Regulators of Mitochondrial Calcium Signaling in *C. elegans*

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Mitochondria are essential organelles for most eukaryotic organisms. They house enzymes important for both metabolism and cell death, two processes that are key to the optimal functioning of an organism. Under conditions of stress, mitochondria are known to adapt by changing morphology, substrate utilization patterns and ATP production or, in extreme stress, signaling for cell death.

Cells commonly use calcium to signal stress to mitochondria. Synapse-like associations with the endoplasmic reticulum and plasma membrane allow efficient transfer of calcium into mitochondria. Since calcium directly binds and stimulates multiple TCA cycle enzymes, ATP generation in mitochondria proceeds in parallel to calcium-activated ATP-consuming processes in the cytosol. Mitochondria also sequester large amounts of calcium in pathological conditions such as ischemia-reperfusion (I-R) injury, during which cells are overloaded with calcium. When this storage capacity is exceeded, formation of a pore in the inner mitochondrial membrane allows free passage of solutes less than 1500 Da, such as calcium, through this otherwise selectively permeable membrane. This phenomenon, known as the mitochondrial permeability transition (mPT), leads to the loss of mitochondrial membrane potential and is a major cause of cell death in I-R injury. Molecular components of the mPT pore (mPTP) have remained elusive to identification.

We hypothesize that the mitochondrial calcium signaling machinery in *C. elegans* is evolutionarily conserved, based on the high degree of identity between known mammalian components and *C. elegans* orthologs. The genetic tractability of *C. elegans* and the ability to use fluorescent reporters in screens of live animals might allow us to identify components of the mPTP, among other regulators of mitochondrial calcium signaling.

We created a strain expressing mitochondrially-targeted GCaMP5, a fluorescent calcium reporter. We observed that mutations in *C. elegans* orthologs of known regulators of mammalian mitochondrial signaling produced changes in this reporter's fluorescence consistent with conserved function. To identify other regulators, we performed an EMS mutagenesis screen and identified mutants with significant changes in reporter fluorescence, possibly indicating changes in basal mitochondrial calcium levels. We are mapping and characterizing these mutants. Also, we are developing functional assays to test for altered mitochondrial calcium signaling in these mutants.

We hope our efforts to further elucidate the mitochondrial calcium signaling pathway will provide insight into its role in physiology and disease as well as help identify new drug targets for I-R injury and other conditions with similar etiology.

Poster
Meeting: Aging, Metabolism, Stress, Pathogenesis and Small RNAs in *C. elegans*
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