the stem cells, the expression level of pro-fusion protein Mitofusin is high and the expression level of mitophagy protein BNIP3 is low. As a result, mitochondria are highly connected and the purifying mtDNA selection is low. In the germline cysts, the expression of Mitofusin is low and the expression of BNIP3 is high, which leads to mitochondrial fragmentation and the removal of deleterious mtDNA through mitophagy.

the pro-fusion factor Mitofusin in the cysts. To test whether the fusion and fission of mitochondria could affect the selection, the authors either overexpressed Mitofusin or reduced the expression of the pro-fission protein Drp1. Both could largely abolish the mtDNA selection in the germline cysts, suggesting that the mitochondrial fragmentation is necessary for the removal of the mutant mtDNA. Interestingly, increasing mitochondrial fission in the germline stem cells or somatic follicle cells was found to be sufficient for inducing the selection.

The question that remained to be addressed is how the mutant mitochondrial genomes are removed once the mitochondria are fragmented. The authors found that the reduction of proton motive force (PMF) generated by the mtDNA encoded proteins was not necessary for the selection; the reduction of mitochondrial ATP was sufficient for the selection. The authors further showed that the mitophagy plays an essential role in the elimination of the mitochondria with mutant mtDNA. Previous study has indicated that the Parkin-mediated mitophagy is not required for the clearance of mutant mtDNA. The authors also found that, although the master autophagy regulator Atg1 was required for the selection, Atg8, the key structural component of autophagosome, was not. Finally, they found that BNIP3 was essential for selection. *Drosophila* BNIP3 is the homolog of human BNIP3L (also known as NIX) whose protein product is required for the clearance of mitochondria during red blood cell maturation [10]. Consistent with its role in selection, BNIP3 was found to be upregulated in the differentiated cysts. The authors found that the blockage of selection by reducing Atg1 or BNIP3 was primarily due to the inhibition of the replication of the wild-type mtDNA instead of prevention of the elimination of the mutant mtDNA. This suggested that mitophagy and mtDNA replication might be coupled and the clearance of the mutant genomes facilitates the proliferation of the wild-type ones. However, it is to be noted that the authors did not directly examine the mitophagy in the germline cysts. This raises a concern whether Atg1 and BNIP3 possess other functions independent of mitophagy.

This study elucidated how a developmental programed mitochondria fragmentation facilitates the purification of mtDNA (Fig. 1). It would be interesting to explore whether increasing mitochondrial fission promotes disease-related mtDNA clearance. A good study always raises more questions. Many details, such as, how the expression of Mitofusin and BNIP3 is regulated, how mitophagy machineries sense the reduction of ATP, how BNIP3 mediates mitophagy in germline cyst, and how turnover of mitochondria is coupled to the mtDNA replication, need further elucidation. Addressing these questions will not only shed new light on how mtDNA selection is regulated, but also promote the development of therapeutic approaches for the mtDNA-related diseases.

Conflict of interest

The author declares that she has no conflict of interest.

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Chao Tong is a professor in Life Sciences Institute, Zhejiang University. Her major research interest is to dissect how cellular organelles such as mitochondria contribute to neuronal health maintenance. Using *Drosophila* as a model system, she aims to identify novel players in organelle biogenesis and clearance and to understand the molecular mechanisms underlying neurodegeneration.