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Understanding Ageing - the Role of Mitochondria in Determination of *Caenorhabditis elegans* Life Span

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ABSTRACT

Mitochondria are organelles found in eukaryotic cells. They are involved in many vital cellular functions. Consequently, mitochondrial dysfunction leads to a variety of human disorders. Many studies of the last 50 years showed that mitochondria are involved in the regulation of physiological ageing. However, the underlying mechanisms are still unknown. We aimed to analyze the mitochondrial role in ageing in *Caenorhabditis elegans* model system. Its short life cycle, powerful genetic tools and known fates of all 959 post-mitotic somatic cells make this nematode an excellent model system for ageing studies. Besides numerous advantages, the small body size of the worm brings along certain technical limitations. We developed a toolkit to analyze mitochondrial morphology, metabolic profile and electron transport chain (ETC) activities on a single-tissue level. In addition, we adapted a method for analysis of mtDNA copy number for use on individual animals.

Each mitochondrion has its own genome that is maintained by mitochondrial DNA polymerase gamma (POLG). By analyzing *polg-1* mutant worms that are deficient in the sole mitochondrial DNA polymerase, we showed that *C. elegans* mtDNA replication mainly takes place in the gonad, the only proliferative tissue in adult worms. Thus mtDNA depletion leads to marked dysfunction of this organ. Severe mtDNA depletion leads to embryonic arrest, whereas mild depletion does not affect development. We showed that mtDNA replication does not take place during embryogenesis; it starts during the L3 larval stage, correlating with germline proliferation. Taken together, mtDNA copies in the somatic tissues mainly stem from the oocyte and stay relatively unchanged during development and early adulthood. Remarkably, somatic tissues are not severely affected in *polg-1* deficient animals despite the marked overall mtDNA depletion in contrast to other model systems, namely flies and mice. Furthermore, we showed that mtDNA copy number exhibits substantial plasticity upon environmental stress.

Mitochondria are the major source of ATP, which they form by oxidative phosphorylation (OXPHOS). Defective OXPHOS often results in severe phenotypes or premature death in several animal models. However, studies in *C. elegans* showed that dysfunction in the mitochondrial respiratory chain is not necessarily lethal. It can rather result in lifespan prolongation in the so-called “Mit (mitochondrial) mutants”. We analyzed molecular mechanisms that underlie the longevity induced by mitochondrial dysfunction. It has been shown that different mechanisms can affect the longevity of Mit mutants. We found that succinate dehydrogenase activity of electron transport chain (ETC) complex II (CII) influences the lifespan of Mit mutants independently of the insulin-like/IGF-1 pathway. We showed that mitochondrial unfolding protein response (UPR^{mt}) is up-regulated in both short- and long-lived Mit mutants. Furthermore, our results suggest that respiration rate is not necessarily linked to longevity. Analysis of several metabolic pathways in Mit mutants revealed that dysfunction of the mitochondrial respiratory chain leads to a common response characterized by up-regulation of the citric acid cycle, glycolysis, and some anaerobic pathways, accompanied by increase in neutral fat storage.

Keywords: *C. elegans*, mitochondria, ageing, development, mtDNA copy number, metabolic changes, electron transport chain activities, succinate dehydrogenase.

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