Quasi-living cell morphology in the absence of EOR-1/PLZF, chromatin reglutors, & WAH-1/AIF1 in a cell that dies via a non-cannonical apoptotic program

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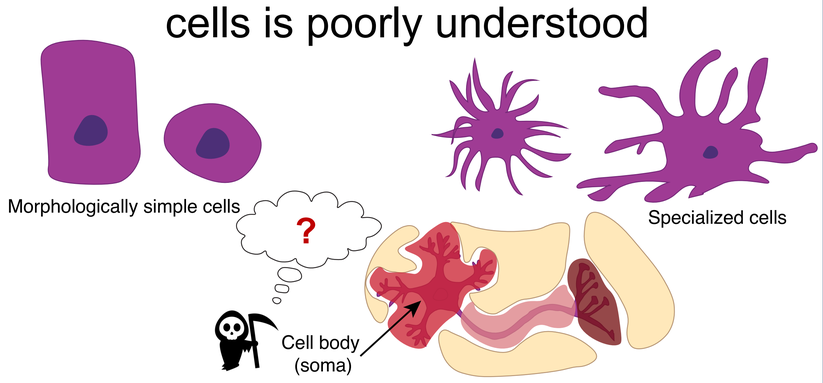
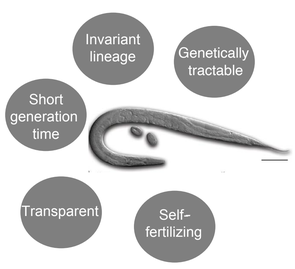
Background

Elimination of morphologically complex cells is poorly understood



- Specialized cells are often morphologically complex with vast subcellular architectures
- How do cells with vast subcellular architecture eliminate themselves
- Specifically, **how is the cell body (soma) eliminated during the death of specalized cells?**

C. elegans is an excellent system to study morphologically complex cell death



H. sapiens

Bcl-2 ⊣ Cyto C → APAF-1 → Caspase-9 → Caspase-3, Caspase-7 → Cell Death

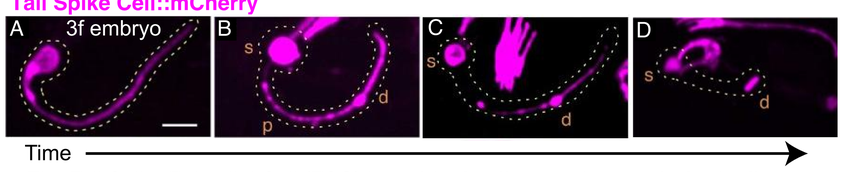
D. melanogaster

BH3-only like: Reaper, Grim, Hid ⊣ DIAP1 ⊣ Caspase-9-like: Dronc (Ark, Apaf-1-related) → DrICE, Dcp-1 → Cell Death

C. elegans

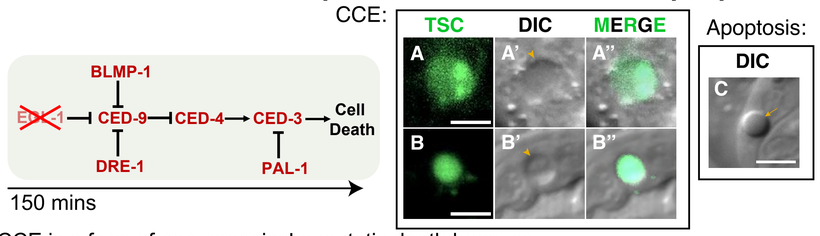
BH3-only like: EGL-1 ⊣ Bcl-2-like: CED-9 ⊣ Apaf-1-like: CED-4 → Caspase-9-like: CED-3 → Cell Death

The Tail Spike Cell (TSC) dies via Compartmentalized Cell Elimination (CCE)



- The Tail Spike Epithelial Cell (TSC) is **an ideal cell to study morphologically complex cell death**
- The 3 morphological compartments of the TSC die in three seperate ways

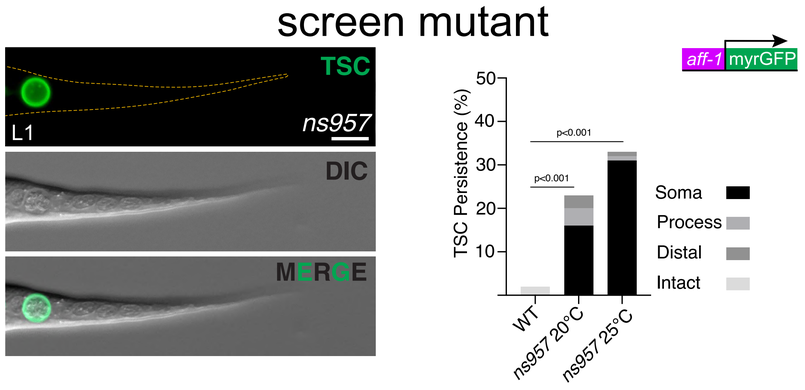
CCE is CED-3 dependent but non-apoptotic



- CCE is a form of non-canonical apoptotic death because:
- CED-3/Caspase 9 executioner dependent and CED-5/Dock180 engulfment dependent
- But EGL-1 independent & weakly dependent on CED-9
- TSC soma seems to not form a refractile apoptotic corpse

Results

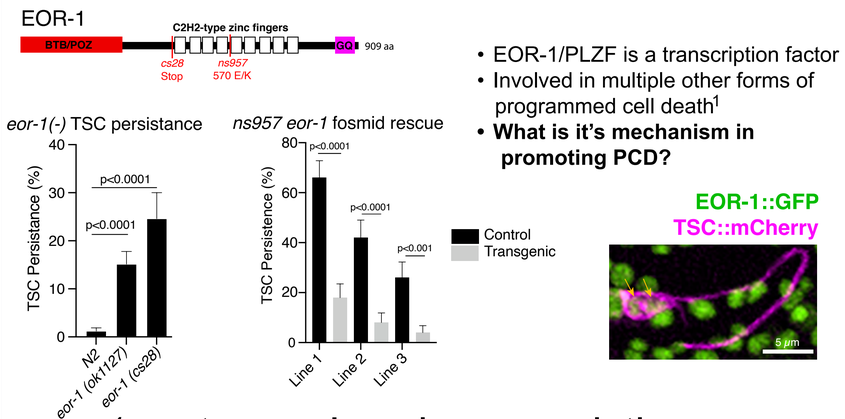
ns957 is a soma-specific forward genetic screen mutant



- A forward genetic screen for CCE mutants recovered a mutant with a persisting, enlarged, soma alone called *ns957*
- *ns957*'s phenotype is temprature sensitive, showing an increase in soma persistance at 25C

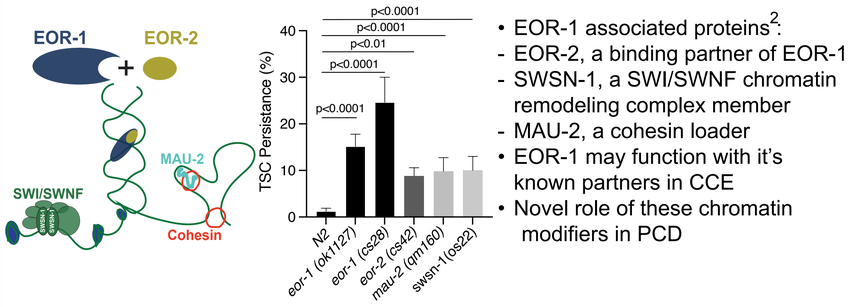
Results Continued

The causative lesion of *ns957* is in *eor-1*



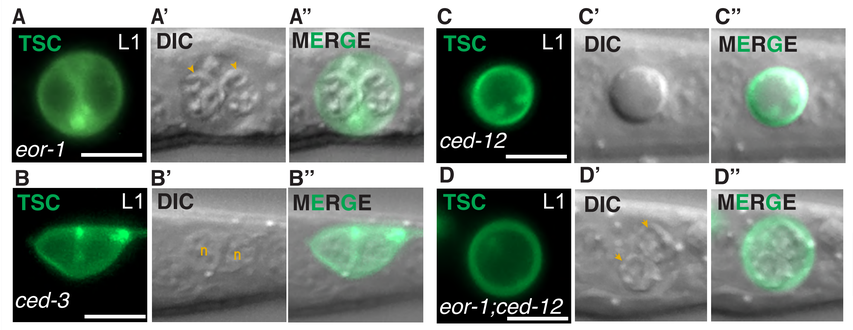
- EOR-1/PLZF is a transcription factor
- Involved in multiple other forms of programmed cell death[1]
- **What is it's mechanism in promoting PCD?**

eor-1 partners also show persisting somas



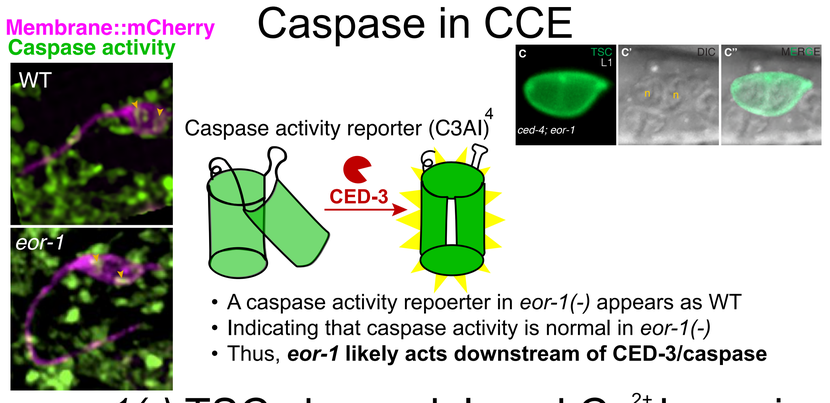
- EOR-1 associated proteins[2]:
  - EOR-2, a binding partner of EOR-1
  - SWSN-1, a SWI/SWNF chromatin remodeling complex member
  - MAU-2, a cohesin loader
- EOR-1 may function with it's known partners in CCE
- Novel role of these chromatin modifiers in PCD

eor-1(-) persisting soma is a quasi-living cell remanent



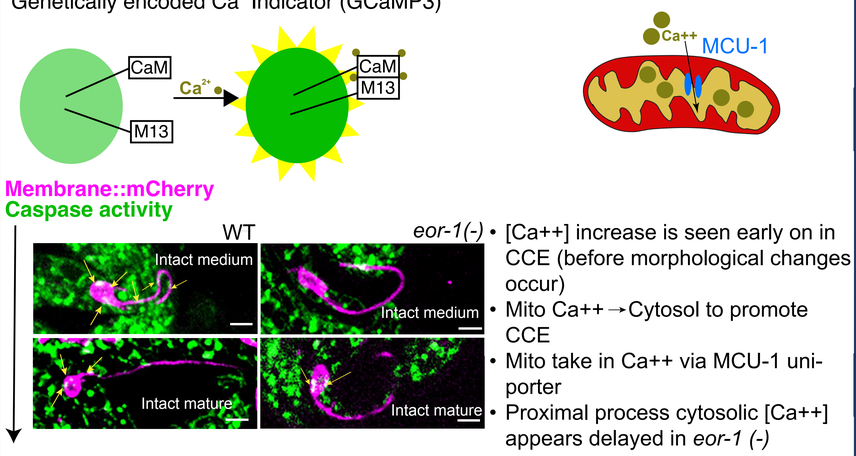
- **Is the *eor-1(-)* soma alive or dead?**
- *ced-3(-)* living soma is oblong with two flat nuclei visible on DIC microscopy
- *ced-12(-)* dead soma appears as a round, unengulfed refractile corpse
- *eor-1(-)* mutants are rounded and enlarged with nuclear condensations present
- *eor-1(-)*; *ced-12(-)* double mutants **appear neither alive or dead**

EOR-1 likely acts downstream of CED-3/ Caspase in CCE



- A caspase activity repoerter in *eor-1(-)* appears as WT
- Indicating that caspase activity is normal in *eor-1(-)*
- Thus, ***eor-1* likely acts downstream of CED-3/caspase**

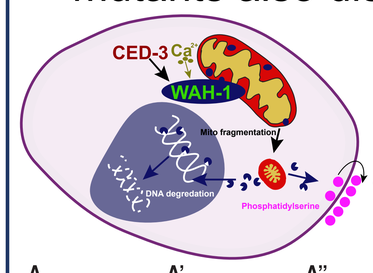
eor-1(-) TSC shows delayed Ca^{2+} dynamics



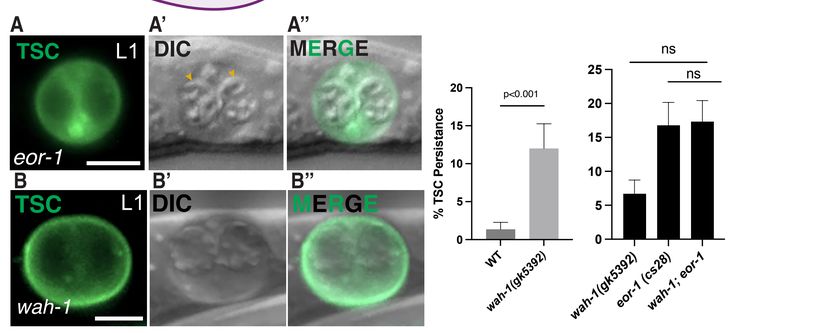
- [Ca++] increase is seen early on in CCE (before morphological changes occur)
- Mito Ca++ →Cytosol to promote CCE
- Mito take in Ca++ via MCU-1 uniporter
- Proximal process cytosolic [Ca++] appears delayed in *eor-1 (-)*

Results Continued

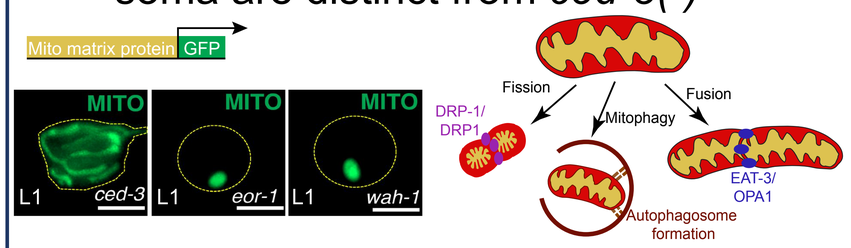
wah-1/aif1, a putative EOR-1 target gene, mutants also display persisting somas



- WAH-1/AIF1 is a mitochondrial oxidoreductase
- Plays a role in mito function (OXPHOS, structure)
- CED-3 cleaves WAH-1 & it translocates to the nucleus to degrade DNA
- **Does *wah-1(-)* cause a persisting TSC soma?**
- What is the developmental role of WAH-1?



Mitochondria in *eor-1(-)* & *wah-1(-)* larval soma are distinct from *ced-3(-)*



- WAH-1 is a mitochondrial proetin that maintains mitochondrial integrity and structure
- Mitochondria appear reticular in *ced-3(-)* TSC soma
- *eor-1(-)* and *wah-1(-)* soma mitochondria are distinct
- **What is the natue of these mitochondria?**
- CCE provides an in vivo & developmental context to study the dual roles of WAH-1: as an executioner & as a protein essential for normal mitochondrial function

Proposed model

- EOR-1/PLZF and its partners EOR-2, MAU-2/MAU2, & SWSN-1 (SMARCC 1/2) all promote cell body specific elimination in CCE
- EOR-1 may do this by regulating the gene expression of WAH-1/AIF1
- In the absence of EOR-1, WAH-1 is unable to be expressed
- Loss of WAH-1 leads to undegraded DNA in CCE causing a "quasi-living" morphology
- WAH-1 also plays a role in mitochondrial dynamics and Ca++ signaling

Outstanding questions

- Is the quasi living soma a transient state?
  - Examine eor-1(-) soma across developemntal stages
- What is the nature of the nuclei in *eor-1(-)* persisting soma?
  - Lamin::GFP
- Does *eor-1(-)* function cell autonomously?
  - Cell specific rescue
- Do *eor-1(-)*, *wah-1(-)* somas externalize phosphatidyl serine?
  - MFG-E8::GFP marker
- What do mitochondrial dynamics look like in *eor-1(-)*, *wah-1(-)* across development?
  - Mitochondrial Matrix::GFP in embryonic TSC
- Where is WAH-1 expressed across in the TSC during CCE?
  - WAH-1::mCherry
- Does DNA degradation failure cause quasi living cell morphology?

References

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