ENPL-1, the *Caenorhabditis elegans* homolog of GRP94, promotes insulin secretion via regulation of proinsulin processing and maturation

Agnieszka Podraza-Farhanieh, Balasubramanian Natarajan, Dorota Raj, Gautam Kao and Peter Naredi
DOI: 10.1242/dev.190082

Editor: Swathi Arur

Review timeline
Original submission: 3 March 2020
Editorial decision: 6 April 2020
First revision received: 4 September 2020
Accepted: 28 September 2020

Original submission

First decision letter

MS ID#: DEVELOP/2020/190082

MS TITLE: ENPL-1, the Caenorhabditis elegans homolog of GRP94, promotes insulin secretion via regulation of proinsulin processing and maturation

AUTHORS: Agnieszka Podraza-Farhanieh, Balasubramanian Natarajan, Gautam Kao, and Peter Naredi

I have now received reviews of your manuscript from 2 experts. The reviewers' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, both reviewers are enthusiastic about your study. Their common concerns are wanting to know if the dauer enhancement phenotype of enpl-1 mutants requires daf-16 and some missing controls. Among the missing controls listed by Reviewer 2, the highest priority ones are 1b, 1c, 1d. Both reviewers offer additional excellent suggestions for improving your study and manuscript.

I invite you to consider the reviewers' suggestions and submit a revised manuscript. Your revised manuscript will be re-reviewed, and acceptance will depend on your satisfactorily addressing the reviewers' concerns. Please note that Development normally permits only one round of â€˜major revision'.

In your revised manuscript, please clearly HIGHLIGHT all changes made in the revised version. You should avoid using 'Tracked Changes' in Word files as these are lost in PDF conversion. I also request a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of the reviewers' criticisms or suggestions, please explain why.

Reviewer 1

Advance summary and potential significance to field

In this paper Podraza-Farhanieh et al. demonstrate a function for the ER chaperone ENPL-1 in insulin secretion. The paper is well written and carefully interpreted. The authors convincingly show that in enpl-1 mutants there is a defect in insulin secretion at the level of dense core vesicle release. Furthermore, they demonstrate that ENPL-1 regulates this process in a dose-dependent manner, and that ENPL-1 physically interacts with proDAF-28 insulin precursor. Finally, proDAF-28 remains in the unprocessed form in enpl-1 mutants, indicating a role for ENPL-1 in processing and maturation. The authors have used a clever two-position protein labeling approach for these experiments, and incorporate biochemical approaches with the genetics and imaging. Their conclusions have important implications for insulin signaling.

Comments for the author

General comments on Figures:
1. Some of the fluorescent images are difficult to assess critically because of insufficiently high resolution. This issue may be due to compression in the PDF review version; if so, the original images may be sufficient for publication.

2. For negative results (such as lack of localization to coelomocytes) it would be helpful to have the companion DIC image with the fluorescent image. Even better would be a fluorescent marker for the cell type/structure.

Other comments:
1. Figure 2D – is there a conclusion to be made from the higher magnification images of animals?

2. Figure 3B – are there error bars on this graph?

3. The negative results on the lack of a role for ER stress are not as convincing as the positive results showing effects at the level of dense core vesicles. The DAF-28 localization in ASI and ASJ is not a direct test of whether high ER stress influences insulin secretion. Furthermore, this experiment lacks the positive control showing how ire-1 mutation causes changes in DAF-28 localization. The XBP-1s experiment shows that reducing ER stress is insufficient to rescue DAF-28 secretion in enpl-1 mutants, but falls short of proving that ER stress does not contribute to the insulin secretion defects. This conclusion could be softened in light of these caveats.

4. Supplemental Figures 2B,C lack a wild-type control.

Typos/suggested edits:
In some places, there is no space between “C.” and “elegans”.
In other places, there seem to be extra spaces between words.

Define spliced XBP-1 (XBP-1s) the first time it occurs in the paragraph rather than later.
In the Discussion:

3rd paragraph - change “asna-1 positive” to “asna-1 positively”
4th paragraph - “previous studied” to “previous studies”
Insert “to” after “proDAF28”, add hyphen to “proDAF28”
“analagous” to “analogous”
“insulin in processed” to “insulin is processed”
5th paragraph – “decreases” to “decreased”
“non-neurons” to “non-neuronal”

Reviewer 2

Advance summary and potential significance to field

This work investigates a previously unknown role for ENPL-1 in dauer regulation and the biogenesis of the insulin-like peptide DAF-28. It has the potential to reveal mechanistic insights into what may be a conserved function of ENPL-1/GRP94 in insulin biogenesis and metabolic regulation.

Comments for the author

This work elucidates a new function for the C. elegans GRP94 ortholog ENPL-1 in dauer regulation. The authors describe the anatomic and subcellular localization of ENPL-1, establish its role in controlling dauer arrest, and present a significant amount of data showing that ENPL-1 promotes the maturation and secretion of the agonist insulin-like peptide DAF-28 through a direct interaction involving the ENPL-1 client binding domain.

The data presented in the manuscript are generally convincing. In my opinion, the main question that is not addressed (and that is particularly important for the readership of Development) is whether the enhancement of dauer arrest caused by enpl-1 mutation is due to reduced DAF-2 signaling per se. It has been shown that disruption of either DAF-2 signaling or ciliated sensory neuron function can enhance the dauer-constitutive phenotype of daf-7 mutants. Since the enpl-1 mutant phenotype is pleiotropic, it is possible that functional defects in amphid neurons contribute to or account for enhancement of dauer arrest. There are also controls missing in some of the experiments, and the manuscript needs to be edited extensively.

Major point:

In Figure 3B, the authors should determine whether the enhanced Daf-c phenotype of daf-7;enpl-1 double mutants requires daf-16, either by constructing a triple mutant or by doing daf-16 RNAi. A lack of suppression by reduction of daf-16 function would call into question the biological significance of the DAF-28 secretion phenotype of enpl-1 mutants.

Minor points:

1. There are a number of controls that are missing:
a. Figure 1C: Lysates from animals not expressing FLAG::ENPL-1 should be included.
b. Figure S2B/C: L4440 control RNAi animals should be included.
c. Figure 4A: An ire-1 control is needed to show DAF-28::GFP accumulation.
d. Figure 4B: A knuSi222 control is needed as a positive control for rescue.
e. Figure 6B: The anti-OLLAS blot is not convincing. A control blot with blocking peptide is needed to show which bands are specific.
f. Figure 7C: An enpl-1 single mutant control is needed. This was shown in Figure 3C, but it should be included in this experiment to demonstrate suppression by tom-1 mutation.

2. p. 2, Introduction, lines 5-6: "Insulin biosynthesis starts...preproinsulin is cleaved..." This statement is not correct. Biosynthesis starts when translation begins in the cytoplasm, after which the nascent signal peptide binds to SRP and cotranslational translocation into the ER begins. Only after these steps is the signal sequence cleaved within the ER.

3. p. 3, lines 1-2: Contrary to what is implied, not all 40 insulin-like peptides are agonists. INS-1 and INS-18 have been shown to function genetically as DAF-2 antagonist peptides.

4. Figure 1A: The neuronal localization in the middle panel is not convincing.

5. Figure 1F: No information is provided about the anti-RME-1 control. This should be included in either Materials and Methods or the figure legend.

6. p. 4, second complete paragraph, lines 5-8: Ogg et al Nature 1997 (not Fielenbach and Antebi) should be cited for enhancement of daf-7 dauer arrest by reduction of DAF-2 signaling.

7. p. 6, second complete paragraph, last sentence: Pierce et al. Genes Dev 2001 (not Chen and Baugh) should be cited for the statement about families of neuropeptides.

8. Figure 5C, anti-OLLAS blot: what is the slowest (fainter) migrating band in the wt sample?

9. p. 8, last sentence: The data do not show that "Unregulated DCV release in tom-1 mutants can bypass this block." In order to do this, DAF-28::GFP secretion in tom-1 kpc-1 double mutants and kpc-1 single mutants would have to be compared.

10. p. 10, first complete paragraph, fourth sentence: The statement about the cause of the semidominant phenotype of daf-28(sa191) mutants is incorrect. Kulalert and Kim have shown (Current Biology 2013) that this phenotype is a consequence of activation of the PERK arm of the UPR in ASI.

11. p. 12, first complete paragraph, line 5: The two Gracheva et al. references need to be distinguished from each other.

12. p. 14, first complete paragraph, line 7: please provide a detailed description of the ENPL-1 CBD deletion mutant.

13. There are several misspellings, grammatical errors, and typographical errors that should be corrected.

First revision

Author response to reviewers' comments

Response to Reviewer #1

General comments on Figures:
1. Some of the fluorescent images are difficult to assess critically because of insufficiently high resolution. This issue may be due to compression in the PDF review version; if so, the original images may be sufficient for publication. –
We fully agree with this comment. The pictures have been retaken at a higher resolution after cleaning the microscope and paired DIC pictures have been added alongside the fluorescence pictures. Figures with retaken pictures: **Fig. 1A,B; Fig. 3C,D,E; Fig. 4B; Fig. 7C,D; Fig. S2B,C**.

2. For negative results (such as lack of localization to coelomocytes) it would be helpful to have the companion DIC image with the fluorescent image. Even better would be a fluorescent marker for the cell type/structure. –
We created a transgenic line expressing coelomocyte specific RFP. However, the bleed-through of red fluorescence interfered with GFP fluorescence from DAF-28::GFP, both with the epifluorescence microscope and the confocal microscope. Hence, we could not use this strategy in our analysis. To address this issue, we used the alternative approach suggested by the reviewer and took GFP fluorescence and DIC pictures of control animals and *enpl-1* mutants at 40X magnification. With this approach we showed DAF-28::GFP-positive coelomocytes in the wild-type strain, strains with increased secretion ability and lack of DAF-28::GFP-positive coelomocytes in *enpl-1(ok1964)* mutants. This is shown in **Fig. 3C,D,E and Fig. 7C,D.**

Other comments:
1. Figure 2D – is there a conclusion to be made from the higher magnification images of animals? –
There is no additional conclusion from the higher magnification images, hence we decided not to add this panel to the figure.

2. Figure 3B – are there error bars on this graph? –

The graph has been corrected and error bars have now been added.

3. The negative results on the lack of a role for ER stress are not as convincing as the positive results showing effects at the level of dense core vesicles. The DAF-28 localization in ASI and ASJ is not a direct test of whether high ER stress influences insulin secretion.
Furthermore, this experiment lacks the positive control showing how ire-1 mutation causes changes in DAF-28 localization. The XBP-1s experiment shows that reducing ER stress is insufficient to rescue DAF-28 secretion in enpl-1 mutants, but falls short of proving that ER stress does not contribute to the insulin secretion defects. This conclusion could be softened in light of these caveats. –

Thank you for this comment. As pointed by Reviewer #1, DAF-28::GFP localization in ASI and ASJ neurons is not a direct test of whether high ER stress influences insulin secretion. The Henis-Korenblit lab had previously shown that *ire-1* mutants, which have high ER stress, display DAF-28::GFP accumulation only in the cell bodies of the amphid neurons and none in their axons and dendrites. That is why we decided to examine this localization of DAF-28::GFP in the *enpl-1* mutants since these mutants also display high ER stress. We however agree with the Reviewer that this is not a direct test, that is why we softened the conclusions in our manuscript.

Furthermore, Reviewer #1 indicated that the lack of rescue from the XBP-1s experiment does not prove that ER stress is not contributing to the insulin secretion defects observed in *enpl-1* mutants. We used this approach in our study because it has been shown that the overexpression of *xbp-1s* in neurons leads to overexpression of many ER chaperones and has a strong effect in protecting animals from the death on tunicamycin. We agree with the Reviewer that the conclusion, which one should take from our study is not that ER stress does not contribute to the insulin secretion defects, but rather that improved ER folding capacity in neurons (caused by expression of *xbp-1s*) does not rescue the secretion block in enpl-1 mutants. We believe that this softens the conclusion and we have changed the title of the results section and the conclusion to reflect this.

We agree that a positive control showing the effect of *ire-1* mutations on DAF-28::GFP localization is necessary to make our point. We note that the effect of *ire-1(-)* on DAF-28::GFP localization has been published by the Henis-Korenblit lab. Therefore, we did not place it in the figure. In order to complete the figure, we attempted to build the *ire-1(v33) svIs69* strain. Unfortunately, we were not able to create this strain because of the tight linkage between the *ire-1* and *svIs69* loci. We have instead made an *xbp-1(tm2457);svIs69* strain and found that the localization defect reported in *ire-1* mutants was identical to the phenotype we observe in *tm2457* animals; no DAF-28::GFP was observed in axons of the neurons in *xbp-1* mutants. Since *xbp-1* acts downstream of *ire-1* in the UPR pathway, its depletion will be similar to the effect of the depletion of *ire-1* and feel that this is an equivalent observation. We have presented the data in **Fig. 4A.**

4. Supplemental Figures 2B,C lack a wild-type control. –
The experiments have been repeated using wild-type animals and *enpl-1(ok1964)* mutants. We performed the experiments with wild-type (N2) and *enpl-1(ok1964)* animals this time rather than by using RNAi knockdown. The original experiments had been done before we started the genetic analysis. We felt that using mutants would be more useful. We have presented the data in **Fig. S2B,C.**

Typos/suggested edits:
In some places, there is no space between "C." and "elegans". –
We have corrected that.
In other places, there seem to be extra spaces between words. –
We have corrected that.
Define spliced XBP-1 (XBP-1s) the first time it occurs in the paragraph rather than later. –
Thank you for that suggestion. We have made that change and it improves the readability of the paragraph.

In the Discussion:
3rd paragraph - change "asna-1 positive" to "asna-1 positively" –
We have corrected that.
4th paragraph - "previous studied" to "previous studies" –
We have corrected that.
Insert "to" after "proDAF28", add hyphen to "proDAF28" –

We corrected that.
“analagous” to “analogous” -
We have corrected that.
“insulin in processed” to “insulin is processed”-
We have corrected that.
5th paragraph – “decreases” to “decreased” -
We have corrected that.
“non-neurons” to “non-neuronal” -
We have corrected that.

Response to Reviewer #2

Major point:

In Figure 3B, the authors should determine whether the enhanced Daf-c phenotype of daf- 7;enpl-1 double mutants requires daf-16, either by constructing a triple mutant or by doing daf- 16 RNAi. A lack of suppression by reduction of daf-16 function would call into question the biological significance of the DAF-28 secretion phenotype of enpl-1 mutants. -
We fully agree with this comment. We have created triple mutant *daf-7(e1372),enpl- 1(ok1964);daf-16(mgDf50)*, as suggested by the Reviewer, and examined the strains for Daf-c dauer formation at 15¤C. Our results show that *daf-16(mgDf50)* suppresses the constitutive dauer formation phenotype of *enpl-1(ok1964);daf-7(e1372)* animals. This result is presented in **Fig. 3B**.

Minor points:

1. There are a number of controls that are missing:
a. Figure 1C: Lysates from animals not expressing FLAG::ENPL-1 should be included. – The wild-type lysates that do not express FLAG::ENPL-1 have been added to **Fig. 1C**.

b. Figure S2B/C: L4440 control RNAi animals should be included. –
The experiments have been repeated using wild-type controls and *enpl-1(ok1964)* mutants. **Fig. S2B,C**.

c. Figure 4A: An ire-1 control is needed to show DAF-28::GFP accumulation. –
We tried to create *ire-1* mutant with DAF-28::GFP, however after many attempts the cross did not succeed most likely because of the tight linkage between the *ire-1* mutation and transgene. Instead, we used a mutant in the downstream target of IRE-1, *xbp-1(tm2457)*, which we found has the same effect on the accumulation of DAF-28::GFP in only in neuronal cell bodies. This is consistent with the fact that XBP-1 acts directly downstream of IRE-1. This data is presented in **Fig. 4A**.

d. Figure 4B: A knuSi222 control is needed as a positive control for rescue. –
We have constructed the strain *uthIs270; enpl-1(ok1964) knuSi222; svIs69*. We find that the rescue transgene *knuSi222* provides near complete rescue of the secretion defect. This is shown in **Fig. 4B.**

e. Figure 6B: The anti-OLLAS blot is not convincing. A control blot with blocking peptide is needed to show which bands are specific. –
In our study (**Fig. 6D**), we showed that ENPL-1 with deleted client binding domain is not able to interact with pro-insulin (pro-DAF-28) tagged with OLLAS. This we believe, shows specificity of antibody towards OLLAS::DAF-28 since no OLLAS reactive band is seen in this experiment. Additionally, the size of the band visible in **Fig. 6B** represents exactly the size of pro-DAF-28, which is 12kDa. These findings demonstrate the specificity of the anti-OLLAS antibody and in our opinion shows that an experiment with a blocking peptide is not required Furthermore, we performed a literature search and found articles where anti-OLLAS antibodies have been successfully used to study various *C. elegans* proteins tagged with OLLAS-tag, both using western blot analysis as well as immunofluorescence staining (Delaney et al., 2019; Ellenbecker et al., 2019; Hefel and Smolikove, 2019; Lee et al., 2017; Lee et al., 2020; Paix et al., 2017). These papers also demonstrate the specificity of the anti-OLLAS antibody which we have used in our study.

f. Figure 7C: An enpl-1 single mutant control is needed. This was shown in Figure 3C, but it should

be included in this experiment to demonstrate suppression by tom-1 mutation. –
We agree with the Reviewer. We have included the suggested panel into **Fig. 7C.**

2. p. 2, Introduction, lines 5-6: "Insulin biosynthesis starts...preproinsulin is cleaved..." This statement is not correct. Biosynthesis starts when translation begins in the cytoplasm, after which the nascent signal peptide binds to SRP and cotranslational translocation into the ER begins. Only after these steps is the signal sequence cleaved within the ER. –
We are sorry for this mistake. We have corrected the indicated sentence.

3. p. 3, lines 1-2: Contrary to what is implied, not all 40 insulin-like peptides are agonists. INS- 1 and INS-18 have been shown to function genetically as DAF-2 antagonist peptides.
Thank you for this comment. We apologize for this incorrect sentence. Reviewer #2 is correct, among all insulin-like peptides in *C. elegans*, there are those which act as DAF-2 agonists and others that act as antagonists. We have changed the sentence.

4. Figure 1A: The neuronal localization in the middle panel is not convincing. –
To clearly demonstrate neuronal localization, we constructed a strain expressing pan-neural nuclear RFP from the *otIs356* transgene in *enpl-1::sfGFP* animals. Confocal analysis of these worms shows ENPL-1::sfGFP accumulation in a perinuclear pattern around neuronal nuclei consistent with the relative location of the endoplasmic reticulum and nuclei. We find that this is true for all the neuronal nuclei we examined in this strain. Two representative confocal images are presented in **Fig. S1A,B.** We have also taken new pictures of worms expressing ENPL-1::sfGFP with paired DIC images to show the localization of the GFP fluorescence in neuronal cell bodies in the nerve ring (**Fig. 1A).**

5. Figure 1F: No information is provided about the anti-RME-1 control. This should be included in either Materials and Methods or the figure legend. –
We added that information into the Materials and Methods.

6. p. 4, second complete paragraph, lines 5-8: Ogg et al Nature 1997 (not Fielenbach and Antebi) should be cited for enhancement of daf-7 dauer arrest by reduction of DAF-2 signaling.
-
We have corrected that citation.

7. p. 6, second complete paragraph, last sentence: Pierce et al. Genes Dev 2001 (not Chen and Baugh) should be cited for the statement about families of neuropeptides. -
We have corrected that citation.

8. Figure 5C, anti-OLLAS blot: what is the slowest (fainter) migrating band in the wt sample? We believe that this was an artefact in the membrane since in other repetitions of this experiment we did not see this fainter band. We have now used another representative western blot in **Fig. 5C** which does not have this slower migrating band.

9. p. 8, last sentence: The data do not show that "Unregulated DCV release in tom-1 mutants can bypass this block." In order to do this, DAF-28::GFP secretion in tom-1 kpc-1 double mutants and kpc-1 single mutants would have to be compared. –
Thank you for this comment. We realized that we finished this paragraph incorrectly leading to the confusion that we meant to imply that *"Unregulated DCV release in tom-1 mutants can bypass this (kpc-1 mutant) block''*. We agree that we haven't presented any data that would show that *tom-1(ok285)* mutation can bypass the secretion defect of *kpc-1(gk8)* mutants. However, we did not intend to present that. What we meant (in our incomplete sentence) was to indicate in this results section was the fact that the *tom-1(ok285)* mutation can bypass the insulin secretion defect of *enpl-1(ok1964)* mutants, because in this double mutant, insulin secretion was restored **Fig. 7C**. We decided to delete this incorrectly worded sentence to avoid confusion.

10. p. 10, first complete paragraph, fourth sentence: The statement about the cause of the semidominant phenotype of daf-28(sa191) mutants is incorrect. Kulalert and Kim have shown (Current Biology 2013) that this phenotype is a consequence of activation of the PERK arm of the UPR in ASI.
We have changed the sentence to reflect the finding that ASI specific activation of PEK-1 in

sa191 mutants causes the semi-dominant Daf-c phenotype.

11. p. 12, first complete paragraph, line 5: The two Gracheva et al. references need to be distinguished from each other. –
We have corrected the presentation of the two citations so that there is no confusion.

12. p. 14, first complete paragraph, line 7: please provide a detailed description of the ENPL- 1 CBD deletion mutant. –
We have added that missing information in materials and methods.

13. There are several misspellings, grammatical errors, and typographical errors that should be corrected. –
We have gone through the manuscript carefully and think that we have caught all the errors.

References:
Delaney, K., Strobino, M., Wenda, J. M., Pankowski, A. and Steiner, F. A. (2019). H3.3K27M-induced chromatin changes drive ectopic replication through misregulation of the JNK pathway in C. elegans. *Nat. Commun.* **10**, 1–15.
Ellenbecker, M., Osterli, E., Wang, X., Day, N. J., Baumgarten, E., Hickey, B. and Voronina, E. (2019). Dynein light chain DLC-1 facilitates the function of the germline cell fate regulator GLD-1 in caenorhabditis elegans. *Genetics* **211**, 665–681.
Hefel, A. and Smolikove, S. (2019). Tissue-specific split sfGFP system for streamlined expression of GFP tagged proteins in the caenorhabditis elegans germline. *G3 Genes, Genomes, Genet.* **9**, 1933–1943.
Lee, C. Y. S., Lu, T. and Seydoux, G. (2017). Nanos promotes epigenetic reprograming of the germline by down-regulation of the THAP transcription factor LIN-15B. *Elife* **6**, 1– 31.
Lee, C. Y. S., Putnam, A., Lu, T., He, S., Ouyang, J. P. T. and Seydoux, G. (2020). Recruitment of mRNAs to P granules by condensation with intrinsically-disordered proteins. *Elife* **9**, 1–31.
Paix, A., Folkmann, A. and Seydoux, G. (2017). Precision genome editing using CRISPR- Cas9 and linear repair templates in C. elegans. *Methods* **121**–**122**, 86–93.
Zheng, S., Chiu, H., Boudreau, J., Papanicolaou, T., Bendena, W. and Chin-Sang, I. (2019). A functional study of all 40 Caenorhabditis elegans insulin-like peptides. *J. Biol. Chem.* **293**, 16912–16922.

Second decision letter

MS ID#: DEVELOP/2020/190082

MS TITLE: ENPL-1, the Caenorhabditis elegans homolog of GRP94, promotes insulin secretion via regulation of proinsulin processing and maturation

AUTHORS: Agnieszka Podraza-Farhanieh, Balasubramanian Natarajan, Dorota Raj, Gautam Kao, and Peter Naredi
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

In this paper Podraza-Farhanieh et al. demonstrate a function for the ER chaperone ENPL-1 in insulin secretion. The paper is well written and carefully interpreted. The authors convincingly

show that in enpl-1 mutants there is a defect in insulin secretion at the level of dense core vesicle release.
Furthermore, they demonstrate that ENPL-1 regulates this process in a dose-dependent manner, and that ENPL-1 physically interacts with proDAF-28 insulin precursor. Finally, proDAF-28 remains in the unprocessed form in enpl-1 mutants, indicating a role for ENPL-1 in processing and maturation. The authors have used a clever two-position protein labeling approach for these experiments, and incorporate biochemical approaches with the genetics and imaging. Their conclusions have important implications for insulin signaling.

Comments for the author

The authors have satisfactorily responded to the previous review in this revision.

Reviewer 2

Advance summary and potential significance to field

This work convincingly establishes a role for the C. elegans ER chaperone ENPL-1 in the biogenesis and secretion of the insulin-like peptide DAF-28. The findings are likely relevant to insulin biogenesis in mammals and diabetes pathogenesis in humans.

Comments for the author

The authors have adequately addressed all of my comments.

Typo: p. 12: "tomosyn" rather than "tomosin"