Loss of the seipin gene perturbs eggshell formation in *Caenorhabditis elegans*

Xiaofei Bai, Leng-Jie Huang, Sheng-Wen Chen, Benjamin Nebenfuehr, Brian Wysolmerski, Jui-Ching Wu, Sara K. Olson, Andy Golden and Chao-Wen Wang
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First decision letter

MS ID#: DEVELOP/2020/192997

MS TITLE: Dietary supplementation with PUFAs rescues the eggshell defects caused by seipin mutations in C. elegans

AUTHORS: Xiaofei Bai, Leng-Jie Huang, Sheng-Wen Chen, Ben Nebenfuehr, Brian Wysolmerski, Jui-Ching Wu, Sara K. Olson, Andy Golden, and Chao-Wen Wang

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 manuscript, as it establishes C. elegans as an excellent model system for studying SEIPIN-related human diseases and sheds light on lipid metabolism in development. The reviewers raise some questions and issues that should be addressed, and Reviewer 1 offers suggestions for a few relatively straight-forward additional experiments (see points 2 and 3 in their review) and for improving the writing and organization of the paper.

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

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns

raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Reviewer 1

Advance summary and potential significance to field

In this paper, the authors present a multi-level, in vivo analysis of C. elegans SEIPIN-1: fertility, cytology (light microscopy, fusion protein, and TEM), and biochemical analysis of lipid composition. The function of this ER-localized protein is poorly understood, but its human homolog is associated with various human diseases. In this study, the authors provide convincing evidence that SEIPIN-1 functions to maintain fatty acid homeostasis and that dietary supplementation with specific amounts of DGLA can rescue specific phenotypes. The work establishes C. elegans as an excellent model system for SEIPIN-1 related diseases and establishes a clear set of assays that can be used to assess related phenotypes – both in terms of lipid metabolism and the development event of forming the embryonic permeability barrier. The electron micrographs are particularly stunning. The authors do a good job of placing their questions in context and citing the relevant literature.

For a broader group of C. elegans researchers, this work identifies lipid droplets as a distinct, scorable component of oocytes that is distinct from the yolk granules. It will also be of interest to C. elegans researchers who are interested the permeability barrier as a scorable component of the oocyte to embryo transition.

Comments for the author

Experimental points to address.
1) Regarding Figure 1. Is there an explanation for the phenotypic variability of av109? Could there be an unrelated lethal in the strain resulting in 25% of the animals having no embryonic viability? Apparent discrepancy between the cwc1 results in 1D (10-20% embryonic lethality) and 1G (90% embryonic lethality) was not explained.

2) Human with seipin-1 mutations have sperm defects and the authors of this paper show that SEIP-1 is also present in C. elegans spermatozoa. Given that the self-fertility of wildtype C. elegans hermaphrodites is limited by their number of sperm, it would interesting to…
a) Test the fertility of seipin-1 mutant males
b) Test whether older seip-1 hermaphrodites will produce either more viable progeny or alternatively a higher percentage of non-viable embryos when crossed with wildtype males.
It would be great if these experiments could be added to the paper for completeness. At minimum the authors should note in the text that impacted sperm could be contributing to the fertility defect and move the SEIP-
1::mScarlet sperm results to Figure 4.

3) In regards to Figure S2C and associated text (line 134). Statement about shrunken and misshapen is somewhat difficult to assess as it appears that the cwc1 embryos have more cells than the controls. Analysis of embryonic in hypotonic solution would be a potentially more informative test – do they lyse? At minimum please address whether the embryos were the same age. The lysis experiment would be a nice addition, but not required if it is difficult for the researchers to be back in lab.

4) Throughout the paper, authors should consider their use of the word “expression” when they are visualizing fusion proteins. Two key contexts to consider. 1) Yolk protein genes are transcribed and translated in the intestine but secondarily exported to the oocytes. SEIP-1 is also present in the intestine, so could some of this protein pool be exported to the germline? 2) Line 221 – genes aren’t transcribed in C. elegans sperm – just in the spermatocytes. So proteins present in the haploid sperm were made earlier. If the authors have the image, it would be great to include an image of SEIP-1::mScarlet in the developing spermatocytes of an L4 hermaphrodite.

Suggestions for improving the paper – organization and writing

Figure 2Ac,d is out of order relative to the text, and the CPG-2 results were hard to appreciate until AFTER seeing the TEM images. Is the excluded space in the anterior (2Ac, 2Ba) due to the polar bodies? Consider moving these results (and 2Ba,b) after the TEM as an independent verification. Define PES in figure legend. If authors are concluding that seip-1(cwc1) embryos lack an embryonic extracellular matrix (EEM), they should explain the clear zones between the embryo and CPGL in 2Cf.

Figure 3: In both 3B and 3D6, it appears that cwc1 mutants may have both abnormally large and abnormally small LDs, but this point wasn't clear in the main text which only emphasized the large LDs. The BODIPY results were convincing.

Supplement 3G-I. Sperm images are small and indistinct. Labelling is likely to be in the membranous organelles (MOs), but it is hard to tell from the images. C. elegans spermatozoa lack ER, but MOs are Golgi derived – so it is notable that there is SEIP-1 fusion protein labeling. Is it possible to show either a male or L4 (sperm producing) hermaphrodite in order to visualize the developing spermatocytes? Or add Hoechst so that the labelling can be visualized relative to the DNA? The experiment is relevant given the human sperm phenotype.

Typos/ Grammar
line 50 – change anchor to anchoring, might to may (same suggestion elsewhere in the introduction)
Line 69 – However, this organism has yet to be employed as a model for human diseases…
line 71 – Lipids and lipid metabolism underlie diverse growth and developmental…
line 75 – structural mimics of …
line 94 – … we report that…
line 252 – As a wide range…
line 377 – test whether SEIP-1 delivers… (delete may, add s)
Line 558 – Formvar coated

Reviewer 2

Advance summary and potential significance to field

Dietary supplementation with PUFAs rescues the eggshell defects caused by seipin mutations in C. elegans Bai et al.

SUMMARY AND RECOMMENDATION
SEIPIN proteins function in lipid droplet biogenesis and are associated with various human diseases. In order to better understand SEIPINs and their role in disease, Bai et al. examined the function of its C. elegans homologue seip-1. The authors found that loss of function mutants of seip-1 results in embryonic lethality. Through careful phenotypic analysis, the authors show that this lethality is associated with disruption of a lipid-rich element of the eggshell. They further show that improper fatty acid regulation due to loss of SEIPIN function in the endoplasmic reticulum likely leads to the eggshell defect. Further, seip-1 mutants are deficient in polyunsaturated fatty acids (PUFAs) and dietary supplementation of PUFAs can suppress mutant embryonic lethality. This is a rigorous and thorough study that brings potentially clinically relevant molecular insights of SEIPIN function. I support accepting this manuscript for publication in Development.

EXPERIMENTAL APPROACH
A single SEIPIN1 ortholog seip-1 was identified in C. elegans and can function in yeast. CRISPR was used to create null alleles of seip-1 for phenotypic analysis. Progeny production as well as viable embryos production was reduced but not eliminated in null mutants. Larval development and longevity of surviving animals seem normal. The authors noted that many embryos had a shrunken and misshapen morphology indicative of eggshell defects. Dyes that are excluded from embryos with normal eggshells were not excluded in mutant embryos. This is further supported by the movement of mCherry::CPG-2 diffulison across the permeability barrier that is not seen in wild type. Transmission electron microscopy analysis of mutant embryos showed a lack of the permeability barrier. Lipid droplets (LDs) are abnormal in various cells in seip-1 mutants consistent with observations in mammalian and yeast cells. The authors used CRISPR to create an

mScarlet::SEIP-1 insertion line. This protein was associated with endoplasmic reticulum. The authors note strong expression in oocytes and sperm. Lipid profiles were abnormal in embryos. Polyunsaturated fatty acids (PUFAs) were notably reduced in seip-1 mutants. Gene expression analysis revealed that various genes involved in fatty acid homeostasis were also perturbed in seip-1 mutants. Since the permeability layer of the eggshell consists of fatty acid derivatives the authors argue for a link to LDs and permeability layer formation. Consistent with this idea, if the authors supplemented the diet of worms with specific PUFAs they could suppress the permeability barrier defects seen in seip-1 mutants. The authors used CRISPR to create a point mutation in C. elegans seip-1 that is similar to a mutation found in the human disease BSCL2 syndrome. This point mutation displayed the same phenotype as loss of function mutants suggesting that the worm could be used to model a human disease and possibly test for genetic suppressors or other therapeutic approaches.

Comments for the author

SPECIFIC MINOR COMMENTS AND QUESTIONS
Results, line 125 and figure 1. Can you explicitly state why broods and embryo viability were separated into two phases? Does lipid droplet deficiency have a bigger impact later in the reproductive span? If so, any ideas why.

Results, line 134 and figure S2C. This is an important phenotype that should be in figure 1 or 2 rather than buried in a supplemental figure.

Results, line 221 and figure S3G-I. There is no ER in sperm. What structures is SEIPIN-1 associated with? Do male worms have reduced fertility? I am not suggesting additional experiments that need to be done. I am just curious.

First revision

Author response to reviewers' comments

Reviewer 1 Advance summary and potential significance to field
In this paper, the authors present a multi-level, in vivo analysis of C. elegans SEIPIN-1: fertility, cytology (light microscopy, fusion protein, and TEM), and biochemical analysis of lipid composition. The function of this ER-localized protein is poorly understood, but its human homolog is associated with various human diseases. In this study, the authors provide convincing evidence that SEIPIN-1 functions to maintain fatty acid homeostasis and that dietary supplementation with specific amounts of DGLA can rescue specific phenotypes. The work establishes C. elegans as an excellent model system for SEIPIN-1 related diseases and establishes a clear set of assays that can be used to assess related phenotypes – both in terms of lipid metabolism and the development event of forming the embryonic permeability barrier. The electron micrographs are particularly stunning. The authors do a good job of placing their questions in context and citing the relevant literature.

For a broader group of C. elegans researchers, this work identifies lipid droplets as a distinct, scorable, component of oocytes that is distinct from the yolk granules. It will also be of interest to C. elegans researchers who are interested the permeability barrier as a scorable component of the oocyte to embryo transition.
We thank Reviewer #1 for their positive feedback.

Reviewer 1 Comments for the author
Experimental points to address.

1)Regarding Figure 1. Is there an explanation for the phenotypic variability of av109?
We cannot explain why a few of the animals do lay a brood of dead embryos. It could be due to a modifier in the background as suggested below.

Could there be an unrelated lethal in the strain resulting in 25% of the animals having no embryonic viability?
We think there might possibly be a modifier in this strain that is contributing to the variability in lethality of this particular mutant. However, the majority of these animals are behaving like the other seip-1 alleles.
Apparent discrepancy between the cwc1 results in 1D (10-20% embryonic lethality) and 1G (90% embryonic lethality) was not explained.
In figure 1C and 1D, the cwc1 allele was compared with the av109 allele grown on MYOB plates, showing similar brood size and embryonic viability phenotypes. However, the cwc1 and cwc2 alleles were normally maintained on standard NGM plates, which consistently display a ~75-80% lethality. The discrepancy was explained in the Materials and Methods of our previous submitted manuscript. In the revised text, we added the sentence "The three deletion strains did appear to have different levels of embryonic viability that we attribute to the protocols and the different wild-type genetic backgrounds in which they were generated and by the media and incubators in which they were grown." (line 671-473). In Figure 1G, the fosmid or plamids were microinjected into the wild-type and cwc1 allele grown on standard NGM plates. We suspect that the discrepancy most likely is due to technical injury on the vulnerable gonad of the cwc1 allele which we did not see for the wild-type.

2) Human with seipin-1 mutations have sperm defects and the authors of this paper show that SEIP-1 is also present in C. elegans spermatozoa. Given that the self-fertility of wildtype C. elegans hermaphrodites is limited by their number of sperm, it would be interesting to…
a) Test the fertility of seipin-1 mutant males.
We agree and did such experiments, the results of which are now shown in Suppl. Fig. 4.

b) Test whether older seip-1 hermaphrodites will produce either more viable progeny or alternatively a higher percentage of non-viable embryos when crossed with wildtype males.
We would be very surprised if we could get male rescue since eggshell formation is driven maternally. We are not sure embryo viability would be the best read-out, perhaps DAPI permeability would be a better readout. The permeability of these embryos was a more penetrant phenotype than the embryonic lethality, suggesting that embryos can be somewhat permeable and yet still survive. We have not tested older mutants to address this specific question however.

It would be great if these experiments could be added to the paper for completeness. At minimum the authors should note in the text that impacted sperm could be contributing to the fertility defect and move the SEIP-1::mScarlet sperm results to Figure 4.
We are not sure what is meant by "impacted"? We have not been emphasizing a fertility defect, despite slightly reduced brood sizes. Though the broods are somewhat smaller, we did not observe infertile animals. A fertility defect implies gamete problems and we did not observe such defects. Our DAPI data suggests that oocytes were fertilized and that embryos were all mitotic.

3) In regards to Figure S2C and associated text (line 134). Statement about shrunken and misshapen is somewhat difficult to assess as it appears that the cwc1 embryos have more cells than the controls. Analysis of embryonic in hypotonic solution would be a potentially more informative test – do they lyse? At minimum, please address whether the embryos were the same age. The lysis experiment would be a nice addition, but not required if it is difficult for the researchers to be back in lab.
We have replaced this panel with embryos of equivalent age. It is clear that these mutant embryos are osmo-sensitive as they shrink within the eggshell compared to wild type.

4) Throughout the paper, authors should consider their use of the word "expression" when they are visualizing fusion proteins. Two key contexts to consider. 1) Yolk protein genes are transcribed and translated in the intestine but secondarily exported to the oocytes. SEIP-1 is also present in the intestine, so could some of this protein pool be exported to the germline? 2) Line 221 – genes aren't transcribed in C. elegans sperm – just in the spermatocytes. So proteins present in the haploid sperm were made earlier. If the authors have the image, it would be great to include an image of SEIP-1::mScarlet in the developing spermatocytes of an L4 hermaphrodite.
We have addressed each of these concerns.

Suggestions for improving the paper – organization and writing

Figure 2Ac,d is out of order relative to the text
This has been fixed. , **and the CPG-2 results were hard to appreciate until AFTER seeing the TEM images**. *We understand this criticism but it made more sense for this story to show the CPG result first.* Is the excluded space in the anterior (2Ac, 2Ba) due to the polar bodies? *No, we have modified the figure and text to explain this better. The excluded space is the embryo and it blebs out a bit at the anterior. The embryo is not a perfect oval.* **Consider moving these results (and 2Ba,b) after the TEM as an independent verification.** *We are trying to argue that the CPG image led us to look at the permeability barrier. This result led us to then try TEM to look more carefully at the layers of the eggshell at much higher resolution.* **Define PES in figure legend.** *Done.* **If authors are concluding that seip-1(cwc1) embryos lack an embryonic extracellular matrix (EEM), they should explain the clear zones between the embryo and CPGL in 2Cf.** *We now state that and say severely compromised and reduced EEM.*

Figure 3: In both 3B and 3D6, it appears that cwc1 mutants may have both abnormally large and abnormally small LDs, but this point wasn't clear in the main text which only emphasized the large LDs. The BODIPY results were convincing. *We have made this point more clearly now, but we are not sure what 3D6 refers to.*

Supplement 3G-I. Sperm images are small and indistinct. Labeling is likely to be in the membranous organelles (MOs), but it is hard to tell from the images. C. elegans spermatozoa lack ER, but MOs are Golgi derived – so it is notable that there is SEIP-1 fusion protein labeling. Is it possible to show either a male or L4 (sperm producing) hermaphrodite in order to visualize the developing spermatocytes? Or add Hoechst so that the labeling can be visualized relative to the DNA? The experiment is relevant given the human sperm phenotype. *We have added such experiments as Suppl. Figure 4. This figure shows that mutant males are fertile and able to produce cross progeny when mated to females.*

Typos/ Grammar *These have all been fixed.*
line 50 – change anchor to anchoring, might to may (same suggestion elsewhere in the introduction)
Line 69 – However, this organism has yet to be employed as a model for human diseases…
line 71 – Lipids and lipid metabolism underlie diverse growth and developmental…
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Reviewer 2 Advance summary and potential significance to field
Dietary supplementation with PUFAs rescues the eggshell defects caused by seipin mutations in C. elegans
Bai et al.

SUMMARY AND RECOMMENDATION
SEIPIN proteins function in lipid droplet biogenesis and are associated with various human diseases. In order to better understand SEIPINs and their role in disease, Bai et al. examined the function of its C. elegans homologue seip-1. The authors found that loss of function mutants of seip-1 results in embryonic lethality. Through careful phenotypic analysis, the authors show that this lethality is associated with disruption of a lipid-rich element of the eggshell. They further show that improper fatty acid regulation due to loss of SEIPIN function in the endoplasmic reticulum likely leads to the eggshell defect. Further, seip-1 mutants are deficient in polyunsaturated fatty acids (PUFAs) and dietary supplementation of PUFAs can suppress mutant embryonic lethality. This is a rigorous and thorough study that brings potentially clinically relevant molecular insights of SEIPIN function. I support accepting this manuscript for publication in Development.
We thank Reviewer #2 for their positive review and feedback.

EXPERIMENTAL APPROACH
A single SEIPIN1 ortholog seip-1 was identified in C. elegans and can function in yeast. CRISPR was used to create null alleles of seip-1 for phenotypic analysis. Progeny production as well as viable embryos production was reduced but not eliminated in null mutants. Larval development and longevity of surviving animals seem normal. The authors noted that many embryos had a shrunken and misshapen morphology indicative of eggshell defects. Dyes that are excluded from embryos with normal eggshells were not excluded in mutant embryos. This is further supported by the movement of mCherry::CPG-2 diffusion across the permeability barrier that is not seen in wild type. Transmission electron microscopy analysis of mutant embryos showed a lack of the permeability barrier. Lipid droplets (LDs) are abnormal in various cells in seip-1 mutants consistent with observations in mammalian and yeast cells. The authors used CRISPR to create an mScarlet::SEIP-1 insertion line. This protein was associated with endoplasmic reticulum. The authors note strong expression in oocytes and sperm. Lipid profiles were abnormal in embryos. Polyunsaturated fatty acids (PUFAs) were notably reduced in seip-1 mutants. Gene expression analysis revealed that various genes involved in fatty acid homeostasis were also perturbed in seip-1 mutants. Since the permeability layer of the eggshell consists of fatty acid derivatives the authors argue for a link to LDs and permeability layer formation. Consistent with this idea, if the authors supplemented the diet of worms with specific PUFAs they could suppress the permeability barrier defects seen in seip-1 mutants. The authors used CRISPR to create a point mutation in C. elegans seip-1 that is similar to a mutation found in the human disease BSCL2 syndrome. This point mutation displayed the same phenotype as loss of function mutants suggesting that the worm could be used to model a human disease and possibly test for genetic suppressors or other therapeutic approaches.

Reviewer 2 Comments for the author
SPECIFIC MINOR COMMENTS AND QUESTIONS
Results, line 125 and figure 1. Can you explicitly state why broods and embryo viability were separated into two phases?
From our experiences with mutants that affect brood size and embryonic viability, we have sometimes observed that these numbers vary based on the age of the mother. For this reason, we typically divide our analysis into young adults and older adults. We can merge these results if the editor thinks necessary since there is not a dramatic difference with our seip-1 mutants. However, there is a difference in embryonic viability in Fig. 1 and 7 when we break down the data by age of the mother. **Does lipid droplet deficiency have a bigger impact later in the reproductive span?** *We have not looked at this phenotype at different ages. That is an interesting suggestion and we hope to determine in the future if this phenotype varies with age.* **If so, any ideas why**.

Results, line 134 and figure S2C. This is an important phenotype that should be in figure 1 or 2 rather than buried in a supplemental figure. *Unfortunately, there is just no room to add this panel to either Fig. 1 or 2.*

Results, line 221 and figure S3G-I. There is no ER in sperm. What structures is SEIPIN-1 associated with? *As shown in our new Suppl. Fig. 4, SEIP-1 appears to co-localize with the FB-MOs of sperm.* **Do male worms have reduced fertility?** *No, but we have added this data to Suppl. Fig. 4 since both reviewers have asked for such data.* **I am not suggesting additional experiments that need to be done. I am just curious.**

Second decision letter

MS ID#: DEVELOP/2020/192997

MS TITLE: Loss of the seipin gene perturbs eggshell formation in C. elegans

AUTHORS: Xiaofei Bai, Leng-Jie Huang, Sheng-Wen Chen, Ben Nebenfuehr, Brian Wysolmerski, Jui-Ching Wu, Sara K. Olson, Andy Golden, and Chao-Wen Wang
ARTICLE TYPE: Research Article

I am happy to tell you that your revised manuscript has been accepted for publication in Development, pending our standard ethics checks. The reviews of this version are appended below.

Reviewer 1

Advance summary and potential significance to field

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For a broader group of C. elegans researchers, this work identifies lipid droplets as a distinct, scorable component of oocytes that is distinct from the yolk granules. It will also be of interest to C. elegans researchers who are interested the permeability barrier as a scorable component of the oocyte to embryo transition.

Comments for the author

Authors did a good job in revising the paper. I look forward to seeing it published!

Reviewer 2

Advance summary and potential significance to field

Dietary supplementation with PUFAs rescues the eggshell defects caused by seipin mutations in C. elegans Bai et al.

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Comments for the author

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