In vivo proximity biotin ligation identifies the interactome of Egalitarian, a Dynein cargo adaptor.

Frederick C. Baker, Hannah Neiswender, Rajalakshmi Veeranan-Karmegam and Graydon B. Gonsalvez

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Editor: Thomas Lecuit

Review timeline

Original submission:	25 June 2021
Editorial decision:	13 September 2021
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Second revision received:	4 October 2021
Accepted:	18 October 2021

Original submission

First decision letter

MS ID#: DEVELOP/2021/199935

MS TITLE: In vivo proximity biotin ligation identifies the interactome of Egalitarian, a Dynein cargo adaptor.

AUTHORS: Frederick C Baker, Hannah Neiswender, Rajalakshmi Veeranan-Karmegam, and Graydon B Gonsalvez

I sincerely apologise for the very long time before being able to come back to you due to the difficulty to find available rerviewers. I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' 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.

The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. I concur with one of the referees that the article ought to be streamlined. Instead of writing it as a Report do reduce the text and bring the article down to 7 Figures maximum, the rest being in Supplement.

Reviewer 1

Advance summary and potential significance to field

The manuscript by Baker et al attempts to address the interactome of Egalitarian (Egl), an adaptor for Dynein transportation, by tagging Egl with TuboID biontin ligase (Trbo) to biotinylate and then identify proximal Egl-interacting partner proteins. The obtaining of the Egl interactome (and dTtc1 interactome) leads the author to draw two additional conceptual hypotheses, 1) dTtc1 being another cargo adaptor for Dynein transport and 2) the co-transport of mRNAs in vesicles of the endomembrane system in Drosophila egg chamber model, and one useful

research tool that uses Trbo linked GFP-binding protein to conveniently analyze the interactomes of GFP-tagged proteins in vivo.

This research brings in novel knowledge to the fast-advancing field of intracellular molecule trafficking. The obtained interactomes of Egl and previously under-investigated, conserved protein dTtc1 provide valuable information/hypothesis to guide future research in cargo trafficking in Drosophila germline and other types of cells. Importantly, the GBP-Trbo interactome toolkit will greatly convenience interactome studies in the future.
Therefore, I suggest the publication of this research after the authors address my 2 minor comments .

Comments for the author

Minor comments:
1. In Fig 4 and related results, the authors showed that egl shRNA caused the disruption of Me31B normal localization/distribution in the egg chambers. Is there a possibility that the observed phenotype was caused by the change/drop in me31B mRNA or protein level? I do note that this is only a comment, so it will be up to the authors whether they like to address it by experimentation.
2. The authors found dTtc1 in Egl interactome, but Egl seems to be absent from dTtc1 interactome when the two interactomes were obtained with similar methods. Would the authors provide some explanation and discussion on this?

Reviewer 2

Advance summary and potential significance to field

Understanding the molecular basis of how the MT minus-end directed motor, Dynein, transports its cargoes is an important, broadly significant cell biological goal. This is achieved through “adaptor proteins” that directly interact with Dynein, and with cargoes, specifying the cargoes’ final intracellular location. A general hypothesis in the field is that, although some adaptors are known, others remain to be identified. A second hypothesis is that, for known adaptors, unknown cargoes remain to be identified.

This paper focuses on the latter – that there are other types of cargo, other than mRNA, that are linked to dynein via the known adaptor, Egalitarian (Egl). Due to the very transitory/weak interactions between Egl Dynein and cargoes maintained after biochemical disruption of cells, the authors used proximity biotin ligation to identify the Egl interactome from the developmentally significant tissue, the Drosophila egg chamber. They also describe a modified technique of purification, based on the nanobody, GFP-binding protein, as a technological advance with broad use for the cell & developmental biology community.

The paper has significance for the field and provides new data that adds to our understanding of Egl function in the Drosophila oocyte. In essence, it is a series of AP-MS experiments, and associated cell biological validation, designed to explore the interactomes for: (i) Egl, (ii) Me31B, (iii) dTtc1, plus some partial characterisation of another Egl interactor, Vap33. Much of the text in Results is, by its nature, discussion of interactions between proteins identified by the AP-MS approach, while the authors provide mainly correlative evidence for functional relationships between Vap33, Me31b and dTtc1. There is stronger evidence in relation to Egl, through use of an Egl-RNAi line, co-expressed with fluorescently-tagged versions of the interactors and the description of a new technique that undoubtedly has potential importance for characterising interactomes of GFP-tagged proteins.

The work is potentially suitable for publication in Development – but, in my view, as a Research Report, rather than an article. There is much repetition between the Results and Discussion; while a simpler organisational approach, with less emphasis on the many co-localisations (Figures 4-7) and greater emphasis on the co-ordinated interactomes, would suit the data.

Comments for the author

In my view, this submission needs to be rewritten as a Research Report, combining Results and Discussion and moving some of the correlatory microscopy images to Supplementary data.
No new experiments would be necessary, but several minor comments (see below) would need to be addressed.

Minor Comments:
The associated Supplementary Table for the triplicate Egl-Trbo experiment contains only the combined data from the experiments – it would be good to point the reader to the full datasets (perhaps online, on their lab website).
The authors mention that DHC was not present in their interactome. They should be more specific – is it that DHC fell outside of their cut-offs? Or was it not present at all in any of their triplicate MS results? It would be good to comment, in either case.

When introducing the reader to P bodies, it would be useful to include a little more information on this class of proteins – particularly drawing attention to the number of P body proteins in flies (which is specified through GO searches in Figures 8&9 as 39 in total) – and therefore whether Egl interact with a small, or large proportion of gene products classified as P body components? This may help to strengthen the hypothesis that P bodies, in general, interact with Egl.
Why was Me31B chosen as initial focus? Is it that reagents were available? Or that Me31B is a "classical" P body component? A little more on the rationale would be welcome.
Include the "n" in relation to the images, image analysis and stats in all cell biological images.
Present a protein alignment of CG14894 with human TTC1 in a supplementary figure (perhaps with a cartoon verion in the main figures), so that the assertion that these are homologues is clear to the reader.

The co-IP of BICD2 with TTC1 in HeLa cells (Supp Figure 1B) needs an appropriate methods section
Figure 9C – what is the x axis? Fold enrichment?
The authors mention P Body components as being enriched in both AP-MS experiments – it would be good to include P Body as a GO enrichment term in revised Figures 8C and 9C.

First revision

Author response to reviewers' comments

Comments for the Editor and Reviewers
We would like to take this opportunity to sincerely thank the Editor and the reviewers for the peer review of our manuscript. We are extremely grateful for your time and effort. Our revised manuscript is attached and we believe we have addressed all comments and critiques. As per the suggestion of Reviewer 2 and the Editor, we have shorted the manuscript to a total of 6 figures and have also revised the text to focus more on the proteomics versus the localization data.

We have also added one additional tool that will further expand the utility of the GBP-nanobody approach. We show using a fly strain expressing GBP-Trbo from a UASt vector that the same strategy can be used in somatic fly tissues in order to purify GFP tagged proteins for proteomics analysis (Supplemental fig. 4).

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We have added this data point (Supplemental figure 1C). We are glad we performed this experiment. Me31b levels are indeed reduced in the egl shRNA background. However, the conclusion regarding localization is still valid because the quantification was done by measuring the oocyte localized signal to the signal in the rest of the egg chamber. We are not directly comparing

fluorescence intensity between the control and Egl depleted strains. Thus, even if the level of Me31b was reduced but the localization pattern was unchanged, this would be reflected in the quantification.

2.The authors found dTtc1 in Egl interactome, but Egl seems to be absent from dTtc1 interactome when the two interactomes were obtained with similar methods. Would the authors provide some explanation and discussion on this?
We have added a discussion of this point to the revised manuscript. This is indeed the case; although we were able to recover peptides corresponding to Egl in the Me31b proteome, we did not recover Egl peptides in the dTtc1 proteome. dTtc1 brought down many more proteins than Egl. Thus, our working hypothesis is that although dTtc1 is part of the Egl/Dynein complex, this might only represent a small percentage of total dTtc1. The majority of dTtc1 in the cell may be present in complexes that does not contain Egl/Dynein. As such, Egl is under-represented in the dTtc1 proteomics using this approach.

Reviewer 2:
In my view, this submission needs to be rewritten as a Research Report, combining Results and Discussion, and moving some of the correlatory microscopy images to Supplementary data.
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The full data sets have been added (Sheet 2 in the respective Supplemental tables).

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This discussion has been added to the text.

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A GO analysis of the Egl interactome has been added (Supplemental fig. 1A). P bodies are indeed enriched within the Egl interactome.

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Present a protein alignment of CG14894 with human TTC1 in a supplementary figure (perhaps with a cartoon version in the main figures), so that the assertion that these are homologues is clear to the reader.
This has been added to the revised manuscript (Supplemental fig. 2 and Fig. 3b).

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We truly apologize for this oversight. We have added this to the methods section.

Figure 9C – what is the x axis? Fold enrichment?
We apologize for this oversight as well. This was accidentally omitted in between our graphing program and Adobe Illustrator. Yes, it is fold enrichment. This has been fixed (now fig. 6C).

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A cellular component analysis for Me31b, which includes the P body GO term, has been added (Supplemental fig.4A). The GO term “P body” was not enriched for the dTtc1 interactome (shown in Fig.6C).

Second decision letter

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The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

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.

Second revision

Author response to reviewers' comments

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Third decision letter

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AUTHORS: Frederick C Baker, Hannah Neiswender, Rajalakshmi Veeranan-Karmegam, and Graydon B Gonsalvez
ARTICLE TYPE: Techniques and Resources Article

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