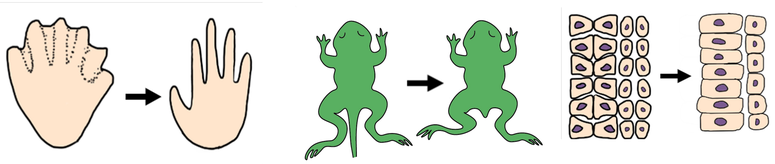
Mitochondrial transport and trapping during compartmentalized cell death: role of caspase and kinesins

Rashna Sharmin[1], Sara Pena Carmona[2], Ginger Clark[1], Karen Juanez[1], Aladin Elkhalil[1], Mark W. Pellegrino[1], Shai Shaham[2], Piya Ghose[1]
1.The University of Texas at Arlington 2.The Rockefeller University

Background



- Programmed cell death is essential for development and homeostasis
- Elimination of specialized cell such as neuron is crucial for development and homeostasis
- Intracellular transport is important in specialized cells such as neuron

- Mitochondria reach synapses to supply energy

- Mitochondria move to the point of injury for regeneration

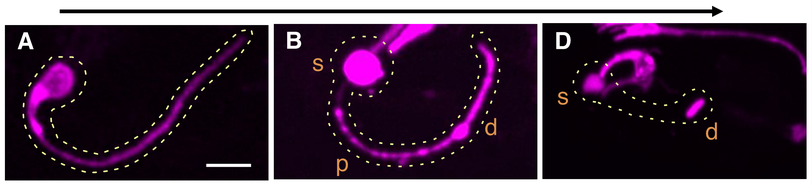
Central Questions

How does intracellular transport of mitochondria effect the death of specialized cells?

C. elegans is an excellent in-vivo genetic tool

- **Genetically tractable**
- **Invariant lineage**
- Short life cycle
- Self fertilizing
- **Transparent: *in vivo* studies**

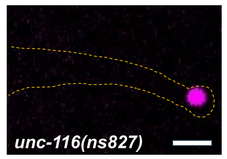
Compartmentalized Cell Elimination (CCE) is a novel, developmental program of "tripartite" killing

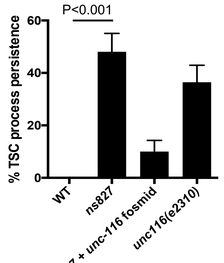


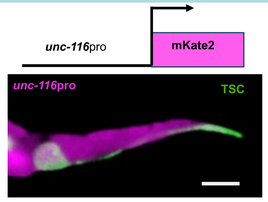
Embryonic TSC epithelial cell

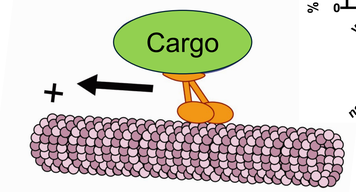
Result and discussion

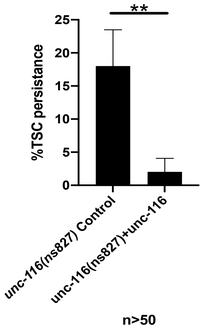
The Kinesin-1 homolog UNC-116 promotes CCE





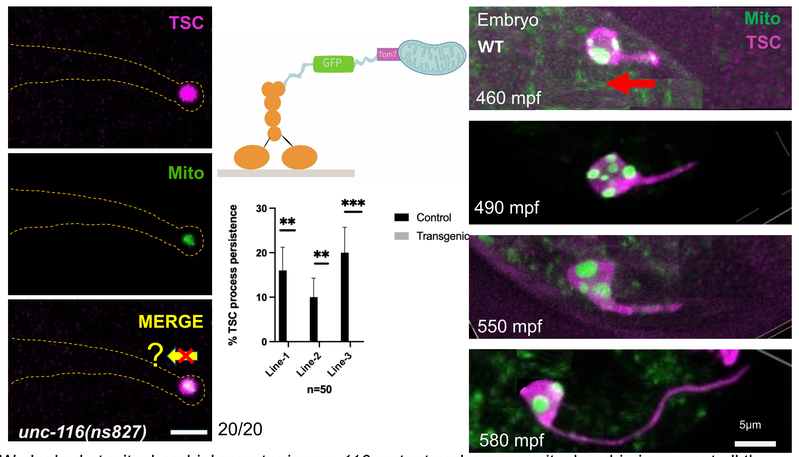






- Through whole genome sequencing and fosmid-rescue the relevant gene was identified as *unc-116*
- UNC-116/ Kinesin-1 is a microtubule '+end' directed motor protein that carries various cargos
- Another allele *unc-116* of showed similar CCE defect
- *unc-116* is expressed in the TSC
- CCE defect of the *unc-116* mutant was rescued TSC specifically

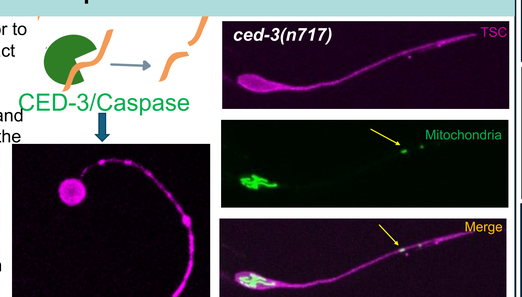
Mitochondria undergo irreversible retrograde transport prior to CCE



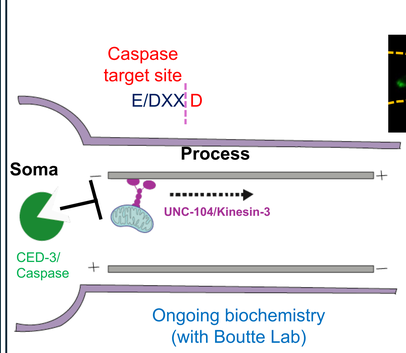
- We looked at mitochondrial reporter in *unc-116* mutant and we see mitochondria is present all the time in the TSC remnant
- Mitochondria-specific UNC-116 rescued *unc-116* phenotype significantly
- As the TSC develop in the embryo mitochondria moves towards soma from the process retrogradely as a preparation for CCE, there is no mitochondria in the TSC process prior to death

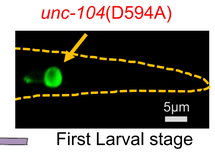
Mitochondria are present in the intact process of *ced-3*/caspase mutants

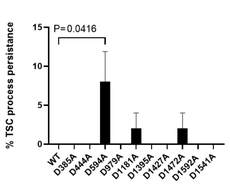
- As mitochondria moves soma-ward prior to CCE, we looked at a strain that has intact TSC to see how mitochondria is doing there
- In *ced-3*/caspase mutant TSC is intact and interestingly mitochondria is present in the process, anterograde mitochondrial movement is allowed in *ced-3*/ caspase mutant
- CED-3/ Caspase is a cysteine protease that cleaves substrate
- May be CED-3/ Caspase is targeting an anterograde mitochondrial motor

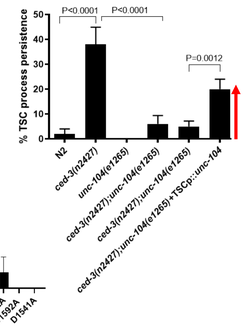


UNC-104/Kinesin 3 may be a CED-3/Caspase substrate



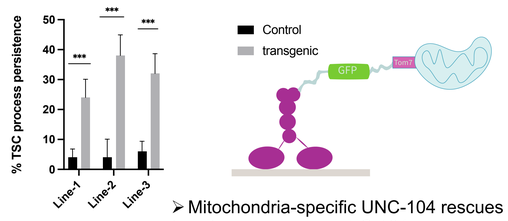


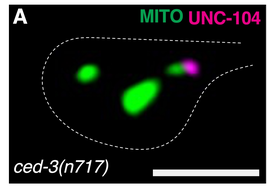




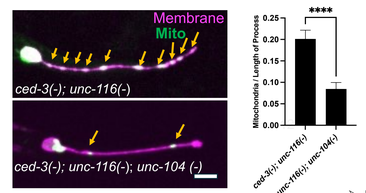
- UNC-104/ Kinesin 3 has 10 putative caspase target sites
- While we mutated all the target sites individually via CRISPR individually one site showed significant CCE defect
- We also found that CED-3/ Caspase is expressed and also active in early embryo may be to cleave UNC-104 as a preparatory step for CCE

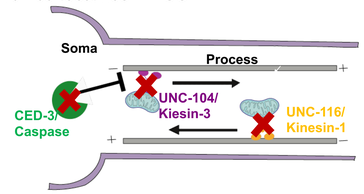
Validating mitochondria as a non-traditional cargo of UNC-104/Kinesin 3





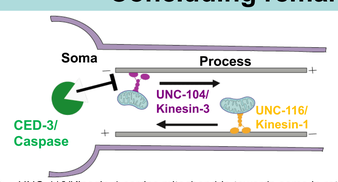
- Mitochondria-specific UNC-104 rescues the *unc-104(-)* mutant CCE defect
- Mitochondria and UNC-104 co-localizes in TSC





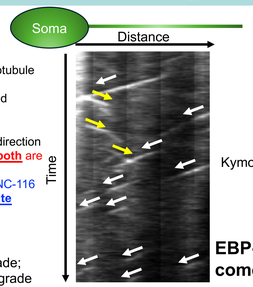
- UNC-104/Kinesin-3 is involved in mitochondrial anterograde transport

Concluding remarks



- UNC-116/Kinesin-1 carries mitochondria towards soma in retrograde manner
- UNC-104/ Kinesin 3 would carry mitochondria towards process but cannot do so as being cleaved by CED-3/caspase
- By cleaving UNC-104 early on CED-3/Caspase is doing novel non-killing function
- Mitochondria is a novel UNC-104 cargo
- TSC process has mixed microtubule polarity

Evidence of mixed microtubule polarity



- Motors travel along microtubule cytoskeleton
- Microtubules have defined polarity (+ and - end)
- Motors move in specific direction
- UNC-104 and UNC-116 **both** are + end directed

Our model: UNC-104 and UNC-116 carry mitochondria in **opposite** direction

White arrows, retrograde;
Yellow arrows, anterograde

Acknowledgements

This work was funded by a Cancer Prevention Research Institute of Texas (CPRIT) Recruitment Grant and a National Institutes of Health National Institute of General Medical Sciences R35 (MIRA) to P. G.

Reference

1. Ghose, P. *et al.* EFF-1 fusogen promotes phagosome sealing during cell process clearance in Caenorhabditis elegans. *Nat. Cell Biol.* **20**, 393–399 (2018).