Canonical nucleators are dispensable for stress granule assembly in *Drosophila* intestinal progenitors

Kasun Buddika, Ishara S. Ariyapala, Mary A. Hazuga, Derek Riffert and Nicholas S. Sokol
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MS TITLE: Canonical nucleators are dispensable for stress granule assembly in intestinal progenitors

AUTHORS: Kasun Buddika, Ishara S Ariyapala, Mary A Hazuga, Derek Riffert, and Nicholas S Sokol
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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.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but raised some critical points that will require amendments to your manuscript. I hope that you will be able to carry these out, because I would like to be able to accept your paper.

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

This manuscript by Buddika et al. describes the identification of a population of stress granules (SGs) in intestinal precursor cells (IPCs) in Drosophila. These granules (called IPSGs) contain canonical markers for SGs and respond to stressors that are expected to induce the formation of SGs. Most of the work in this field is done in homogenous cell populations. the rationale for using this system is as a model for adult stem cell pools in vivo. This, in and of itself, does not

significantly advance the SG field. However, the following findings are significant. First, IPSGs are found primarily in mitotic IPCs but do not appear to form in their terminally differentiated neighbors. At this point, it is not clear why there is a difference. It would be nice to expand more on this in the discussion. Second, formation of IPSGs in response to stress does not appear to require canonical nucleates of SG formation. This is a significant finding to the SG field. While this manuscript does not provide mechanistic insight into this process, it does open up new lines of research.

Comments for the author

In my opinion, this paper is appropriate for publication in JCS as is with only a few relatively minor revisions.

1. The most significant of these is that Figure 8 does not really add much to the story. It is an interesting finding and certainly may be worth pursuing the future. However, it deviates from the main point to the paper (i.e. that IPCs have SGs that can form in the absence of canonical SG nucleators).
2. The usefulness of SGs as markers for stem cell populations is questionable.
3. It would be useful to show a simple diagram early in the results (Fig. 1 or Fig. S1) showing the developmental relationship between the various cell types (including markers).
3. In Figure S1, it would be nice to include a diagram of the Rin gene structure showing the CRISPR strategy for HA insertion.
4. I believe the legend for Fig. S3 is wrong. FMRP is red and HRP green.
5. Lines 192-196 refer to experiments with 2 mM arsenite or rapamycin. Neither supplemental figure seem to show this (both legends indicate 1 mM).
6. It would be useful to include quantitive data for Fig. 3 showing the degree of colocalization.

Reviewer 2

Advance summary and potential significance to field

This manuscript presents data related to the formation of stress granules in Drosophila tissues upon ex vivo exposure to sodium arsenite, a stress that is found to be very effective in triggering this coalescence in tissue culture cells, as well as rapamycin (which is less used and somewhat problematic because of more pleiotropic effects).

The results presented here are of interest because, although it is not the first time, the manuscript reports the formation of stress granules in Drosophila tissues where it has not been reported before, in particular in the intestinal stem cells.
Many factors are investigated and some mechanisms such as the fact that translation is stalled when stress granules are formed and resumes when they dissolve.
The reversibility and presence of RNAs and RNA binding proteins in the structures presented in the paper strongly support that they are stress granules. The microscopy is very strong.

Comments for the author

1.It is interesting that upon KRB, tiny stress granules are present. However, do they represent the basal 'non stressed' situation or does KRB trigger a certain level of stress that triggers the formation of tiny SGs. How does SG formation look like when the tissues are bathed in full medium such as Schneider's? indeed KRB has been shown to lead to the formation of stress granules in Drosophila cells and the reviewer does not consider it to be a 'basal condition'. The reviewer is keen on having the basal conditions well described.
2. The authors conclude that upon arsenite treatment, the stress granules in intestinal stem cells (IPSG) that form do not depend on the canonical markers G3BP, ATaxin2 and Rox8. When depleted, stress granules are still formed.
Yet, they show that G3BP (Rasputin) and Ataxin2 are sufficient for stress granule formation. How can a factor be sufficient but be dispensable (so not necessary). The reviewer cannot put their head around these seemingly contradictory results. The reviewer is aware of necessary factors that are not sufficient but not of sufficient factors that are not necessary.

Please explain.

3. The results above suggest that the stress granules in intestinal stem cells likely form by an unconventional mechanism due to other factors. However, this is not dissected in the slightest. What is also not done is to show that, upon depletion of G3BP and Ataxin 2, the stress granules that form are still reversible and still contain RNAs and possibly are the same as when these two factors are present.

4. Altogether, despite the fact that the paper is of good quality and data rich, it is essentially descriptive. Being descriptive is not wrong per se, but here the conclusions of these descriptions are not completely different from what is now well established, i.e, that there are many pathways to form stress granules and that all in all, they all are a bit different (including a study from Nancy Kedersha a couple of years ago and one from Paul Anderson recently, which should be discussed). Of course, the novelty here is that the heterogeneity is within one tissue (stem cells and enterocytes) and in vivo.
So, although the reviewer is left a little frustrated that the mechanisms are not better dissected (For instance, do the effects of specifically not forming stress granules in one cell type or the other affect gut homeostasis? Are the RNA (and protein) signatures of the stress granules in stem cells different from those formed in other cells??), the reviewer is overall positive.

First revision

Author response to reviewers' comments

We thank the reviewers for their comments. We have made a number of changes to the text of the main manuscript.

Reviewer 1 Advance Summary and Potential Significance to Field

This manuscript by Buddika et al. describes the identification of a population of stress granules (SGs) in intestinal precursor cells (IPCs) in Drosophila. These granules (called IPSGs) contain canonical markers for SGs and respond to stressors that are expected to induce the formation of SGs. Most of the work in this field is done in homogenous cell populations. the rationale for using this system is as a model for adult stem cell pools in vivo. This, in and of itself, does not significantly advance the SG field. However, the following findings are significant. First, IPSGs are found primarily in mitotic IPCs but do not appear to form in their terminally differentiated neighbors. At this point, it is not clear why there is a difference. It would be nice to expand more on this in the discussion. Second, formation of IPSGs in response to stress does not appear to require canonical nucleates of SG formation. This is a significant finding to the SG field. While this manuscript does not provide mechanistic insight into this process, it does open up new lines of research.

Thank you for this assessment!

As the reviewer highlights, the differential SG formation in intestinal progenitor cells versus terminally differentiated cells is an interesting observation. One simple reason could be that that SG proteins are weakly expressed in differentiated cells compared to progenitor cells. Such low abundance of SG markers will hinder the identification of SGs in differentiated cells. Although not studied in the current manuscript, phosphorylation of eukaryotic initiation factor 2a (eIF2a) is a key event in SG formation in other cell types. Based on previous studies, P-eIF2a is present in intestinal progenitor cells at high levels at least during aging (Wang et al., PLoS Genet. 2015). Therefore, the ability to phosphorylate eIF2a could also be causative of differential SG formation in progenitor vs terminally differentiated cells.

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1. The most significant of these is that Figure 8 does not really add much to the story. It is an interesting finding and certainly may be worth pursuing the future. However, it deviates from the main point to the paper (i.e. that IPCs have SGs that can form in the absence of canonical SG nucleators).

Thank you for this suggestion. To address this comment, we have decided to delete Figure 8 and remove the text referring to those results.

2. The usefulness of SGs as markers for stem cell populations is questionable.

At least in the intestinal epithelium, SG proteins are reasonably good markers of progenitor cells. To some extent, the utility of each SG protein shown in Figure 1 as a progenitor marker depends on the quality of the antibody to detect it. LIN-28::GFP is very good – high quality anti-GFP antibodies detect abundant LIN-28 expression specifically in ISCs and EBs – while Ago1 is not as good because of the relatively poor quality of the anti-Ago1 antibody.

3. It would be useful to show a simple diagram early in the results (Fig. 1 or Fig. S1) showing the developmental relationship between the various cell types (including markers).

Thank you for this suggestion. A schematic has been added to Figure S1.

3. In Figure S1, it would be nice to include a diagram of the Rin gene structure showing the CRISPR strategy for HA insertion.

Thank you for this suggestion. A schematic has been added to Figure S1.

4. I believe the legend for Fig. S3 is wrong. FMRP is red and HRP green.

Thank you for catching this mistake!! The legend has been fixed.

5. Lines 192-196 refer to experiments with 2 mM arsenite or rapamycin. Neither supplemental figure seem to show this (both legends indicate 1 mM).

Thank you for catching this mistake also!! The text has been rewritten.

6. It would be useful to include quantitive data for Fig. 3 showing the degree of colocalization.

As the reviewer has suggested, we calculated the Pearson's correlation coefficient (Pearson's R values) for each of the staining combinations shown in Fig. 3 and included those data in the main text.

Reviewer 2 Advance Summary and Potential Significance to Field

This manuscript presents data related to the formation of stress granules in Drosophila tissues upon ex vivo exposure to sodium arsenite, a stress that is found to be very effective in triggering this coalescence in tissue culture cells, as well as rapamycin (which is less used and somewhat problematic because of more pleiotropic effects).

The results presented here are of interest because, although it is not the first time, the manuscript reports the formation of stress granules in Drosophila tissues where it has not been reported before, in particular in the intestinal stem cells. Many factors are investigated and some mechanisms such as the fact that translation is stalled when stress granules are formed and

resumes when they dissolve. The reversibility and presence of RNAs and RNA binding proteins in the structures presented in the paper strongly support that they are stress granules. The microscopy is very strong.

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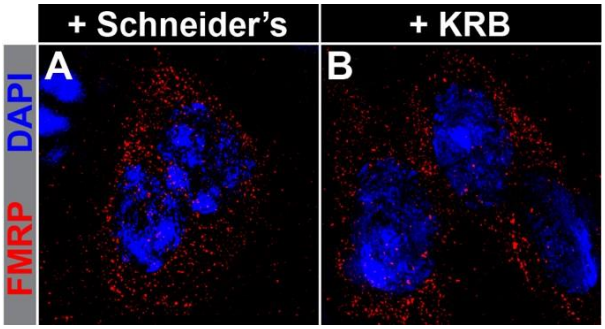
The reviewer is keen on having the basal conditions well described.

In Figure 2, we quantified FMRP puncta size of both untreated and KRB treated intestinal progenitor cells. Compared to untreated, we found that during the 1-hour KRB treatment FMRP puncta size is not significantly altered. Therefore, we conclude that the KRB treatment regime that we have used here does not induce SGs. We are aware that this is different from reports in the literature that KRB induces SGs in Drosophila S2 cells, but at least one reason that might explain this difference is that the reported KRB treatment of S2 cells is 3 hours while our treatment is only 1 hour. Our rationale for quantifying FMRP puncta size of untreated vs KRB treated was to address this concern. Additionally, we performed superesolution microscopic analysis of progenitor cells treated with Schneider's and KRB media to show that the basal punctateness is present in progenitors exposed to either treatment (see below). Although we have not reported it in the manuscript, we have observed that arsenite or rapamycin diluted in Schneider's media induces SGs while Schneider's alone does not.



2. The authors conclude that upon arsenite treatment, the stress granules in intestinal stem cells (IPSG) that form do not depend on the canonical markers G3BP, ATaxin2 and Rox8. When depleted, stress granules are still formed. Yet, they show that G3BP (Rasputin) and Ataxin2 are sufficient for stress granule formation. How can a factor be sufficient but be dispensable (so not necessary). The reviewer cannot put their head around these seemingly contradictory results.

The reviewer is aware of necessary factors that are not sufficient but not of sufficient factors that are not necessary. Please explain.

As the reviewer mentions, based on our data we propose that G3BP (Rasputin) and ATX2 are sufficient but not required for IPSG formation. Although this data seems confusing, we think that several reasons could account for this result. For instance, both G3BP and ATX2 contain long stretches of internally disorganized regions (IDRs). Therefore, one possibility could be that overexpression of these proteins increase local density of IDRs, thus allowing SG-like granule assembly. Another reason for this could be that increased G3BP or ATX2 protein concentration alone alter the protein-protein interactions in these cells. However, at the same time, G3BP, ATX2 and TIA1 appear to be redundant with one another such that loss of any individual component does not lead to the absence of SGs.

3. The results above suggest that the stress granules in intestinal stem cells likely form by an

unconventional mechanism due to other factors. However, this is not dissected in the slightest. What is also not done is to show that, upon depletion of G3BP and Ataxin 2, the stress granules that form are still reversible and still contain RNAs and possibly are the same as when these two factors are present.

We agree with the reviewer. To address this concern, we assessed whether IPSGs formed in triple mutant (mutant for *atx2*, *rin* and *rox8*) progenitor cells are reversible like wild type cells. Based on these results we extended Fig. 7 to now show that, like wild type cells, triple mutant IPSGs recover normally. We believe that this additional data will further strengthen the model we propose.

4. Altogether, despite the fact that the paper is of good quality and data rich, it is essentially descriptive. Being descriptive is not wrong per se, but here the conclusions of these descriptions are not completely different from what is now well established, i.e, that there are many pathways to form stress granules and that all in all, they all are a bit different (including a study from Nancy Kedersha a couple of years ago and one from Paul Anderson recently, which should be discussed). Of course, the novelty here is that the heterogeneity is within one tissue (stem cells and enterocytes) and in vivo. So, although the reviewer is left a little frustrated that the mechanisms are not better dissected (For instance, do the effects of specifically not forming stress granules in one cell type or the other affect gut homeostasis? Are the RNA (and protein) signatures of the stress granules in stem cells different from those formed in other cells??), the reviewer is overall positive.

We agree with the general sentiment communicated by the reviewer. We tried very hard to find conditions under which SGs failed to form. We also note that the genetic approach we took, including making triple mutant MARCM clones, was non-trivial and labor intensive. Nevertheless, we believe that the results described in the paper are significant and likely to be of interest to other researchers investigating the heterogeneity of mRNPs *in vivo*.

Second decision letter

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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.