ER network stability promotes organized microtubule disassembly during Compartmentalized Cell Elimination

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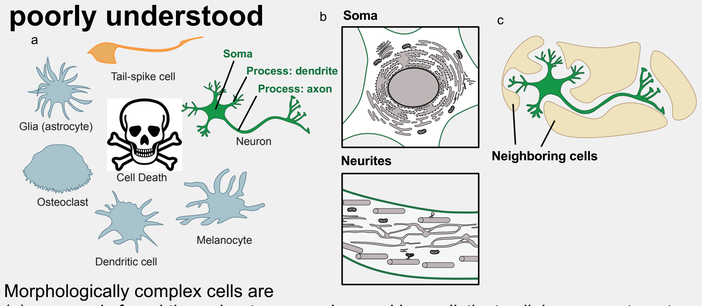
Abstract

Here we report that MTs have stereotyped dynamics throughout the development and death of the TSC. Through forward genetic screens, we found that genes promoting endoplasmic reticulum (ER) network stability, *atnl-1*/Atlastin and *lnp-1*/Lunapark, which encode the homologs of human Atlastin GTPase and Lunapark, promote process dismantling during CCE. We find that *atnl-1*/Atlastin and *lnp-1*/lunapark promote the function of the conserved MT-severing ATPase SPAS-1/Spastin in facilitating CCE. We propose that the stable ER network and ER network stability proteins anchor SPAS-1/Spastin to allow for precisely targeted and organized MT disassembly, which leads to the highly defined demise of the TSC process during CCE. Our findings shed new light on the localized elimination of complex cells and provide a mechanism for how MTs are linked to pruning and neurodegeneration through an unexpected connection with the ER.

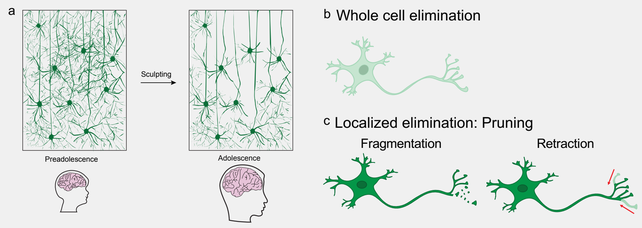
Background

Elimination of morphologically complex cells is poorly understood



Morphologically complex cells are
(a) commonly found throughout an organism and have distinct cellular compartments.
(b) have differing subcellular distribution of organelles between compartments.
(c) each compartments are surrounded by different neighbors and microenvironments.

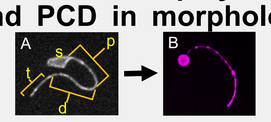
Developmental pruning is essential for proper neuronal function



(a) Efficient brain function requires sculpting and removal of unnecessary connections.
(b) Neuron elimination: whole cell
(c) Neurite specific elimination or pruning; fragmentation and retraction.

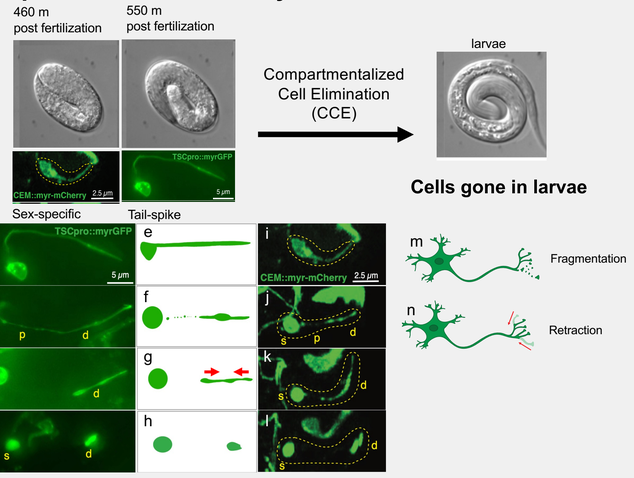
Objective & Approach

Understand the molecular players and mechanism of pruning and PCD in morphologically complex cells.



(a) Morphologically complex cells have distinct compartments: (s) soma, (p) proximal process, (d) distal process and (t) process tip
(b) Morphologically complex cells display complex elimination

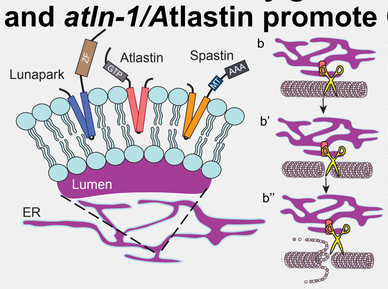
Model system: Relatively long-lived morphologically complex cells in the embryo



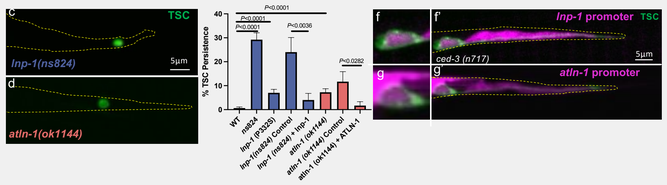
Embryonic cell death: (a-j) Compartmentalized Cell Elimination (CCE) observed in tail-spike cell (TSC) and (i-l) sex-specific cephalic male neurons (CEM), schematic (e-h). (a,e,i) intact cell, (b,f,j) beading of proximal process, (c,g,k) distal process retraction,(d,h,l) prior to phagocytosis (b,f,j) resemble fragmentation during pruning (m) and (c,g,k) resemble retraction during pruning (n).TSCpro, tail-spike cell promoter; myrGFP, myristoylated GFP. CCE occurs in two different cells, potentially universal and maybe a broad phenomenon.

Results

ER network stability genes *lnp-1*/Lunapark and *atln-1*/Atlastin promote CCE

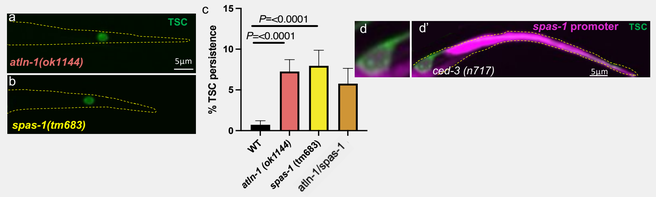


(a) Schematic of ER stability proteins Lunapark, Atlastin and Microtubule (MT) severing ATPase Spastin. Lunapark and Atlastin are localized at ER 3-way junctions. Spastin is a MT severing ATPase that is a binding partner of Atlastin. (b,b',b'') Schematic demonstrating the function of Spastin in cleaving MTs.



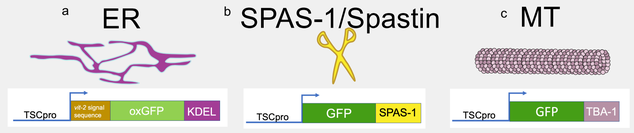
Persisting TSC process in larvae (c) *lnp-1(ns824)* and (d) *atln-1(ok1144)* mutants. (e) Quantification of TSC persistence and cell specific rescue of *lnp-1*/Lunapark and *atln-1*/Atlastin. Both *lnp-1*/Lunapark and *atln-1*/Atlastin function cell autonomously. *lnp-1*/Lunapark and *atln-1*/Atlastin data are mean ± s.e.m. Statistics: two-tailed unpaired t-test. (e,e') *lnp-1*/Lunapark *and* (f,f') *atln-1*/Atlastin are expressed in TSC.

MT severing SPAS-1/Spastin which associates with ATLN-1/Atlastin promotes CCE



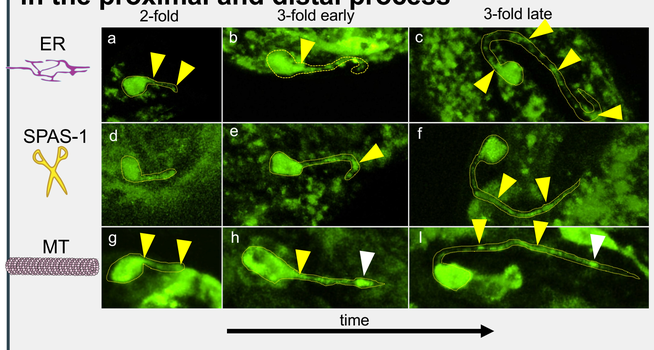
Persisting TSC process in larvae (a) *atln-1*(*ok1144*) and (b) *spas-1 (tm683)* mutants (c) Quantification of TSC persistence of *atln-1*(ok1144), *spas-1 (tm683)* and *atln-1/spas-1* double mutant. Data are mean ± SEM. Statistics: two-tailed unpaired t-test. *atln-1/spas-1* double mutant show no additive effect, possibly working in the same genetic pathway (d,d') *spas-1*/Spastin is expressed in TSC.

Design of markers generated and used



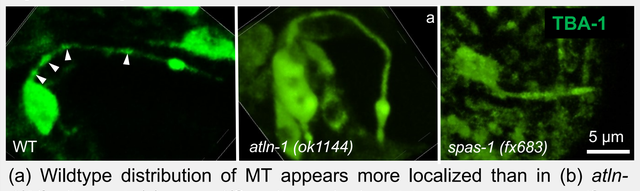
Schematic of markers generated: (a) TSC promoter driven oxGFP with KDEL sequence. (b) TSC promoter driven GFP SPAS-1. (c) TSC promoter driven GFP TBA-1.

ER, SPAS-1 and MTs show differential distribution in the proximal and distal process



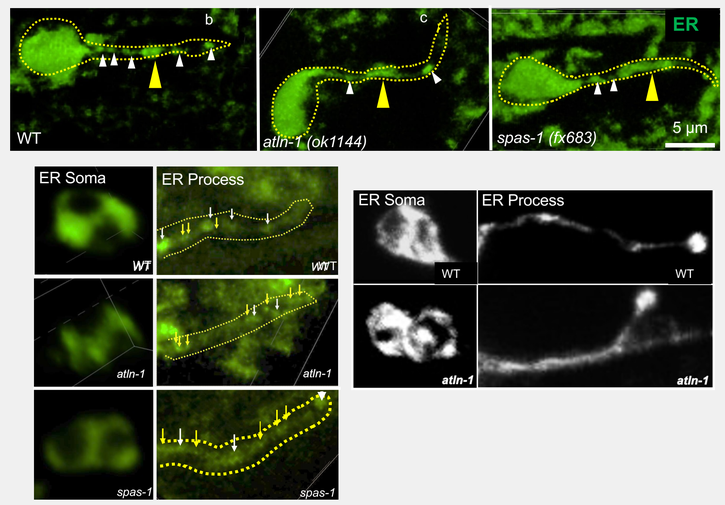
(a-i) ER, SPAS-1 and MT markers observed in different embryonic stages up to the beginning of cell death. (a,b) Early ER process appears more uniform with soma-process junction enrichment while in (c) 3-fold late a more discrete localization with enrichment distally. (d-f) SPAS-1 and (g,h) TBA-1 similarly become more localized with time and (i) enriched distally. ER, SPAS-1 and MTs appears highly dynamic across development and prior to death.

MT appear more stabilized in *atln-1*/Atlastin and *spas-1*/Spastin mutants



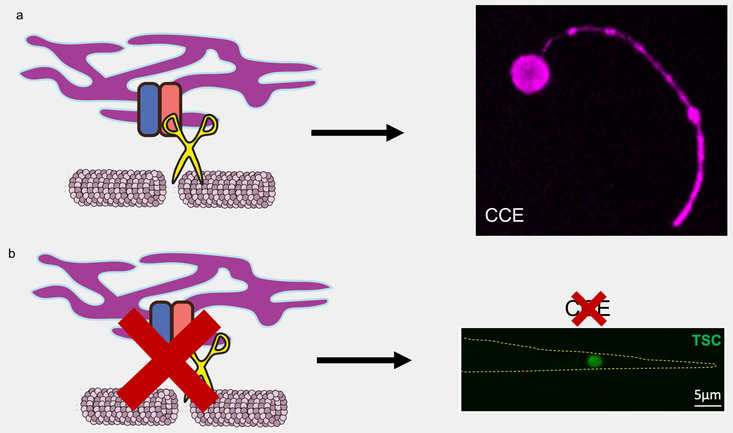
(a) Wildtype distribution of MT appears more localized than in (b) *atln-1*/Atlastin and (c) *spas-1*/Spastin mutant which appears more uniform suggesting more stable MTs.

ER distribution appears less enriched in *atln-1*/Atlastin and *spas-1*/Spastin mutants



(a) WT ER appears discretely localized as shown by the white arrows with enrichment in the distal node (yellow arrow). (b) *atln-1*/Atlastin mutant appears less discretely localized including the distal node showing possible abnormal ER distribution. (c) *spas-1*/SPAS-1 mutant show some discrete localization with possible abnormal distal node enrichment. ISIM images show similar trend of ER localization.

Proposed Model



We propose that (a) ER network stability proteins position SPAS-1/Spastin at specific microtubule points allowing for dismantling and CCE to take place. (b) ER instability leads to SPAS-1/Spastin position failure and CCE can no longer take place, leading to TSC persistence

Future direction

- Time-lapse image microscopy of ER, SPAS-1, and microtubule markers in wildtype and mutant strains.
- *spas-1* cell specific rescue
- Does ER structure affect SPAS-1 localization?
- Does ATLN-1 associate with LNP-1 and SPAS-1 directly?
- How are MT influenced by SPAS-1 and ER?
- Broader role of ER in CCE?
- MT dynamics in CCE
- Tri-color imaging and marker validation:
  - ER
  - SPAS-1
  - ATLN-1
  - MT

References

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- Juanez K, Ghose P. Repurposing the Killing Machine: Non-canonical Roles of the Cell Death Apparatus in *Caenorhabditis elegans* Neurons. Front Cell Dev Biol. 2022 Feb 14

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