Endoplasmic Reticulum Exit Sites scale with somato-dendritic size in neurons.

Ruben Land, Richard Fetter, Xing Liang, Christopher Tzeng, Caitlin Taylor, and Kang Shen

Corresponding author(s): Kang Shen, Stanford University

Review Timeline:

Submission Date:	2023-03-10
Editorial Decision:	2023-04-06
Revision Received:	2023-07-06
Accepted:	2023-07-29

Editor-in-Chief: Matthew Welch

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E23-03-0090
TITLE: Neurons alter endoplasmic reticulum exit sites to accommodate dendritic arbor size

Dear Dr. Shen:

Thank you for submitting your work to MBoC. I agree that it is interesting that you have distinguished that biosynthetic fate depends on instrinsic neuronal properties rather than feedback from arbor size. The reviewers have suggested important additional experiments and controls, as well as areas of clarification. In your resubmission, please address all the reviewers' comments point by point. In particular, please clarify in the text and titles whether your (or other) data argue that the correlation between soma size and ERES number has a causal relationship (and in which direction).

Sincerely,

Avital Rodal
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Shen,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 90 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

MBoC offers the option to publish your work with immediate open access. Open access can increase the discoverability and usability of your research. If you would like to publish your paper with immediate open access but did not select this option during initial submission, please contact the editorial office at mbc@ascb.org.

Thank you for submitting your manuscript to MBoC. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The manuscript by Land et al. describes experiments investigating how early secretory specializations, namely ER exit sites (ERES) scale with neuron size. Intuitively, larger neurons place more demand on the secretory network for synthesis and delivery of integral membrane proteins and secreted factors over a larger volume/surface area. Indeed, this is borne out in the experiments described here; neurons with larger somatodendritic volumes (e.g. PVD neurons) also possess more ERES, quantified using transgenic worms expressing GFP-SEC16. The authors ask whether various genetic manipulations designed to perturb the symmetry of the final cell division generating PVD neurons (thus generating different initial somatic volumes) impact ERES number. Initial (i.e. immediately following final cell division to generate PVD) ERES number scales with somatic cell volume suggesting the COPII proteins that form ERES are passively inherited. Mutants with altered dendritic outgrowth/elaboration phenotypes do not impact ERES number. As PVD somas subsequently grow (and dendrites elaborate), ERES number increases. If subsequent growth is prevented, through genetic loss of TOR signaling, ERES number remain stable. In some manipulations (starvation) somatic volume decreases, with a concomitant decrease in ERES number. Thus, the number of ERES tightly scales with overall somatic volume. While the experiments appear to be carefully carried out with multiple genetic manipulations providing consistent results, I found the significance and overall conclusions of the work to be incremental.

Major points:
The title of the manuscript suggesting that ERES number supports dendrite size is misleading. While this may be true, forward trafficking through ERES supports a broad range of cellular functions. Lack of dendritic elaboration/maintenance is not surprising when forward secretory trafficking through ERES is disrupted (I'm sure there are numerous cellular dysfunctions). Data throughout the manuscript show that ERES number simply scales with soma size across a range of genetic manipulations. Thus, the manuscript could have equally been titled "Neurons alter ERES to accommodate soma size", or given the correlative nature of the experiments, "Soma size predicts ERES number"

The authors only look at somatic ERES. Given the potential for local protein synthesis/secretion, are ERES observed in the growing/established dendrites as a potential local source of secretory proteins? In this regard, local ERES trafficking could more directly support dendrite size/function, but this is not addressed.

In Fig. 3 how are boundaries drawn between mother/sister cells? From the images it is unclear how these boundaries were delineated and how initial cell volumes were quantified.

The discussion leads with "we show that ER-exit site (ERES) number is predictive of neuron size and complexity" however given the correlative nature of the experiments, one could also say the opposite, that cell size predicts ERES number.

Minor point
In Fig. 4 Merge and mCh panels switched in WT

Reviewer #2 (Remarks to the Author):

This well written and interesting paper describes new findings that link dendritic complexity to neuron-specific genetic regulation of cell size and ER secretory capacity. This work exploits the ready tractability of the model organism C. elegans for genetic analysis and live cell imaging. Initial results suggest that dendritic arbor complexity is correlated with the number of ER exit sites (ERES) associated with the Golgi apparatus. Additional experiments manipulate the fate of the highly branched PVD nociceptive neuron to investigate the mechanisms that establish and maintain ERES number. Genetic mutants (e.g, lin-5) that alter the asymmetry of the initial cell division that gives rise to PVD revealed a correlation between the size of the PVD soma and ERES number. A mutation that disables the mec-3 transcription factor and drastically restricts PVD dendritic branching also results in a failure to maintain ERES number in postmitotic PVD soma. Finally, TOR, a conserved regulator of growth promoting lipid and protein biosynthesis, is shown to function downstream of MEC-3 to control ERES number and dendritic complexity. Interestingly, nutrient availability, a known regulator of TOR activity, is also demonstrated to control ERES number in a mechanism that depends on TOR. This work is notable because if forges a new link between the genetic specification of neuronal morphology with established components of cell biological and biosynthetic pathways that control neuronal growth. Additional experiments are needed, however, to support the central hypothesis that ERES number is directly related to dendritic complexity.

Major Revisions:
1. Figure 4C shows a strong correlation of ERES with the size of the PVD soma. For example, individual worms with a lin-5 conditional allele show a range of sizes for the PVD soma that correlate with ERES number. The model proposed in this paper also predicts that PVD dendritic complexity should be similarly linked to ERES number but these data are not provided. This experiment should be conducted.
2. Similarly, results presented in Figure 6 argue that nutrients function with LET-363/TOR to promote ERES number in PVD. Does starvation during development also impair PVD dendritic branching as the proposed model suggests?

Additional concerns
1. A conditional PVD floxed allele of let-363/TOR was used for experiments in Fig 5 and 6 but no independent data are presented to confirm that "floxing" actually worked. For example, for mouse conditional mutants, its standard practice to use an indicator allele (Rosa26) to detect cell-specific cre activity. Aternatively, knockins of fluorescent markers at the target locus are activated by Cre to confirm site specific recombination.
2. A transgenic GFP reporter was used to detect a role for mec-3 for in vivo expression of let-363/Tor. This reporter may not be reliable, however, because it is limited to an upstream promoter that may not contain key regulatory domains and is also over-expressed. This experiment would be more convincing if it used smFISH, which is well established for C. elegans, or a GFP reporter knockin at the endogenous let-363 gene.

Minor Revisions:

1. The authors use PVD dendritic branch number as a proxy for neuron size and correlate this with ERES number, but some of the wording makes it unclear whether they think it is branches specifically that require the large ERES number or just size of the dendrite or neuron. It would be interesting for the authors to address this point in the discussion, or correlate these factors (neuron size, branch number, dendrite size) with ERESs to make a more concrete assessment. For example, PDE is larger than AVM and PVM but has the same number of ERESs. Is this because their size difference isn't that great or because the branch number isn't different. Could the authors look at the next largest neuron after PVD/FLP (perhaps the unbranched PVC or AVA) to get an additional datapoint for the size vs branching question?
2. A summary cartoon illustrating the proposed model would be a useful addition that would help readers.
3. General comment about X-Y plots and histograms. Results would be more easily visualized if color were used to distinguish data classes (e.g., neuron types, genotype, etc.). In current plots, data points are depicted as different black and white shapes (circles, triangles, squares, etc) and are difficult to distinguish.
4. Figure 1b. SEC-16 puncta for AVM, PVM, PDE are not distinguishable...likely because these results are limited to whole numbers and only 1 or 2 SEC-16 puncta are detected. The apparent fusion of these data into a single "mark" could be ameliorated by using a jitter plot.
5. What criteria were used to identify ERES in panels 1d-f?
6. Figure 1. Black and white arrows in panels d-f are barely visible.
7. Figure 4. unc-86 (4b) is not mentioned in the legend.
8. Figure 5a. The data point for - 1 hr is positioned to the left of the Y-axis. This is confusing and can be rectified by moving the Y-axis label further to the left.
9. Pg 9. reference to (Fig 3b and top right 3c) should be (Fig 4b and top right 4c)
10. The timing of the experiments in Figure 6d-e is hard to follow. Could you make a graphic showing when animals are on and off the food?
11. FLP has an elevated number or ERESs relative to AVM/PVM/PDE. Do the authors also think this is due to an asymmetric division with its sister AIZ or through a separate mechanism? I am not requesting any new experiments here but would be interested in any thoughts the authors have on FLPs development vs PVDs.
12. figure S1: Why is the AMAN-2::GFP Golgi marker used instead of SEC-16::GFP. Can you explain in the text why a Golgi marker is a safe proxy for ERESs?
13. typos in the discussion: In mec-3(e1338) mu-tants, as well as starvation conditions.... unc-86(e1416) and mec-3(e1338) are con-sistent with the differential expression
14. The methods section refers to FRAP experiments that are not included in the paper

We thank the reviewers for their thoughtful and constructive comments and suggestions. In response, we have conducted additional experiments and have addressed their comments point-by-point below. Changes to the original manuscript are indicated in red text and modified portions of figures have been indicated below.

Reviewer 1

Major points

1. The title of the manuscript suggesting that ERES number supports dendrite size is misleading. While this may be true, forward trafficking through ERES supports a broad range of cellular functions. Lack of dendritic elaboration/maintenance is not surprising when forward secretory trafficking through ERES is disrupted (I'm sure there are numerous cellular dysfunctions). Data throughout the manuscript show that ERES number simply scales with soma size across a range of genetic manipulations. Thus, the manuscript could have equally been titled "Neurons alter ERES to accommodate soma size", or given the correlative nature of the experiments, "Soma size predicts ERES number"

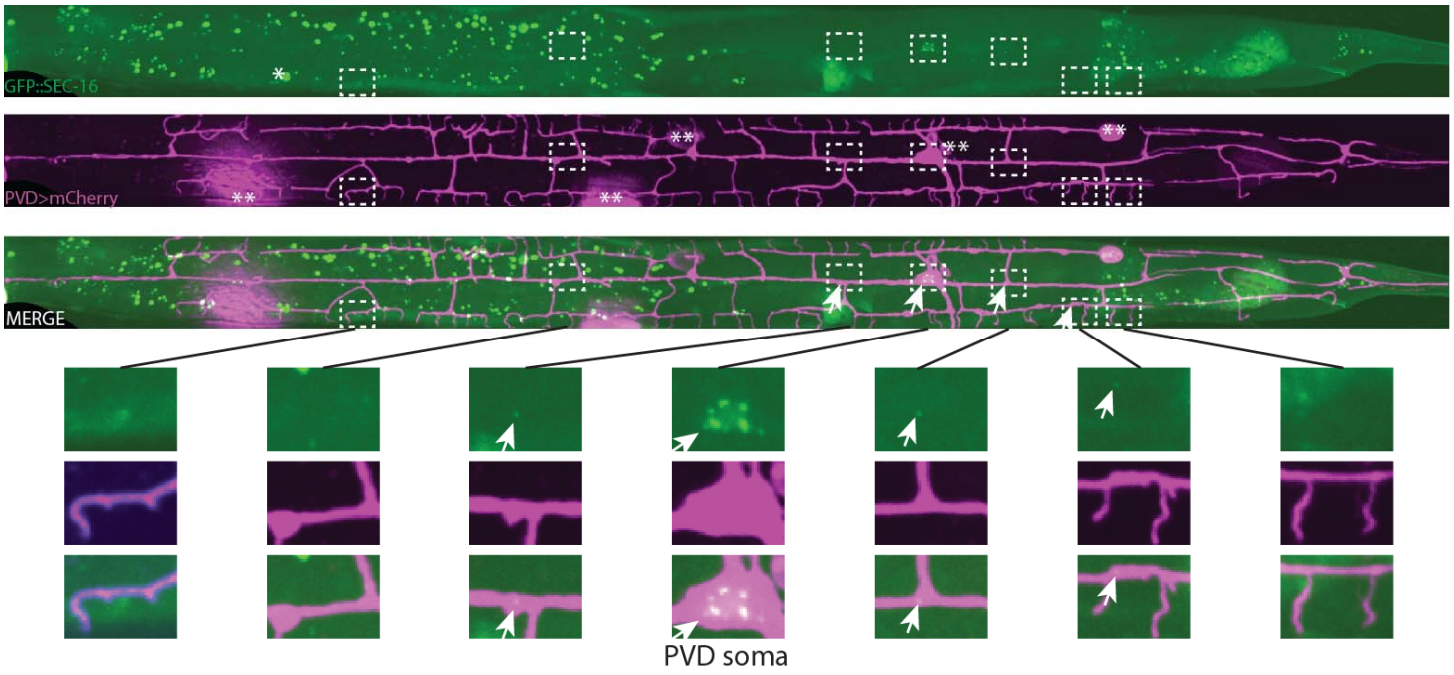
Response 1:

> We have retitled the manuscript “Endoplasmic Reticulum Exit Sites scale with somato-dendritic size in neurons.”

2. The authors only look at somatic ERES. Given the potential for local protein synthesis/secretion, are ERES observed in the growing/established dendrites as a potential local source of secretory proteins? In this regard, local ERES trafficking could more directly support dendrite size/function, but this is not addressed.

Response 2:

> We agree that local protein secretion in peripheral dendrites is possible and may be important for dendrite outgrowth and neuron function. However, signal from endogenous ERES markers in distal dendrites is very rare in all animals, and the overwhelming majority of ERES markers are seen in the neuron soma (as shown in the accompanying figure below). It is possible that peripheral ERESs exist more commonly than we observe, but are below our threshold of detection. Given this dearth of detection in the periphery, we focused our analyses on somatic ERESs. Overexpression of ERES and Golgi markers does not remedy this issue because overexpression can lead to mis-localization.



The above figure is a representative confocal fluorescence microscopic image of PVD's full morphology labeled with mCherry driven by the PVD-specific promoter Pser2prom3, and an endogenous ERES marker, GFP::SEC-16 (white arrows), which is flipped on in PVD by a flippase driven by the lineage-specific promoter Punc-86. Sections of the image labeled with a white dashed box were enlarged and shown below.
*Green puncta labeled with a single asterisk are examples of autofluorescent gut granules, which make up the majority of green puncta seen in this image and fall outside of PVD, as can be clearly observed in the original unprojected 3D image stack. Genuine signal from GFP::SEC-16 is indicated by white arrows.
**Note that mCherry signal indicated with a double asterisk is due to co-injection markers used to identify animals of interest, and are not part of PVD morphology.

3. In Fig. 3 how are boundaries drawn between mother/sister cells? From the images it is unclear how these boundaries were delineated and how initial cell volumes were quantified.
The discussion leads with "we show that ER-exit site (ERES) number is predictive of neuron size and complexity" however given the correlative nature of the experiments, one could also say the opposite, that cell size predicts ERES number.

Response 3:

We have amended the Neuron Soma Measurements methods section and the first paragraph of the discussion to include the following text shown in red, in order to address these points:

a. Z-stack still images taken on the 3i spinning disc microscope, as described above, were projected into a single plane using ImageJ software. Regions of interest (ROI's) were drawn manually around the projected soma, using the neuron plasma membrane marker mCherry::PH(PLCγ), or cytoplasmic mScarlet to identify the cells of interest (COI), which develop with highly stereotyped polarities and positions. In Fig. 3 and Fig. 4

timelapse imaging experiments, plasma membrane localized mCherry was used to identify the cytokinesis cleavage furrow resulting in PVD's birth, and ROIs were drawn around PVD and PVD sister as described above, in the time frame showing the conclusion of cytokinesis (determined by the point at which the two sisters become and remain clearly separated by membrane mCherry). The areas of these ROI's were measured in μm^2 as an estimation of the maximal soma cross-section of each COI in the xy plane.

b. In this study, we show that ER-exit site (ERES) number is predictive of neuron size and complexity, and identify mechanisms by which large, complex neurons establish and maintain elevated ERES numbers. REVISED TO: In this study, we show that ER-exit site (ERES) number and somato-dendritic size are coordinately regulated, and identify mechanisms by which large, complex neurons establish and maintain elevated ERES numbers.

Minor point

In Fig. 4 Merge and mCh panels switched in WT

Response:

In FIG 4 WT panels, we have switched Merge and mCh channels in Fig. 4 WT panels.

Reviewer 2

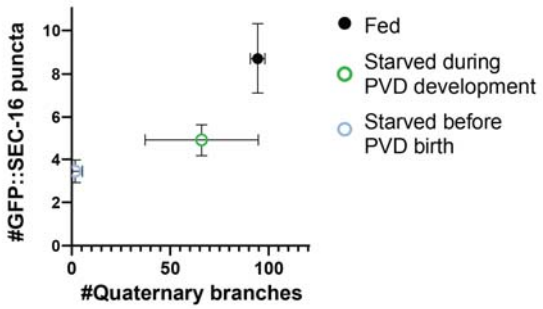
Major revisions

1. Figure 4C shows a strong correlation of ERES with the size of the PVD soma. For example, individual worms with a lin-5 conditional allele show a range of sizes for the PVD soma that correlate with ERES number. The model proposed in this paper also predicts that PVD dendritic complexity should be similarly linked to ERES number but these data are not provided. This experiment should be conducted.

2. Similarly, results presented in Figure 6 argue that nutrients function with LET-363/TOR to promote ERES number in PVD. Does starvation during development also impair PVD dendritic branching as the proposed model suggests?

Response 1 & 2:

We conducted additional experiments to address points 1 & 2, to assess the relationship between dendrite elaboration and ERES number under both nutrient deprivation and fed conditions, and have added the data as Figure 6e (see below). These data suggest that ERES number and dendrite elaboration are correlated with each other, and that both are regulated by the amount of nutrients available during PVD growth. We have added a description of these experiments to the methods section in red, and have included a schematic of the starvation experiments as Figure S3 (see response to Minor Revision #10).



Added to figure legend of Figure 6:

(e) Quantification of PVD quaternary dendrite number vs. ERES number in either animals that were removed from food 7h before PVD birth and starved for 1 day (pink, n=9), animals that were removed from food just after PVD birth and starved for 1 day (green, n=9), or animals that were fed for the duration of the experiment until 1 day after PVD birth at the L4 larval stage (black, n=7). Points shown are mean values for each condition with standard deviations indicated by error bars. Pearson correlation between quaternary dendrite number and ERES number across conditions: r=0.7122, R2=0.5072, p(two-tailed)<.0001. Color coding corresponds to diagram of starvation timelines in Figure S3.

We have also added the following text to the results section as the second-to-last paragraph:

Given the nutrient requirement for PVD's high ERES number and the correlation between dendrite size and ERES number in Figure 1, we predicted that dendrite size would scale with ERES number at different levels of nutrient availability. To test this prediction, we quantified ERES number and total quaternary dendrite number while varying the amounts of nutrients available to PVD during outgrowth (Fig. S3). In the first condition, to maximally reduce available nutrients for PVD growth, animals were removed from food before PVD birth. In the second condition, to partially reduce available nutrients during PVD growth, animals were removed from food shortly after PVD birth during dendrite outgrowth (as in Fig. 6a-d). In the third condition, to test for the requirement of nutrients on ERES number in mature PVD, animals were removed from food at the L4 stage, after PVD reached maturity. In the fourth and control condition, animals were left on food throughout the experiment. Consistent with our prediction, ERES number and quaternary dendrite number tend to scale with one another in response to the amount of nutrients available during PVD development (Fig. 6e).

Additional Concerns

1. A conditional PVD floxed allele of let-363/TOR was used for experiments in Fig 5 and 6 but no independent data are presented to confirm that "floxing" actually worked. For example, for mouse conditional mutants, its standard practice to use an indicator allele (Rosa26) to detect cell-specific cre activity. Aternatively, knockins of fluorescent markers at the target locus are activated by Cre to confirm site specific recombination.

Response 1:

Previous work in the lab shows that the integrated CRE line used in this paper (wyIs897) exhibits cell-specific CRE activity in PVD. As shown in the figure below, Pnhr-81::CRE in the wyIs897 line eliminates expression of the endogenously tagged gene loxP-UNC-116-GFP-loxP in PVD. This data has been added as Figure S2.

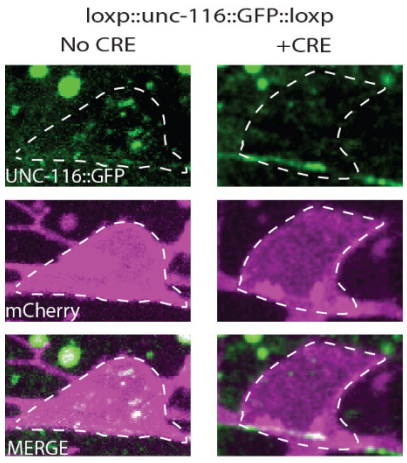


Fig. S2 (related to Fig. 5): The CRE line used in this study, wyIs897, disrupts expression of floxed genes in PVD. Confocal fluorescence microscopic images of PVD soma in animals with the endogenously tagged gene loxP-UNC-116-GFP-loxP, without (left) or with (right) an integrated array (wyIs897) expressing CRE in PVD lineage cells. This is the same CRE array used to disrupt LET-363 expression in Figure 5 and Figure 6. punc-86::mCherry::PLCdeltaPH is expressed via an extrachromosomal array and labels PVD membrane.

2. A transgenic GFP reporter was used to detect a role for mec-3 for in vivo expression of let-363/Tor. This reporter may not be reliable, however, because it is limited to an upstream promoter that may not contain key regulatory domains and is also over-expressed. This experiment would be more convincing if it used smFISH, which is well established for C. elegans, or a GFP reporter knockin at the endogenous let-363 gene.

Response 2:

We attempted to engineer an endogenous GFP knock-in into the *let-363* locus to generate a 1:1 translational reporter, for improved quantification of LET-363/ceTOR levels in WT and *mec-3* PVD. Using CRISPR, we knocked in the following construct: P2A::NLS::GFPnovo, at the c-terminus of the let-363 gene, in the final exon directly upstream of the stop codon. We successfully generated strains that were heterozygous for this insertion, however, homozygotes arrested in larval stages and did not reproduce, exhibiting phenotypes similar to the constitutive *let-363(ok3018)* mutants. We concluded that this insertion, in spite of the P2A cleavage site, disrupted either LET-363 regulation or function (perhaps because of the small peptide sequence present even after cleavage (Wang et al., 2015), and are therefore unable to use these animals as reliable measures of endogenous LET-363 expression levels. We agree that this quantification is important for developing a more rigorous model of neuron size regulation and hope to follow up on this point in future work.

Minor Revisions

1. The authors use PVD dendritic branch number as a proxy for neuron size and correlate this with ERES number, but some of the wording makes it unclear whether they think it is branches specifically that require the large ERES number or just size of the dendrite or neuron. It would be interesting for the authors to address this point in the discussion, or correlate these factors (neuron size, branch number, dendrite size) with ERESs to make a more concrete assessment. For example, PDE is larger than AVM and PVM but has the same number of ERESs. Is this because their size difference isn't that great or because the branch number isn't different. Could the authors look at the next largest neuron after PVD/FLP (perhaps the unbranched PVC or AVA) to get an additional datapoint for the size vs branching question?

Response 1:

We have written an additional section to include as the third paragraph of the discussion:

a. Whether high ERES numbers in *C. elegans* neurons correspond specifically to increased dendritic complexity (i.e. degree of branching), or more generally to increased cell surface area or volume is an open question. Given the technical challenges of using fluorescence microscopy to measure cell membrane areas or volumes of entire extended neurons with complex morphologies, we used terminal branch number as a proxy for cell surface area. While the assumptions made by this approximation are reasonable for the cell types examined, the same assumptions may not hold for other cell types. For example, the CAN neuron has a very simple morphology, but its processes have large diameters that result in very large estimations of CAN total cell volume (Froehlich et al., 2021). Anecdotally, we have observed a large cell body in the region where CAN soma is located, that has high ERES numbers (data not shown). We surmise that high ERES number results from the elevated biosynthesis required for large neuron surface areas in general, rather than being specifically related to high levels of neuronal branching. However, we cannot rule out the possibility that the morphological specifications and specialized proteins required for elaborate dendritic branching specifically impact secretory rate and/or ERES number.

2. A summary cartoon illustrating the proposed model would be a useful addition that would help readers.

Response 2:

We have added the following summary cartoon of the model to the manuscript as Figure 7, and reference Fig. 7 in the final paragraph of the results section.

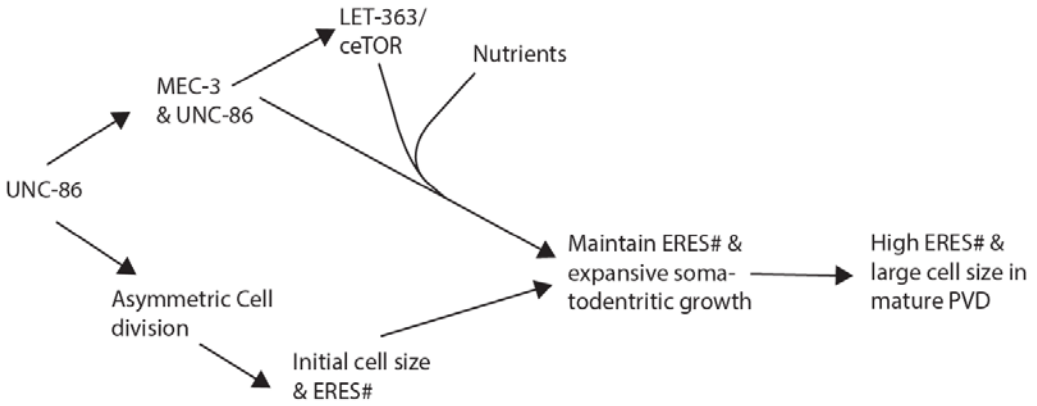


Fig. 7: Proposed model for PVD neuron size control. A schematic summary of the molecular pathways that regulate somato-dendritic size and ERES number in PVD.

3. General comment about X-Y plots and histograms. Results would be more easily visualized if color were used to distinguish data classes (e.g., neuron types, genotype, etc.). In current plots, data points are depicted as different black and white shapes (circles, triangles, squares, etc) and are difficult to distinguish.

Response 3:

We have edited potentially confusing figures such that data classes (genotypes, starvation conditions etc) have different, consistent colors.

4. Figure 1b. SEC-16 puncta for AVM, PVM, PDE are not distinguishable...likely because these results are limited to whole numbers and only 1 or 2 SEC-16 puncta are detected. The apparent fusion of these data into a single "mark" could be ameliorated by using a jitter plot.

Response 4:

We believe the reviewer may have been referring to Figure 1c, and we have edited figure 1c to improve visibility of individual data classes. We have also added the following text to the figure legend of figure 1c:

X-values have been nudged slightly to increase visibility of data points for PDE (x-0.25) and AVM(x+0.25).

5. What criteria were used to identify ERES in panels 1d-f?

Response 5:

We have added the following text to the Electron Microscopy methods section:

Criteria for identification of ERESs in Electron Micrographs: ER-exit sites can be unambiguously identified by serial EM reconstruction of the PVD cell body. ERESs show the characteristic

tubulovesicular structures located where ER lumen is expanded and ER membrane is free of ribosomes, adjacent to the Golgi stacks.

6. Figure 1. Black and white arrows in panels d-f are barely visible.

Response 6:

We have enlarged the size of black and white arrows in Figure 1d-f.

7. Figure 4. unc-86 (4b) is not mentioned in the legend.

Response 7:

We have made the following revisions to the figure legend of Figure 4:

Representative images of wild type (WT) and mutant worms at PVD birth and 0.5 hour after birth. *unc-86* mutants (top right) consistently disrupt asymmetry of PVD birth, displaying symmetric cell division of PVDmother. lin-5 mutants sometimes increase asymmetry of division (bottom left) and sometimes reduce asymmetry of division (bottom right). Scale bar = 5µm. (c) Quantification of cross-sectional soma area at birth vs. ERES number 30 minutes after birth of PVD and PVDsister in WT and mutants. Pearson correlation between soma size and ERES number: r=0.8404, R2=0.7062, p<.0001, n=58 cells from 29 animals (WT: n=8, lin-5: n=11, unc-86: n=10).

8. Figure 5a. The data point for - 1 hr is positioned to the left of the Y-axis. This is confusing and can be rectified by moving the Y-axis label further to the left.

Response 8:

We have moved the Y-axis label further left in Figure 5a.

9. Pg 9. reference to (Fig 3b and top right 3c) should be (Fig 4b and top right 4c)

Response 9:

We have made this correction in the text in the indicated location.

10. The timing of the experiments in Figure 6d-e is hard to follow. Could you make a graphic showing when animals are on and off the food?

Response 10:

We have added the following graphic as a supplemental figure, Figure S3, related to Figure 6 to clarify the timing of the experiments. We have also color-coded figures in Fig. 6 according to the diagram in Fig. S3, and added the following text to the Figure 6 legend:
Color coding corresponds to diagram of starvation timelines in Figure S3.

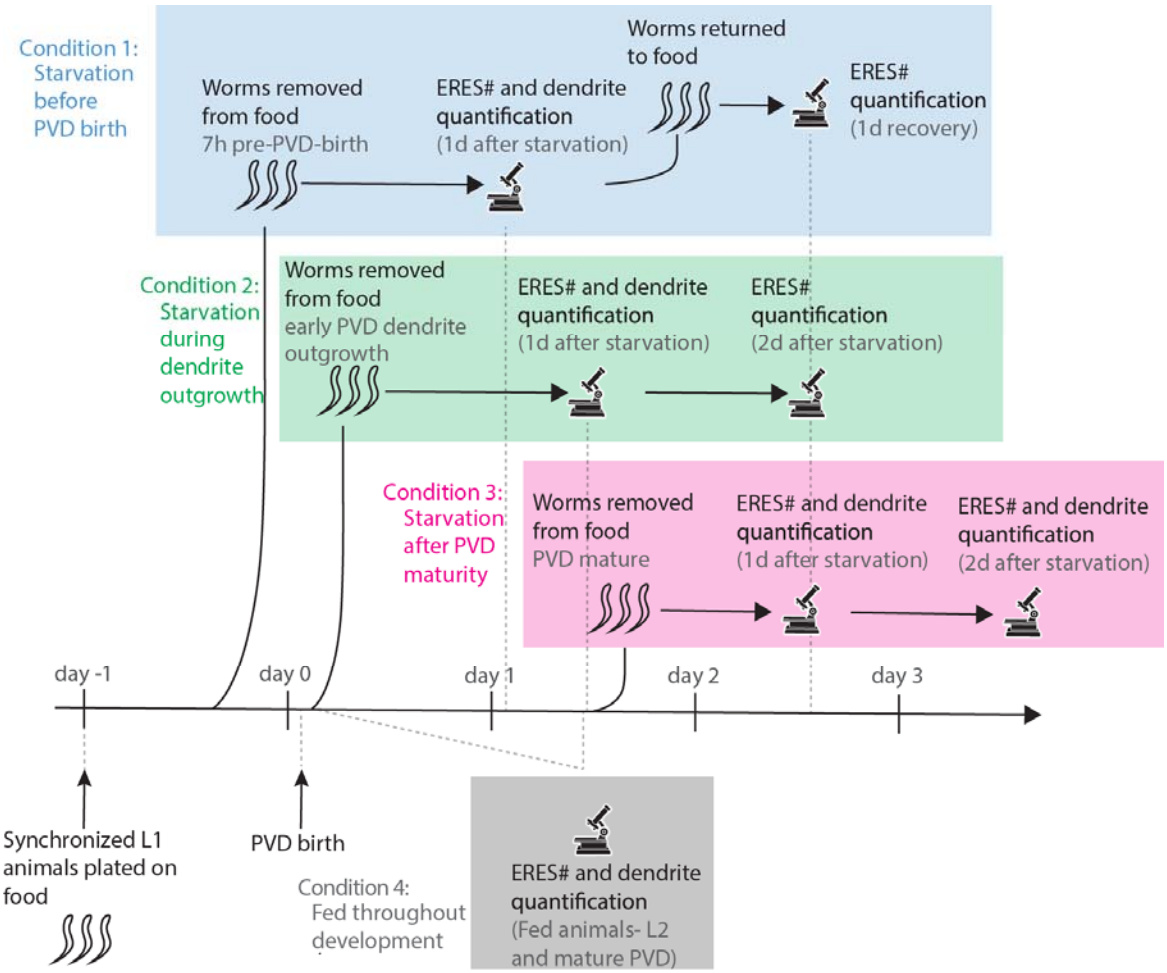


Fig. S3 (related to Fig. 6): Diagram of starvation timelines. A graphic illustration of the experimental procedure for the starvation experiments shown in Figure 6.

11. *FLP has an elevated number or ERESs relative to AV*M/PVM/PDE. Do the authors also think this is due to an asymmetric division with its sister AIZ or through a separate mechanism? I am not requesting any new experiments here but would be interested in any thoughts the authors have on FLPs development vs PVDs.

Response 11:

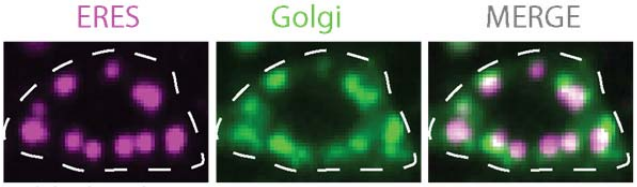
FLP is born as the sister of neuron AIZ, rather than as the sister of an apoptotic sister as in the case of PVD. We have not determined whether this is an asymmetric division or not. Notably, while FLP is born and begins to grow much earlier than PVD, the quaternary branches of FLP do not emerge until the L3 stage, when PVD's quaternary branches also emerge. Thus, FLP's early growth is not as rapid as that of PVD. It may be that FLP is born with fewer ERESs and then upregulates ERESs later in development during increased growth, and this would be an intriguing question for future experimentation. We hypothesize that the asymmetric division resulting in PVD's birth is only one mechanism of generating high ERES number (especially given that starved worms with a small soma and few ERESs can recover normal ERES numbers after being put back on food- Fig. 6). Large initial soma size may be especially important for neurons that require both high initial growth rates and large final cell size, given that a high biosynthetic

rate is required both for high growth rates and for maintaining large cell surfaces. Due to the speculative nature of these comments, we have decided not to include them in the manuscript.

12. figure S1: Why is the AMAN-2::GFP Golgi marker used instead of SEC-16::GFP. *C*an you explain in the text why a Golgi marker is a safe proxy for ERESs?

Response 12:

Our data show consistent colocalization between Golgi and ERES markers, with one to two ERES puncta per one to two Golgi puncta. Electron micrographs are consistent with the close correspondence between ERES and Golgi stack numbers. We have included an additional panel (shown below) in Figure S1 to show the close correspondence between ERES markers and Golgi markers, and have added the indicated text to the figure legend of Figure S1.



Added to figure S1

(d) ERES marker SEC-23::TAG-RFP (left) in WT PVD soma, along with the Golgi marker, AMAN-2::GFP (middle).

13. typos in the discussion: In mec-3(e1338) mu-tants, as well as starvation conditions.... unc-86(e1416) and mec-3(e1338) are con-sistent with the differential expression

Response 13:

We have corrected these typos in the discussion.

14. The methods section refers to FRAP experiments that are not included in the paper

Response 14:

We have removed the FRAP methods from the paper.

Additional changes:

1. **We found an error in Figure 4d, which was missing data points from PDE.**
    a. The missing data points were added and statistics were recalculated, though the difference was very small.
2. We removed original Figure 1E to make space to conform with the figure dimension requirements.
3. Additions and corrections of additional typos are shown in red text in the manuscript.

Works Cited:

Froehlich JJ, Rajewsky N, Ewald CY. 2021. Estimation of C. elegans cell- and tissue volumes. *microPublication Biol* **2021**. doi:10.17912/MICROPUB.BIOLOGY.000345

Wang Y, Wang F, Wang R, Zhao P, Xia Q. 2015. 2A self-cleaving peptide-based multi-gene expression system in the silkworm Bombyx mori. *Sci Reports 2015 51* **5**:1–10. doi:10.1038/srep16273

RE: Manuscript #E23-03-0090R
TITLE: "Endoplasmic Reticulum Exit Sites scale with somato-dendritic size in neurons."

Dear Kang:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Sincerely,
Avital Rodal
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Shen:

Congratulations on the acceptance of your manuscript! Thank you for choosing to publish your work in Molecular Biology of the Cell (MBoC).

Within 10 days, an unedited PDF of your manuscript will be published on MBoC in Press, an early release version of the journal. The date your manuscript appears on this site is the official publication date.

Your paper is among those chosen by the Editorial Board for Highlights from MBoC. Hightlights from MBoC appears in the ASCB Newsletter and highlights the important articles from the most recent issue of MBoC.

All Highlights papers are also considered for the MBoC Paper of the Year. In order to be eligible for this award, however, the first author of the paper must be a student or postdoc. Please email me to indicate if this paper is eligible for Paper of the Year.

Your copyedited and typeset manuscript will be scheduled for publication in the next available issue of MBoC. Our production team will notify you when the page proofs of your paper are ready for your review.

MBoC offers the option to publish your paper with immediate open access. Open access can increase the discoverability and usability of your research. If you would like to publish your paper with immediate open access but did not select this option during initial submission, please contact the MBoC Editorial Office (mbc@ascb.org).

We invite you to submit images related to your accepted manuscript to be considered for the journal cover. Please contact mboc@ascb.org to learn how to submit candidate cover images.

Authors of Articles and Brief Communications are also encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

We look forward to publishing your paper in MBoC.
Sign up for MBoC's new content alerts here https://tinyurl.com/3bcsyk3e

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The authors have satisfied my initial concerns and I feel the manuscript is ready for publication.

Reviewer #2 (Remarks to the Author):

The authors have thoughtfully addressed my concerns.