Unravelling the Mitochondrial Calcium Uniporter: Lessons from the Tiny Worm with Big Implications

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ABSTRACT

Mitochondria are central regulators of cellular metabolism, survival, and signaling, integrating environmental cues and cellular needs to maintain energy balance. Among their specialized functions, calcium (Ca^{2+}) uptake into the mitochondrial matrix is pivotal for coordinating oxidative phosphorylation, modulating reactive oxygen species (ROS), and initiating or preventing apoptotic pathways. This critical process is mediated by the mitochondrial calcium uniporter complex (MCUcx), a highly conserved molecular machinery across eukaryotes. Dysregulated mitochondrial calcium handling has been implicated in neurodegenerative disorders, cardiovascular disease, cancer, and metabolic syndromes, highlighting the biomedical importance of MCUcx regulation. *Caenorhabditis elegans* (*C. elegans*) offers a powerful genetic and physiological platform to dissect MCUcx biology, as it combines evolutionary conservation with tractable genetics and well-defined organismal phenotypes. The nematode harbors the core MCUcx components: the pore-forming subunit MCU-1 and the regulatory subunits EMRE-1, MICU-1, and MICU-3. Despite their evolutionary conservation, the precise contributions of these proteins to mitochondrial integrity and organismal health remain incompletely understood. Studying the MCUcx in *C. elegans* therefore provides a unique opportunity to integrate genetic dissection with physiological readouts, illuminating conserved mechanisms of calcium signaling and mitochondrial regulation at the cellular and whole-organism levels.

This study was designed to achieve a comprehensive genetic characterization of the MCUcx in *C. elegans*. Specifically, the objectives were: [1] to determine how loss of individual MCUcx subunits influences mitochondrial bioenergetics, oxygen consumption, and stress responses; [2] to assess the impact of MCUcx disruption on organismal phenotypes, including development, reproduction, motility, and lifespan; [3] to explore genetic interactions among MCUcx components through double- and triple-mutant analyses, identifying compensatory or synergistic relationships; and (4) to contextualize findings from *C. elegans* within translational frameworks, drawing parallels to human disease while highlighting broader implications for cellular resilience and host–microbe interactions.

To accomplish this, loss-of-function mutants for MCU-1, EMRE-1, MICU-1, and MICU-3 were employed to investigate subunit-specific roles in mitochondrial function and physiology. Mitochondrial assays included direct measurements of matrix calcium uptake, quantification of ATP levels, and oxygen consumption assays to evaluate bioenergetic capacity. Additional stress markers included ROS accumulation and sensitivity to hypoxia and oxidative stress. Physiological readouts encompassed developmental timing, brood size, locomotory activity, neuromuscular performance, and lifespan. Kaplan-Meier survival analysis was applied to lifespan curves, while ANOVA and regression models supported quantitative comparisons across genotypes. To probe genetic interactions, double and triple mutants were generated, enabling dissection of epistatic and compensatory relationships among MCUcx components. All experiments were performed with multiple biological replicates to ensure reproducibility and statistical robustness.

Our results establish the MCUcx as a critical determinant of mitochondrial function and organismal health in *C. elegans*. MCUcx mutants displayed normal developmental timings and brood size and affected more

subtle aspects of development. However, MCUcx mutants displayed profoundly impaired mitochondrial calcium uptake, swimming activity, stress tolerance and aging compared to wild-type controls, indicating compromised mitochondrial performance. Notably, genetic interaction studies uncovered compensatory dynamics, and the removal of certain regulatory subunits modulated the severity of outcomes, pointing to complex intra-complex regulation. Collectively, these data highlight that MCUcx integrity is indispensable for sustaining both cellular metabolism and whole-organism resilience.

This work represents the first systematic genetic dissection of the MCUcx in *C. elegans*, linking subunit-specific roles to mitochondrial performance and organismal health. By demonstrating that disruption of MCUcx subunits undermines energy production, stress responses, and longevity, our findings underscore the evolutionary conservation of mitochondrial calcium signaling. These insights carry broad translational significance for human health, as defects in MCUcx activity are increasingly recognized in neurodegeneration, metabolic diseases, and cancer. Furthermore, because mitochondria trace their origins to ancient microbial endosymbionts, the study of calcium-regulatory mechanisms in *C. elegans* bridges fundamental mitochondrial biology with themes of host-microbe interactions, cellular resilience, and ecological adaptation. Our results emphasize how mitochondrial regulation exemplifies natural evolutionary strategies for maintaining homeostasis in the face of stress. By deepening our understanding of these mechanisms, we can uncover unifying principles of cellular adaptation that transcend species boundaries. Such principles hold the potential to inspire innovative applications ranging from therapeutic interventions in human health to the development of sustainable solutions that harness natural antimicrobial processes and plant-microbe interactions. Ultimately, this integrative perspective paves the way toward biotechnologies with wide-reaching biomedical, agricultural, and ecological relevance.

Keywords: Caenorhabditis Elegans, Mitochondrial Calcium Uniporter (MCU), Cellular Resilience and Adaptation, Neurodegenerative and Metabolic Disorders, Natural Antimicrobial Strategies.

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