The spectrin-based membrane skeleton is asymmetric and remodels during neural development in *C. elegans*

Ru Jia, Yongping Chai, Chao Xie, Gai Liu, Zhiwen Zhu, Kaiyao Huang, Wei Li and Guangshuo Ou

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First decision letter

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MS TITLE: Spectrin-based Membrane Mechanics Is Asymmetric and Remodels during Neural Development

AUTHORS: Ru Jia, Yongping Chai, Chao Xie, Gai Liu, Zhiwen Zhu, Kaiyao Huang, Wei Li, and Guangshuo Ou
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.organd click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, both reviewers gave favourable reports but reviewer 1 raised a few points that will require amendments to your manuscript. I agree the title should be changed and that you should provide more details concerning figure 4. I hope that you will be able to carry these out, because I would like to be able to accept your paper.

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.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to

all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

Spectrin is a major component of the cortical actin cytoskeleton underneath the plasma membrane. This work by Jia and colleagues characterizes in vivo function of spectrins during neuronal development in C. elegans. A nice combination of genetics and live imaging of neuroblasts and developing neurons demonstrated important roles of two spectrin genes in neuroblast migration and dendrite formation. They analyzed two spectrin genes, spc-1 and unc-70. Mutations in spc-1 or unc-70 impair long-range migration of neuroblasts and cause accumulation of actin filaments in the lateral and rear cortices instead of leading edge in normal migrating neuroblasts.
Consistently, these spectrins localized to lateral and rear cortices. During dendrite elongation, abnormal branching of dendrites occurred in the spectrin mutants. During this process, spectrin redistributes from the cell body to the dendrites, suggesting that spectrin normally inhibits lateral branching. Finally, they found ankyrin (unc-44) as a binding partner of SPC-1 spectrin and showed that unc-44 mutant are also defective in neuroblast migration and dendrite formation. Overall, this is a high-quality work reporting novel in vivo functions of spectrin in neuronal development.

Comments for the author

Major points

1. The title of this manuscript contains “spectrin-based membrane mechanics”, but this is an overstatement. This work contains no data for “mechanics”. Therefore, the title should be revised to reflect fair conclusion of the study.

2. In Fig. 3B, specific labels for dendrites and axons should be added for non-neurobiologists. Also, a statement of how dendrites and axons were distinguished should be added.

3. In Fig. 4, UNC-70 and UNC-44 are shown as binding proteins for SPC-1::GFP in the mass spec analysis without showing what other proteins were identified in the pool and how they were identified as positives as compared with controls. UNC-70 and UNC-44 are quite obvious “hits”. The authors should provide additional data for how a control experiments were set up how these “hits” were identified from the pool of other positives, and what other specific and non-specific proteins were identified in the analysis.

4. The data suggest that SPC-1 and UNC-70 have similar functions in neuronal development. No additional experiments are needed, but the authors should discuss whether SPC-1 and UNC-70 act similarly or have some distinct functions.

Minor points

1. Legend for Fig. 1F. “GFP-tagged F-actin” is not an accurate description.
“GFP-labeled F-actin” may be acceptable.

2. In Fig. 3D, only spc-1 mutant was analyzed by live imaging. How about unc-70 mutant?

Reviewer 2

Advance summary and potential significance to field

Jia et al. investigate the role of the spectrin membrane skeleton in neuroblast migration using the C. elegans Q cell and its progeny as a model. The authors present interesting and convincing in vivo data on the asymmetric and dynamic localization of three proteins associated with the membrane skeleton: SPC-1/alpha spectrin, UNC-70/beta spectrin, and UNC-44/ankyrin. The use of knock-in

GFP tags and cell-specific CRISPR/Cas9 mutagenesis reduces the possibility of technical artifacts and allows the authors to claim that spectrin acts cell-autonomously to support neuroblast migration and neurite outgrowth. Specifically, the authors present a model of Q cell migration where SPC-1, UNC-70, and UNC-44 localize to the rear of the migrating cell to exclude actin polymerization in the cell posterior (or to the dendrite shaft to presumably prevent ectopic branching at the tip). The authors also make the interesting observation that SPC-1 dynamically reorganizes during cellular development, from cytokinesis to migration to neurite outgrowth. This observation provides a nice contrast to the canonical view of the spectrin-ankyrin as a static, structural skeleton.

The idea of spectrin and ankyrin acting as a membrane "seal" to prevent ectopic actin polymerization is not new in itself, but the authors convincingly show this role in neuronal migration and neurite formation using a relevant, in vivo developmental context. This advancement supports previous in vitro work and allows for the future interrogation of how asymmetric spectrin and ankyrin are established in vivo, including how the spectrin skeleton is dynamically rearranged for different developmental functions. Overall this is an excellent paper to be published in JCS.

First revision

Author response to reviewers' comments

June 20, 2020

Michael Way, Ph.D.
The Journal of Cell Science

Dear Michael,

Thank you for your letter of June 15, 2020. I now submit our revised manuscript entitled "Spectrin-based Membrane Skeleton Is Asymmetric and Remodels during Neural Development" for publication in *the Journal of Cell Science*.

We are glad that both referees are positive about our work. As detailed in response to reviewer #1, we have carefully addressed each point. In particular, we change "mechanics" to "skeleton" in the title, and we provide additional information on our mass spectrometry experiments. We feel that our revised manuscript is ready for publication. We thank you for handling this manuscript and look forward to hearing from you.

Sincerely,

Guangshuo

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RESPONSE TO THE REFEREES

Reviewer 1 Advance Summary and Potential Significance to Field:
Spectrin is a major component of the cortical actin cytoskeleton underneath the plasma membrane. This work by Jia and colleagues characterizes in vivo function of spectrins during

neuronal development in C. elegans. A nice combination of genetics and live imaging of neuroblasts and developing neurons demonstrated important roles of two spectrin genes in neuroblast migration and dendrite formation. They analyzed two spectrin genes, spc-1 and unc-70. Mutations in spc-1 or unc-70 impair long-range migration of neuroblasts and cause accumulation of actin filaments in the lateral and rear cortices instead of leading edge in normal migrating neuroblasts. Consistently, these spectrins localized to lateral and rear cortices. During dendrite elongation, abnormal branching of dendrites occurred in the spectrin mutants. During this process, spectrin redistributes from the cell body to the dendrites, suggesting that spectrin normally inhibits lateral branching. Finally, they found ankyrin (unc-44) as a binding partner of SPC-1 spectrin and showed that unc-44 mutant are also defective in neuroblast migration and dendrite formation. Overall, this is a high-quality work reporting novel in vivo functions of spectrin in neuronal development.

We thank the referee for the positive assessment of our study.

Reviewer 1 Comments for the Author:
Major points

1. The title of this manuscript contains “spectrin-based membrane mechanics”, but this is an overstatement. This work contains no data for “mechanics”. Therefore, the title should be revised to reflect fair conclusion of the study.
We appreciate the referee for pointing out the overstatement. We have changed “mechanics” to “skeleton” in the revised title.

2. In Fig. 3B, specific labels for dendrites and axons should be added for non-neurobiologists. Also, a statement of how dendrites and axons were distinguished should be added.
We have labeled the dendrites and axons for Fig. 3B. The location and morphology of the *C. elegans* dendrites and axons have been systematically documented at the single-cell resolution. Such information is available from the database “wormatlas”. In the figure legend of 3B, we have added the information about how dendrites and axons were distinguished as below:

“Dendrites and axons were labeled and distinguished based upon the information from wormatlas.org.”

3. In Fig. 4, UNC-70 and UNC-44 are shown as binding proteins for SPC-1::GFP in the mass spec analysis without showing what other proteins were identified in the pool and how they were identified as positives as compared with controls. UNC-70 and UNC-44 are quite obvious “hits”. The authors should provide additional data for how a control experiments were set up, how these “hits” were identified from the pool of other positives, and what other specific and non-specific proteins were identified in the analysis.

We thank the Reviewer for pointing out the issue. As the Reviewer indicated that UNC-70 and UNC-44 are obvious hits based upon literature, we confirmed that these interactions occur in *C. elegans*. We have now provided an Excel file summarizing the comprehensive list of the proteins that the mass spectrometry analysis identified from the SPC-1::GFP experiments. As shown in this file, UNC-70 and UNC-44 are among the top hits. We have performed GFP-affinity purification and mass spectrometry for other proteins that are associated with the plasma membrane (e.g., MIG-13, a transmembrane protein essential for Q neuroblast migration). However, we did not uncover UNC-70 and UNC-44, suggesting that our experimental pipeline can detect specific interactions. We have revised the results on line 20 page 9:

“Another top hit was UNC-44 (Supplementary Table S4), an ortholog of human ankyrin protein , which was another known binding partner of the membrane skeleton (Lorenzo, 2020). We used the same GFP-affinity purification and mass spectrometry to identify binding partners for other proteins that are associated with the plasma membrane [e.g., MIG-13, a transmembrane protein essential for Q neuroblast migration (Zhu, 2016)]; however, we did not uncover UNC-70 and UNC-44, suggesting that the experimental pipeline can detect specific interactions.”

4. The data suggest that SPC-1 and UNC-70 have similar functions in neuronal development. No additional experiments are needed, but the authors should discuss whether SPC-1 and UNC-70 act similarly or have some distinct functions.
We thank the Reviewer for suggesting additional discussion. This issue is similar to Minor points #2. Fig. 1B shows that neuronal migration is similarly affected in *spc-1* and *unc-70* mutant adults. Fig. 3B-C show that dendrites are similarly defective in *spc-1* and *unc-70* mutant adults. Indeed, we only have time-lapse imaging data of neuroblast migration from *spc-1* mutant larvae. Considering that SPC-1 and UNC-70 form the spectrin complex and that both mutations cause similar phenotypes in neuronal migration and uncoordinated animal behavior, we suggest that SPC-1 and UNC-70 play the same function during neuronal development. We discuss the issue in the revised Discussion on line 7, page 13:

"SPC-1 and UNC-70 are subunits in the spectrin complex, and both mutations similarly reduce neuronal migration (Fig. 1B) and disrupt dendrite outgrowth (Fig. 3B-C), leading to uncoordinated animal behavior. These data suggest that SPC-1 and UNC-70 may play a similar function during neuronal development."

Minor points

1. Legend for Fig. 1F. "GFP-tagged F-actin" is not an accurate description. "GFP-labeled F-actin" may be acceptable.
We have corrected Fig. 1F legend to "GFP-labeled F-actin"

2. In Fig. 3D, only spc-1 mutant was analyzed by live imaging. How about unc-70 mutant?
As discussed in the Major points #4 above, we suggest that neuronal development (e.g., dendrite outgrowth in Fig. 3D) may be similarly disrupted in the *unc-70* mutant. In support of this, Fig. 3B-C showed that dendrites are similarly defective in *spc-1* and *unc-70* mutant adults.

Reviewer 2 Advance Summary and Potential Significance to Field:
Jia et al. investigate the role of the spectrin membrane skeleton in neuroblast migration using the C. elegans Q cell and its progeny as a model. The authors present interesting and convincing in vivo data on the asymmetric and dynamic localization of three proteins associated with the membrane skeleton: SPC-1/alpha spectrin, UNC-70/beta spectrin, and UNC-44/ankyrin. The use of knock-in GFP tags and cell-specific CRISPR/Cas9 mutagenesis reduces the possibility of technical artifacts and allows the authors to claim that spectrin acts cell-autonomously to support neuroblast migration and neurite outgrowth. Specifically, the authors present a model of Q cell migration where SPC-1, UNC-70, and UNC-44 localize to the rear of the migrating cell to exclude actin polymerization in the cell posterior (or to the dendrite shaft to presumably prevent ectopic branching at the tip). The authors also make the interesting observation that SPC-1 dynamically reorganizes during cellular development, from cytokinesis to migration to neurite outgrowth. This observation provides a nice contrast to the canonical view of the spectrin-ankyrin as a static, structural skeleton.

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We appreciate that the referee is also positive for our study.

Second decision letter

MS ID#: JOCES/2020/248583

MS TITLE: Spectrin-based Membrane Skeleton Is Asymmetric and Remodels during Neural Development

AUTHORS: Ru Jia, Yongping Chai, Chao Xie, Gai Liu, Zhiwen Zhu, Kaiyao Huang, Wei Li, and Guangshuo Ou
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.