

# Involvement of autophagy and mitochondrial dynamics in determining the fate and effects of irreparable mitochondrial DNA damage

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**M**itochondrial DNA (mtDNA) is different in many ways from nuclear DNA. A key difference is that certain types of DNA damage are not repaired in the mitochondrial genome. What, then, is the fate of such damage? What are the effects? Both questions are important from a health perspective because irreparable mtDNA damage is caused by many common environmental stressors including ultraviolet C radiation (UVC). We found that UVC-induced mtDNA damage is removed slowly in the nematode *Caenorhabditis elegans* via a mechanism dependent on mitochondrial fusion, fission, and autophagy. However, knock-down or knockout of genes involved in these processes—many of which have homologs involved in human mitochondrial diseases—had very different effects on the organismal response to UVC. Reduced mitochondrial fission and autophagy caused no or small effects, while reduced mitochondrial fusion had dramatic effects.

The mitochondrial genome (mtDNA) is ~200,000 times smaller than the nuclear genome, but is present in most human cells at very high copy number (1000s to 100,000s). However, the copy number can be much lower (e.g., 10s to 100s in primordial germ cells). Copy number in some organisms, including *C. elegans*, is considerably lower (10s to 100s). mtDNA is very susceptible to damage from genotoxicants and exhibits a high rate of mutation. However, cellular dysfunction and disease are detected only above a threshold level of heteroplasmy (occurrence of more than one mtDNA sequence in a cell).

mtDNA damage and repair have recently received more attention due to the increasing number of diseases associated with mtDNA mutations. While most oxidative mtDNA lesions are repaired relatively effectively due to the presence of base excision repair in mitochondria, the fate of several other types of damage is unclear. In particular, DNA damage caused by many important environmental stressors including UV radiation, polycyclic aromatic hydrocarbons that result from combustion of organic fuels, certain mycotoxins, and others is not repaired in mtDNA due to a lack of nucleotide excision repair. We reported that while not repaired, UVC-induced mtDNA damage is slowly removed in *C. elegans*, and that this removal is dependent on genes coding for proteins involved in mitochondrial fusion, fission, and autophagy. This finding establishes mitochondrial autophagy as a critical pathway for the removal of otherwise irreparable mtDNA damage.

The UVC-induced damage, which likely blocks both DNA and RNA polymerases, correlates with decreased ATP levels, decreased oxygen consumption, and developmental arrest. Developmental arrest in *C. elegans* can serve as a whole-organism marker of mitochondrial function. UVC-induced developmental arrest is strongly exacerbated and irreversible in mitochondrial fusion mutants compared with the wild type. To our surprise, fission mutants show no exacerbation of developmental arrest, and autophagy mutants (including the *C. elegans* homologs of the Parkinson disease-related mitophagy genes *PINK1* and *PARK2*) show minor or no exacerbation.

**Keywords:** mitochondrial fusion, mitochondrial fission, autophagy, mitophagy, mitochondrial DNA damage, ultraviolet radiation, *Caenorhabditis elegans*

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Why are fusion mutants so much more sensitive than autophagy mutants, given that autophagy is presumably the rate-limiting step in removal of damaged mtDNAs, and fusion (unlike fission) would not seem to be required to segregate mitochondria for degradation? One possible explanation was provided by the Nunnari laboratory, which showed that fusion is required for normal autophagy. A second possibility derives from the importance of fusion-mediated functional complementation for mitochondrial function in the face of stress. Individual mitochondria carrying mtDNAs too damaged to be used as templates might benefit from fusion because it would allow transcription and replication from undamaged mtDNAs from other mitochondria. This interpretation is supported by the fact that exposure to ethidium bromide, a powerful inhibitor of mtDNA replication and transcription, exacerbates UVC-induced developmental arrest as does the fusion mutation. This also suggests that cells with low mtDNA copy number, such as primordial germ cells, may be particularly susceptible to the effects of persistent mtDNA damage. This vulnerability may be exacerbated by the fact that very early in development, mtDNAs are simply

allocated, and not replicated, which could stochastically lead to specific daughter cells carrying very high proportions of damaged mtDNAs. We note that because of its low mtDNA copy number, *C. elegans* is a useful excellent model for the study of effects of sublethal levels of mtDNA damage in low-copy number human cells. Such effects may be harder to identify in most cell cultures because copy number is so much higher. Relatedly, we hypothesize that loss of fission had no effect on development due to the continued—perhaps even enhanced—complementation resulting from networked mitochondria.

Finally, why does blocking autophagy not exacerbate larval development more severely? We hypothesize that in the presence of fusion-mediated complementation, the redundancy inherent in multiple copies is sufficient to permit relatively normal mitochondrial function even without autophagy. However, these experiments were relatively short-term (3 days, the normal period of development to adulthood in *C. elegans*), and an important future direction of research will be to investigate the long-term effects of such damage. It is also striking that *PINK1* and *PARK2* mutants show little or no exacerbation of developmental

arrest. This might relate to research by the Berman group showing that the current model of mitochondrial dysfunction-mediated mitophagy may not apply in cells with active oxidative phosphorylation, which would be the case for many if not all of the cell types in later-stage *C. elegans*. Additional important questions remain. While we observed increased autophagy after UVC exposure, we were not able to determine whether mitophagy was increased nor whether the removal of damaged mtDNAs was specific for damaged mtDNAs, or indiscriminate.

Overall, our results support a model in which functional buffering by the capacity for fusion-mediated complementation and mtDNA replication protect against the effects of irreparable mtDNA damage. From a health perspective, this buffering is clearly good news—yet also reveals potential Achilles' heels. What if damage occurs in the context of low copy number and absence of replication (e.g., early in development), or if removal processes are hindered genetically? Mutations in many genes in these pathways exist and are associated with disease, raising the possibility that such diseases result from a combination of genetic differences, environmental exposures, and timing of exposure.