DLC-1 facilitates germ granule assembly in C. elegans embryo

Nicholas Day, Mary Ellenbecker, Xiaobo Wang, and Ekaterina Voronina

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Review Timeline:

- Submission Date: 2021-06-02
- Editorial Decision: 2021-06-29
- Revision Received: 2022-01-31
- Accepted: 2022-02-27

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 #E21-05-0275
TITLE: DLC-1 facilitates germ granule assembly in C. elegans embryo

Dear Dr. Voronina:

Thanks for submitting your paper to MBoC. The paper has been carefully reviewed and has potential for publication after you revise the manuscript in response to the reviews below. The most important issues that should be addressed are 1) determining if the impacts of loss of dlc-1 are directly altering P-granules or indirect, through altered polarity regulation and 2) resolving if the phenotypes is due to loss of the whole motor activity or really specific to the DLC. Please also address each comment in a detailed response.

Sincerely,

Amy Gladfelter
Monitoring Editor
Molecular Biology of the Cell

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Reviewer #1 (Remarks to the Author):

Dynein Light Chain (DLC-1) and its DLC homologs function with intermediate and heavy chain co-factors within a dynein motor.
However, studies have shown that DLC facilitates the assembly of numerous complexes through its LC8 protein interaction domain. For example, the authors of this study have previously shown that DLC-1 interacts with and serves as a co-factor for the germline RNA-binding proteins FBF-2 and GLD-1. Given the growing list of DLC-1 identified through multiple high-throughput screens, the authors use an in silico motif scan to predict additional LC8 interaction motifs that extend beyond the historical QT motif identified when fewer LC8 interactions were known. The current list of DLC-1 interacting proteins contains several components of germline P granules. Depletion of dlc-1 causes P granules to disperse, suggesting that DLC-1 promotes P-granule assembly - possibly through its propensity to homodimerize and bring together interacting P-granule proteins. While DLC-1::GFP is expressed throughout the germline, the authors use a proximity ligation assay and GST pull-downs to show that DLC-1 does interact with multiple P-granule proteins, supporting their model for DLC-1 function. The author's findings are novel and have broad implications to the dynamics liquid-liquid phase separation field as LC8 has a propensity for binding protein motifs in intrinsically disordered sequences.

While enthusiastic about these findings, some things need further addressed. Foremost, the authors may be missing essential links between DLC-1 and P granules that should at least be discussed if not addressed experimentally.

First, Dynein Light, Heavy, and Intermediate Chains interact within the dynein motor in yeast and other models. While the authors propose that DLC-1 assembles P granules independent of the dynein motor, RNAi depletion of any of these results in the same P-granule dispersal phenotype (Updike & Strome 2009, Table 1 dhc-1, dlc-1, and dyci-1). Wouldn't this suggest that the phenotype is dependent on the dynein motor?

Second, DLC-1 is discussed and categorized with other nuclear pore complex factors in the Updike 2009 study based upon its homology with yeast Dlc2, a known nucleoporin (Stelter et al. 2007). Stelter et al. show that at least 25% of Dlc2 can be found within NPCs. Several papers have now shown the structure of Dlc2 within NPCs, binding Nup159 (a cytoplasm-facing FG-Nup with homology to human NUP214 and C. elegans NPP-14) through multiple regularly spaced LC8 (QT) binding sites (Romès 2012, Nyarko 2013, a good review in Allegretti 2020). Previous papers from (corresponding - Voronina et al. 2010), (Sheth et al. 2010) and (Updike 2009, 2011), show the importance of these FG-Nups on P-granule localization. A quick search of C. elegans NPP-14 shows (potentially) up to 7 LC8 (QT) binding sites. A brief mention of this may be warranted. Although likely beyond the scope of this paper, the authors should consider testing if DLC-1 and NPP-14 interact at the cytoplasmic surface of NPCs (similar to Dlc2 and Nup159), and whether DLC-1 depletion compromises NPCs and, secondarily, the P granules anchored to them.

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- On page 7, lines 135 to 147, the methods need to be clarified and details added. It may not be possible for someone to replicate this pipeline as it is stated. Additionally, the analysis and enrichment statement on page 9, line 187, should make a reference to the data.

- PLA... Starting on page 10, more information should be provided to explain PLA. For example, the acronym is not defined on line 214. While the Day et al 2020 paper is referenced, a brief recap of the method would be appreciated. It would also add clarity to include references to the Sigma-Aldrich Duolink PLA system if that is what is being used.

- Page 11 line 227. Why are PGL-3 and PGL-1 affinities are flipped in PLA vs. the pull-down. Does this have something to do with the transgenes used or antibody concentrations?

- At the end of page 12 and beginning of 13, the germ/soma analysis could be tightened up to add clarity. For example, are you capturing the surge in somatic PGL observed around the 30-50 cell stage? PGL is subsequently cleared out by autophagy, but you may want to look at just z2/z4 stage embryos instead of P4 embryos to ensure that you're past the PGL surge. Additionally, I couldn't find information on the GFP control strain. Is it a transgene, and is a germline promoter being used? It looks like GFP in 4A is in P granules.

- On lines 281 and 282, it is concluded that DLC-1/PGL RNPs are enriched in the germ cells. Why is this stated? Fig 3S shows DLC-1 expression in z2/z3, and of course, PGLs are there, so shouldn't the germ cell enrichment be expected? Clarify why this is significant.

- PLA in Figures 2, 3, and 4 are barely visible, even when zoomed up. Can those panels be made brighter?

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- The GFP::PGL-3 strain is not included in the strain list.

- Figure 5S. Clarify that germline = adult germline. This will help avoid confusion from Fig 5D that shows PGL phenotypes in dlc-1 RNAi embryos.

Reviewer #2 (Remarks to the Author):

Studies of P granules assembly have contributed significantly to our understanding of the mechanisms that control RNP granule assembly, and this manuscript describes a role for the LC8 protein DLC-1 in this process in the C. elegans embryo. Day et al show that DLC-1 interacts with a subset of P granule proteins in GST-pull down assays and depletion of DLC-1 causes a severe defect in the assembly of PGL-1, PGL-3 and MEG-3 into granules in early and late embryos. Proximity ligation assays support the close proximity between DLC-1 and both PGL-1 and PGL-3, particularly in germline cells. Overall, the data are compelling, clearly described and reasonably interpreted and this study makes a novel contribution to our understanding of the mechanisms...
that control P granule assembly.

One lingering issue that should be addressed prior to publication is the extent to which dlc-1 loss disrupts cell polarity/asymmetric division generally versus playing a specific role in P granule assembly. This could be done by assessing whether PAR polarity is established normally and/or whether other cytoplasmic asymmetries form normally (for example, MEX-5 or PIE-1) in the one cell embryo. If these asymmetries are normal in dlc-1 mutant embryos, the conclusion that DLC-1 plays a specific role in P granule organization would be significantly strengthened. If there are more general defects in polarization, either mbk-2;dlc-1 or mex-5/6;dlc-1 mutants could be analyzed to determine whether dlc-1 acts downstream of these P granule destabilizers to promote P granule assembly.

Minor comment:
The description in the Results of the selection of Motifs A, B and C could be made more concise and divided into 2-3 paragraphs, with some details moved to the Methods section.

It would be helpful to add some details regarding how the GST pull downs were performed to the Methods section, rather than directing the reader to previous studies.
We wish to thank the reviewers for their careful and helpful reviews of our manuscript. We are pleased to resubmit this much-improved manuscript, which we hope has fully addressed the reviewers’ initial concerns. The manuscript has been revised, and includes new data (Figure 9). All changes related to the reviewers’ original comments and suggestions are indicated below. The original review comments are italicized and our responses are in blue, below.

Sincerely,
Ekaterina Voronina

Reviewer #1 (Remarks to the Author):

*Dynein Light Chain (DLC-1) and its DLC homologs function with intermediate and heavy chain co-factors within a dynein motor. However, studies have shown that DLC facilitates the assembly of numerous complexes through its LC8 protein interaction domain. For example, the authors of this study have previously shown that DLC-1 interacts with and serves as a co-factor for the germline RNA-binding proteins FBF-2 and GLD-1. Given the growing list of DLC-1 identified through multiple high-throughput screens, the authors use an in silico motif scan to predict additional LC8 interaction motifs that extend beyond the historical QT motif identified when fewer LC8 interactions were known. The current list of DLC-1 interacting proteins contains several components of germline P granules. Depletion of dlc-1 causes P granules to disperse, suggesting that DLC-1 promotes P-granule assembly - possibly through its propensity to homodimerize and bring together interacting P-granule proteins. While DLC-1::GFP is expressed throughout the germline, the authors use a proximity ligation assay and GST pull-downs to show that DLC-1 does interact with multiple P-granule proteins, supporting their model for DLC-1 function. The author’s findings are novel and have broad implications to the dynamics liquid-liquid phase separation field as LC8 has a propensity for binding protein motifs in intrinsically disordered sequences.*

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*First, Dynein Light, Heavy, and Intermediate Chains interact within the dynein motor in yeast and other models. While the authors propose that DLC-1 assembles P granules independent of the dynein motor, RNAi depletion of any three of these results in the same P-granule dispersal phenotype (Updike & Strome 2009, Table 1 dhc-1, dlc-1, and dyci-1). Wouldn’t this suggest that the phenotype is dependent on the dynein motor?*

*In our hands, RNAi depletion of dhc-1 and dyci-1 resulted in a failure of oocyte production or a failure of early embryonic cell divisions. The absence of large P granules in one-cell embryos with massively endoreplicated DNA in these conditions might be secondary to the failure of cytokinesis. By contrast, dlc-1 knockdown embryos continue cell divisions until ~100-cell stage. Therefore, we suggest that P granule dispersal after knockdown of dhc-1 and dyci-1 on one
hand and dlc-1 on the other hand is resulting from distinct causes. We now emphasize this in Results and Discussion (lines 357-365; 494-500).

Second, DLC-1 is discussed and categorized with other nuclear pore complex factors in the Updike 2009 study based upon its homology with yeast Dlc2, a known nucleoporin (Stelter et al. 2007). Stelter et al. show that at least 25% of Dlc2 can be found within NPCs. Several papers have now shown the structure of Dlc2 within NPCs, binding Nup159 (a cytoplasmic-facing FG-Nup with homology to human NUP214 and C. elegans NPP-14) through multiple regularly spaced LC8 (QT) binding sites (Romes 2012, Nyarko 2013, a good review in Allegretti 2020). Previous papers from (corresponding - Voronina et al. 2010), (Sheth et al. 2010) and (Updike 2009, 2011), show the importance of these FG-Nups on P-granule localization. A quick search of C. elegans NPP-14 shows (potentially) up to 7 LC8 (QT) binding sites. A brief mention of this may be warranted. Although likely beyond the scope of this paper, the authors should consider testing if DLC-1 and NPP-14 interact at the cytoplasmic surface of NPCs (similar to Dlc2 and Nup159), and whether DLC-1 depletion compromises NPCs and, secondarily, the P granules anchored to them.

While DLC-1’s potential connection to nuclear pore function is intriguing, we decided against pursuing it in this study. The nucleoporins predicted to interact with DLC-1 based on homology to the yeast proteins (as suggested by the reviewer) or based on the results of our motif search (data not shown) are NPP-3, NPP-12, and NPP-14. However, none of the large-scale or targeted genetic studies testing the importance of nucleoporins for P granule integrity (Updike and Strome, 2009; Voronina and Seydoux, 2010; Sheth et al., 2010) recovered either of these presumptive DLC-1 interactors as contributors to P granule integrity. Additionally, a recent publication (Maheshwari et al., 2021, 10.1093/g3journal/jkab264, Table S1) reported that dlc-1(RNAi) results in multinucleated oocytes or enlarged nuclei, but does not affect NPC distribution. Based on the lack of genetic evidence supporting the function of presumptive DLC-interacting nucleoporins in P granule nucleation or assembly, we decided against pursuing this direction of research.

- On page 7, lines 135 to 147, the methods need to be clarified and details added. It may not be possible for someone to replicate this pipeline as it is stated. Additionally, the analysis and enrichment statement on page 9, line 187, should make a reference to the data.

Thank you for the suggestion, we have added further detail on bioinformatics in Materials and Methods section. Additionally, we clarified that the enrichment of DLC-1-binding RBP was relative to the previous candidate screen in the lab (lines 172-174).

- PLA... Starting on page 10, more information should be provided to explain PLA. For example, the acronym is not defined on line 214. While the Day et al 2020 paper is referenced, a brief recap of the method would be appreciated. It would also add clarity to include references to the Sigma-Aldrich Duolink PLA system if that is what is being used.
We now define the abbreviation (line 196), provide a summary of PLA approach, add references, and include additional detail in Materials and Methods.

- Page 11 line 227. Why are PGL-3 and PGL-1 affinities are flipped in PLA vs. the pull-down. Does this have something to do with the transgenes used or antibody concentrations?

Thank you for raising this question. First, we do not intend to suggest differential affinity of PGL-1 and PGL-3 for DLC-1. Establishing affinity of the interaction is challenging with GST pulldowns that test the interaction at a single concentration of binding partners. To reduce confusion, we have replaced the PGL-1 pulldown image with one of the replicates that detected a more robust PGL-1 band in the eluate. Second, PLA assay only detects differences in the abundance of PGL-1 and PGL-3 complex with DLC-1 in the germline, while the abundance in the embryos is not significantly different. The differences in germline DLC-1/PGL interactions may simply result from differences in the expression levels of PGL-1 and PGL-3. Another more exciting possibility is that the interaction of PGLs with DLC-1 is regulated by differential accessibility of binding sites on PGLs that might be due to post-translational modification or interactions with additional binding partners. We now bring up these possibilities in the Discussion (lines 439-446).

(A) - At the end of page 12 and beginning of 13, the germ/soma analysis could be tightened up to add clarity. For example, are you capturing the surge in somatic PGL observed around the 30-50 cell stage? PGL is subsequently cleared out by autophagy, but you may want to look at just z2/z4 stage embryos instead of P4 embryos to ensure that you’re past the PGL surge.
(B) - On lines 281 and 282, it is concluded that DLC-1/PGL RNPs are enriched in the germ cells. Why is this stated? Fig 3S shows DLC-1 expression in z2/z3, and of course, PGLs are there, so shouldn't the germ cell enrichment be expected? Clarify why this is significant.

These two issues are related. As we observed noticeable DLC/PGL proximity signal in the somatic cells of the early embryos, we aimed to assess whether PGLs are in complex with DLC-1 everywhere in the embryo or if there is a preference for complex formation in the germ cells. This question is best answered in the P4 stage embryos, during the somatic surge of PGLs. Detecting a significant enrichment of DLC/PGL proximity in germ cells despite somatic surge of PGLs suggests the interaction may be more relevant for germ cell function of PGLs. Following somatic clearance of PGLs, at the z2/z3 stage, expression of PGLs is limited to the germ cells, so any enrichment of PLA signal in the germ cells is simply due to PGL expression pattern, as the reviewer has noted. This logic is now communicated more clearly in the Results (lines 258-262).

Additionally, I couldn't find information on the GFP control strain. Is it a transgene, and is a germline promoter being used? It looks like GFP in 4A is in P granules.

The GFP transgene in the FLAG::DLC-1; GFP control strain is described in Table S3 (UMT422), and it is driven by gld-1 germline promoter. The GFP transgene is uniformly expressed in the
adult germline (Supplemental Fig. S1Aii) and throughout embryos (Supplemental Fig. S2Aii-Dii).
We regret any confusion concerning the labeling in Fig. 4: P granules in Fig. 4A-C are labeled with an antibody to the endogenous PGL-1 (K76 antibody from DSHB), not GFP. We have now pseudocolored K76 staining yellow and annotated each color channel within the Figure for clarity.

- **PLA in Figures 2, 3, and 4 are barely visible, even when zoomed up. Can those panels be made brighter?**
  Thank you for the suggestion, we have adjusted the brightness of the PLA panels in Figures 2, 3, and 4 and changed PLA channel pseudocolor to magenta in Figures 2 and 4 for better contrast.

- **Figure 2D and 3P. It’s hard to judge the significance of the data, especially with large error bars on a bar graph. Would it be possible to include individual data points here like was done in figure 4?**
  Thank you for the suggestion, the data in Figures 2D and 3P has been replotted as dot-plots with average/SD. The difference between the control and experimental PLA densities was evaluated for significance statistically, as noted in each Figure legend.

- **The GFP::PGL-3 strain is not included in the strain list.**
The GFP::PGL-3 strain UMT77 is included in the Supplemental Table 3.

- **Figure 5S. Clarify that germline = adult germline. This will help avoid confusion from Fig 5D that shows PGL phenotypes in dlc-1 RNAi embryos.**
  Thank you for the suggestion, we have made the change in the figure legend (now Supplemental Figure S3) and throughout the text when referring to the adult germline.

Reviewer #2 (Remarks to the Author):

*Studies of P granules assembly have contributed significantly to our understanding of the mechanisms that control RNP granule assembly, and this manuscript describes a role for the LCB protein DLC-1 in this process in the C. elegans embryo. Day et al show that DLC-1 interacts with a subset of P granule proteins in GST-pull down assays and depletion of DLC-1 causes a severe defect in the assembly of PGL-1, PGL-3 and MEG-3 into granules in early and late embryos. Proximity ligation assays support the close proximity between DLC-1 and both PGL-1 and PGL-3, particularly in germline cells. Overall, the data are compelling, clearly described and reasonably interpreted and this study makes a novel contribution to our understanding of the mechanisms that control P granule assembly.*

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1 plays a specific role in P granule organization would be significantly strengthened. If there are more general defects in polarization, either mbk-2;dlc-1 or mex-5/6;dlc-1 mutants could be analyzed to determine whether dlc-1 acts downstream of these P granule destabilizers to promote P granule assembly.

We thank the reviewer for this suggestion. We have determined the localization of GFP::PAR-2 and GFP::PIE-1 in dlc-1 mutant or RNAi-treated embryos. We did observe some embryos with abnormal distribution of PAR-2 or PIE-1, however P granule assembly in these embryos was not affected (although localization was abnormal; data not shown). By contrast, in all embryos with dispersed or absent P granules, apparent localization of GFP::PAR-2 and GFP::PIE-1 was normal. We conclude that P granule dispersal in dlc-1 mutants does not result from a general loss of cytoplasmic asymmetries supporting a specific role for DLC-1 in P granule assembly. These results are now included in a new Figure 9 and described in the Results (lines 394-413).

Minor comments:
The description in the Results of the selection of Motifs A, B and C could be made more concise and divided into 2-3 paragraphs, with some details moved to the Methods section.

We thank the reviewer for this suggestion. We have shortened this section of the Results and divided it into 2 paragraphs, with details moved into the Methods.

It would be helpful to add some details regarding how the GST pull downs were performed to the Methods section, rather than directing the reader to previous studies. We have now added the details of GST pulldowns in the corresponding Methods section.
Dear Dr. Voronina:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell. The reviewers are pleased with the effort in the revised manuscript and agree it makes an important contribution to the field. Congratulations!

Sincerely,
Amy Gladfelter
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Voronina:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

Your paper is among those chosen by the Editorial Board for Highlights from MBoC. Highlights 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.

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We are pleased that you chose to publish your work in MBoC.

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

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Reviewer #1 (Remarks to the Author):

The authors have sufficiently addressed all of my previous comments. The document and its presentation of the data are substantially improved. This impressive study opens up the unexplored role of DLC in germ-granule assembly and establishes the effectiveness of the PLA technique to visualize protein interactions in C. elegans. I'm enthusiastic about these important findings.
Reviewer #2 (Remarks to the Author):

The authors have addressed my previous comments in the revised manuscript and I am supportive of publication.