Changes in subcellular structures and states of pumilio1 regulate the translation of target *Mad2* and cyclin B1 mRNAs

Natsumi Takei, Yuki Takada, Shohei Kawamura, Keisuke Sato, Atsushi Saitoh, Jenny Bormann, Wai Shan Yuen, John Carroll and Tomoya Kotani
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MS TITLE: Changes in subcellular structures and states of Pumilio1 regulate the translation of target *Mad2* and *Cyclin B1* mRNAs

AUTHORS: Natsumi Takei, Yuki Takada, Shohei Kawamura, Atsushi Saitoh, Jenny Bormann, Wai Shan Yuen, John Carroll, and Tomoya Kotani
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.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, all three experts thought that the work was potentially quite interesting and significant but all also raised a number of concerns that must be dealt with before the manuscript can be reconsidered. Please address these issues as thoroughly as possible. I would be pleased to see a revised manuscript. We would then return it to the reviewers.

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

In this manuscript Takei et al. aim to understand on a molecular level the temporal and spatial control of translation during oocyte development and progression through meiosis. Their prior work had shown that the cyclin B1 mRNA is found in granules in immature oocytes. Translational activation of cyclin B1 is coordinated with dissolution of these granular structures and is required for progression through meiosis. The RNA-binding protein Pumilio1 (Pum1) binds to cyclin B1 mRNA and determines the timing of its translational activation during oocyte maturation. In this current work they show that, similar to cyclin B1 RNA, the Mad2 RNA is found in granular structures and also associates with Pum1. However, the Mad2 and cyclin B1 mRNAs form distinct granules surrounded by Pum1. These Pum1 aggregates dissolve upon oocyte maturation, possibly through phosphorylation. They propose that changes in subcellular structures and states of the RNA-binding protein Pum1 regulate the translation of target mRNAs and progression of oocyte maturation. While this is potentially an interesting model that links translational activation with dissolution of RNA granules, the manuscript fails to concretely link Pum1 aggregation state to RNA granules and translation of the corresponding RNAs. Additionally, other conclusions reached need more quantification and controls to be better supported. These issues should be addressed in a revised manuscript.

Comments for the author

With regards to the first concern mentioned above, the authors nicely use a series of Pum1 mutants that exhibit different levels of aggregation. These would be important to connect Pum1 aggregation to RNA translation. The authors show that these mutants affect progression through meiosis looking at polar body extrusion and spindle formation, however, they do not provide crucial experiments linking these to Mad2 and cyclin B1 RNA translation. How does changing the aggregation level of Pum1 (using the deltaN deltaQN, deltaC mutants) affect the appearance/number of RNA granules? Some data on the effect of the deltaC mutant on Mad2 and cyclin B1 protein levels are provided (Fig 6D), however comparison to uninjected oocytes is not an appropriate control. Injections with GFP, Pum1 and all Pum1 mutants should be included. Given the importance of these data, quantitations and statistical analysis should be shown. If the authors want to connect translation changes to defects in polar body extrusion and spindle formation then rescue experiments would be important. For example, does forced expression of Mad2 and/or cyclin B1 in the presence of Pum1(deltaC) rescue the observed defects? I understand that establishing such a connection might not be feasible given the number of RNAs potentially regulated by Pum1, however in the absence of such data the conclusions reached cannot be substantiated.

In Fig 6E-H the authors use a different method (injection of a Pum1 antibody) to prevent Pum1 aggregate dissolution. However, similar omissions, as above, exist for these experiments. There is no indication as to how this treatment affects Mad2/cyclin B1 RNA granules and corresponding protein levels. The interpretation of these experiments is also difficult given that there is no characterization or discussion of the epitope recognized by this antibody. How is its inhibitory action mediated and is it consistent with the results of the truncation mutants used above?

In Fig 7, the authors provide evidence for the role of phosphorylation in regulating Pum1 aggregation, but again do not provide any link as to how these changes in phosphorylation affect Mad2/cyclin B1 RNA granules and translation.

The authors show that Pum1 binds to Mad2 and cyclinB1 RNAs, but rather counterintuitively it doesn’t colocalize with them. It rather appears to ‘surround’ these RNAs. While it is possible that Pum1 granules contain multiple RNAs which occupy distinct locations in the granules, such a conclusion would have to be strengthened by more controls. Given the density of both Pum1 and RNA FISH signals, the authors should address to what extent the apparent association between the two results from random overlap. For example, what degree of overlap would be observed if signals

of similar density were randomized? What extent of overlap is observed between Pum1 and non-target RNAs, such as actin or tubulin? Additionally these conclusions would be strengthened if the Pum1 antibody specificity was better supported. Is all the immunofluorescence signal observed reflecting Pum1 presence? Alternatively, the authors could provide similar quantitations of Pum1-RNA overlap when using GFP-Pum1 (as shown in Fig S2B) to support the specificity of these conclusions.

Throughout this work the authors infer that changes in protein levels are due to increased translation, but they have not formally ruled out that they might result from changes in protein stability. The lack of increase in levels upon puromycin treatment is indicative that translation might be involved but this experiment alone is not conclusive. It would be nice to see a more direct proof of translation e.g. through polysome association or through ruling out effects on protein stability.

The authors should better explain why they think the structures identified by FISH correspond to granules made up of multiple RNAs. From the images provided it seems that either single RNAs are undetectable or that there is RNA degradation. PCR analysis should be used to distinguish between those possibilities.

More minor points:
There are a couple of points that the manuscript makes, whose relevance is not clear. Firstly, the authors describe the existence of two Mad2 isoforms, but do not discuss further any connections with the remaining conclusions. Is the long Mad2 isoform regulated differently by Pum1?
Secondly, in Fig 2B the authors point out that the Mad2 and cyclin B1 granules are distributed close to each other. Supporting such a statement would require careful quantitation and controls (e.g. measuring minimal distances between granules and comparing that to distances from a control RNA such as actin or tubulin). It is though not clear how the closeness of the two granules relates to the remaining findings.

More detailed description is needed about the regions selected for FRAP experiments. The panels in Fig 4B and 5B appear to show regions with diffuse, non-granular signal. When looking at Pum1 in immature oocytes, are granules of similar size selected or are regions of more diffuse signal included in the ROIs?
Scale bars are needed in the panels (Fig 4B and 5B). Also, the recovery rates (Fig 4C and 5C) are derived by fitting the FRAP data to a double exponential, however only the raw data are shown in Figs 4B and 5B.
The actual fit and quality of the fit should be shown.

Fig S1: Figure should state and show which loading control was used to normalize the Mad2 protein signal.
Values from both stated replicates should be shown in the graph.

Page 9 (lines 6-9): the statement “Taken together, the results indicate that Pum1 aggregates dissolve during oocyte maturation and suggest that the change in the property of Pum1 from insoluble and immobile to soluble and mobile is crucial for temporal regulation of target mRNA translation” should be toned down. The results show a correlation between Pum1 aggregation and RNA translation. Whether the change in Pum1 status is crucial cannot be inferred.

Fig 4A, 4E, 6E: statistical analysis should be included.

Fig 7B: It is stated that arrowheads indicate non-specific bands. It is not clear what is the basis for this conclusion. It rather seems that these bands correspond to the phosphorylated-Pum1 detected in the panels of Fig 7A.

Reviewer 2

Advance summary and potential significance to field

In this paper, Takei et al reported the role of the RNA-binding protein PUM1 on translation of MAD2 and cyclin B1 mRNAs during meiosis. They propose an aggregation-dissolution model for the spatial and temporal activation of translation by PUM1. This paper is interesting and should be of broad interest for the community.

Comments for the author

1- Many RNA-binding proteins non-specifically aggregate when mutated or truncated, especially in the RNA-binding domain. Therefore, the increase in the number of stable large aggregates following GFP-PUM1-dC may represent non-specific aggregation. If they are RNA-free non-specific aggregates, it is difficult to establish a correlation between the presence or not of these aggregates and translation. What is the level of expression of the mutants compared to endogenous PUM1? Do they contain (colocalize) with MAD2 or Cyclin B1 RNAs?
2- Fig 2. The authors speculated that mRNAs coding for cyclin B1 and MAD2 should be assembled into the same granules and that it is intriguing that they are not. It was previously shown that RNA-granules contain in average only few RNA molecules and that each granule contains different RNAs (Mikl et al., EMBO Reports 12, 1077. 2011).
3- Fig 7. The phosphorylation experiments are interesting but seem preliminary.
Especially, the western blots with subtle differences between phosphorylated and non-phosphorylated proteins are difficult to interpret. Other approaches should be used to strengthen their conclusions. Many additional questions remain: Is PUM1 a known target of Plk4? Does inhibition of other kinases involved in meiosis (Cdk1, MAPK…) generate a different output?

Reviewer 3

Advance summary and potential significance to field

In the manuscript " Changes in subcellular structures and states of Pumilio1 regulate the translation of target Mad2 and Cyclin B1 mRNAs" the authors report studies focused on the temporal and spatial control of mRNA translation in oocytes. The authors demonstrate that the binding of RNA binding protein Pumilio 1 to MAD 2and Cyclin B1 mRNA regulates their translation and progression of oocytes maturation.
The subject is interesting and up-to-date. In general the MS is well written, the experiments well designed and the conclusion are of interest for the scientific community.

Comments for the author

Minor revisions:-
-Discussion section The meaning and potential impact of the results should be better discussed. In particular the authors should consider that mRNA localization is a conserved post-transcriptional process crucial for a variety of systems not only for oocytes; for example, in somatic cells, the asymmetrical distribution of mRNAs in the cytoplasm may facilitate protein import into organelles and several mechanisms for mRNA localization have been identified. These comments could be beneficial for the discussion. There are related papers that might be mentioned/discussed too:
-Russo A et al. cis-acting sequences and trans-acting factors in the localization of mRNA for mitochondrial ribosomal proteins Biochimica et Biophysica Acta (2008)

First revision

Author response to reviewers' comments

Response to the reviewers' comments

Reviewer 1
With regards to the first concern mentioned above, the authors nicely use a series of Pum1 mutants that exhibit different levels of aggregation. These would be important to connect Pum1 aggregation to RNA translation. The authors show that these mutants affect progression through meiosis looking at polar body extrusion and spindle formation, however, they do not provide crucial experiments linking these to Mad2 and cyclin B1 RNA translation. How does changing the aggregation level of Pum1 (using the deltaN, deltaQN, deltaC mutants) affect the appearance/number of RNA granules? Some data on the effect of the deltaC mutant on Mad2 and cyclin B1 protein levels are provided (Fig 6D), however comparison to uninjected oocytes is not an appropriate control. Injections with GFP, Pum1 and all Pum1 mutants should be included. Given the importance of these data, quantitations and statistical analysis should be shown. If the authors want to connect translation changes to defects in polar body extrusion and spindle formation then rescue experiments would be important. For example, does forced expression of Mad2 and/or cyclin B1 in the presence of Pum1(deltaC) rescue the observed defects? I understand that establishing such a connection might not be feasible given the number of RNAs potentially regulated by Pum1, however in the absence of such data the conclusions reached cannot be substantiated.

> According to the reviewer's comment, we first analyzed the appearance and number of Cyclin B1 and Mad2 RNA granules in oocytes expressing GFP, GFP-Pum1, GFP-Pum1deltaQN, GFP-Pum1deltaN and GFP-Pum1deltaC by in situ hybridization. The results showed that RNA granules were disassembled at 4 h after resumption of meiosis in oocytes expressing GFP and Pum1 mutants except Pum1deltaC, while those were maintained in oocytes expressing Pum1deltaC (New Fig. 6D and E). Second, we analyzed the amount of Cyclin B1 and Mad2 proteins in oocytes expressing GFP, GFP-Pum1, GFP-Pum1deltaQN and GFP-Pum1deltaN by immunoblotting and compared them with that in uninjected oocytes. The results showed that the protein synthesis was not prevented by the expression of GFP and Pum1 mutants except Pum1deltaC (New Fig. 6F). We quantified the results and have added the data in New Fig. S5A with statistical analysis. Third, we performed rescue experiments by observing the formation of bipolar spindle in meiosis I. The previous study reported that Cyclin B1 synthesis is required for promoting bipolar spindle formation after germinal vesicle breakdown (Polanski et al., 1998). Consistent with the attenuation of Cyclin B1 synthesis, meiosis I spindles remained round shape in oocytes expressing Pum1deltaC at 9 h after resumption of meiosis (New Fig. 6G), while control oocytes including those expressing GFP formed elongated bipolar spindles (New Fig. 6G). These defects were completely rescued by the injection of Cyclin B1 mRNA carrying SV40 3'UTR, which lacks Pumilio-binding element (New Fig. 6G). These results indicated that the inhibition of protein synthesis by expression of GFP-Pum1deltaC is indeed a cause of abnormal progression of meiosis. We have described these results in Results section (p.10, line 25-p.11, line 2).

In Fig 6E-H the authors use a different method (injection of a Pum1 antibody) to prevent Pum1 aggregate dissolution. However, similar omissions, as above, exist for these experiments. There is no indication as to how this treatment affects Mad2/cyclin B1 RNA granules and corresponding protein levels. The interpretation of these experiments is also difficult given that there is no characterization or discussion of the epitope recognized by this antibody. How is its inhibitory action mediated and is it consistent with the results of the truncation mutants used above?

> According to the reviewer's comments, we analyzed the synthesis of Cyclin B1 and Mad2 proteins in oocytes injected with anti-Pum1 antibody by immunoblotting and quantified the results. The synthesis of Cyclin B1 and Mad2 was attenuated in oocytes injected with the antibody but not in oocytes injected with control IgG (New Fig. 7D). The quantitative data has been added in New Fig. S5B. Furthermore, we have demonstrated that phosphorylation of Pum1 was prevented in oocytes injected with anti-Pum1 antibody (New Fig. 7F). Since this antibody recognizes amino acid residues from 225 to 275 of Pum1, we discussed the importance of phosphorylation around this region in triggering dissolution of Pum1 aggregates. In addition, we previously showed that overexpression of GFP-Pum1deltaC prevented disassembly of cyclin B1 RNA granules and phosphorylation of Pum1 in

zebrafish oocytes (Saitoh et al., 2018). We have added the discussion on the possibility that inhibition of Pum1 phosphorylation may be a cause of inhibition of dissolution of Pum1 aggregates in both experiments in Discussion section (p.15, lines 17-25). We could not perform in situ hybridization of oocytes and statistical analysis of immunoblot in this experiment, due to COVID-19, since many female mice are required for performing the injection experiments and we had used large numbers of females for in situ hybridization and immunoblotting of oocytes injected with Pum1-mutant mRNAs (New Fig. 6) and oocytes treated with okadaic acid (OA) and Plk4 inhibitor (New. Fig. 8). We could not maintain mice more than those in this situation. We hope the reviewer understands this situation and the new data described above and below should strengthen the link between Pum1 aggregation state and assembly/disassembly of RNA granules.

In Fig 7, the authors provide evidence for the role of phosphorylation in regulating Pum1 aggregation, but again do not provide any link as to how these changes in phosphorylation affect Mad2/cyclin B1 RNA granules and translation.

> According to the reviewer's comments, we first analyzed the appearance and number of Cyclin B1 and Mad2 RNA granules in oocytes treated with OA and Plk4 inhibitor by in situ hybridization. The results showed that RNA granules were almost completely disassembled in oocytes treated with OA, while those were maintained in oocytes treated with OA and Plk4 inhibitor (New Fig. 8E and F). Second, we analyzed the synthesis of Cyclin B1 and Mad2 proteins in oocytes treated with OA and Plk4 inhibitor by immunoblotting. The results showed that the protein synthesis was promoted by treatment with OA, but it was inhibited by treatment with Plk4 inhibitor (New Fig. 8G). We quantified the results and have added the data in New Fig. S6B with statistical analysis. These results strongly link Pum1 aggregation state to assembly/disassembly of RNA granules and translation of Pum1-target mRNAs.

The authors show that Pum1 binds to Mad2 and cyclinB1 RNAs, but rather counterintuitively it doesn't colocalize with them. It rather appears to 'surround' these RNAs. While it is possible that Pum1 granules contain multiple RNAs which occupy distinct locations in the granules, such a conclusion would have to be strengthened by more controls. Given the density of both Pum1 and RNA FISH signals, the authors should address to what extent the apparent association between the two results from random overlap. For example, what degree of overlap would be observed if signals of similar density were randomized? What extent of overlap is observed between Pum1 and non-target RNAs, such as actin or tubulin? Additionally, these conclusions would be strengthened if the Pum1 antibody specificity was better supported. Is all the immunofluorescence signal observed reflecting Pum1 presence? Alternatively, the authors could provide similar quantitations of Pum1-RNA overlap when using GFP-Pum1 (as shown in Fig S2B) to support the specificity of these conclusions.

> To confirm the specificity of immunostaining signals, we produced recombinant protein consisting of GST and N-terminus region of mouse Pum1 (amino acids 1-399). This GST-Pum1NN recombinant protein includes the region recognized by the anti-Pum1 antibody (amino acids 225-275). No signal was detected when the anti-Pum1 antibody was incubated with the GST-Pum1NN recombinant protein (Fig. S2C), indicating that the signals of immunostaining were specific to Pum1. Then, we confirmed the specificity of Mad2 and Cyclin B1 mRNA distribution by analyzing the distances between randomly distributed dots and Pum1 aggregates using Monte Carlo simulation. The average of resulting distances was 1.8-fold longer than the experimental distance between the center of RNA granules and Pum1 aggregates. We further analyzed the distribution of alpha-tubulin mRNA and Pum1 by in situ hybridization followed by immunostaining. We found that alpha-tubulin mRNA was not surrounded by Pum1 and instead it uniformly distributed in the cytoplasm (Fig. S2D). These results strengthened the conclusion that Mad2 and Cyclin B1 RNA granules were surrounded by Pum1.

Throughout this work the authors infer that changes in protein levels are due to increased translation, but they have not formally ruled out that they might result from changes in protein stability. The lack of increase in levels upon puromycin treatment is indicative that translation might be involved but this experiment alone is not conclusive. It would be nice to see a more direct proof of translation e.g. through polysome association or through ruling out effects on protein stability.

> According to the reviewer's comments, we analyzed the stability of Mad2 by expressing GFP-Mad2 followed by puromycin treatment. The rate of destruction of GFP-Mad2 was similar in immature and mature oocytes (Fig. S1C), indicating that the stability of Mad2 was not changed in immature and mature oocytes. We described the results in Results section (p.5, line 32-p.6, line 3.).

The authors should better explain why they think the structures identified by FISH correspond to granules made up of multiple RNAs. From the images provided it seems that either single RNAs are undetectable or that there is RNA degradation. PCR analysis should be used to distinguish between those possibilities.

> According to the reviewer's comments, we analyzed the amount of Mad2 mRNA by quantitative PCR. The amount of mRNA in immature and mature oocytes (18 h after resumption of meiosis) was not changed (Fig. S2B). The results indicate that the decrease in the number of Mad2 RNA granules is caused by granule disassembly.

More minor points:

There are a couple of points that the manuscript makes, whose relevance is not clear. Firstly, the authors describe the existence of two Mad2 isoforms, but do not discuss further any connections with the remaining conclusions. Is the long Mad2 isoform regulated differently by Pum1? Secondly, in Fig 2B the authors point out that the Mad2 and cyclin B1 granules are distributed close to each other. Supporting such a statement would require careful quantitation and controls (e.g. measuring minimal distances between granules and comparing that to distances from a control RNA such as actin or tubulin). It is though not clear how the closeness of the two granules relates to the remaining findings.

> To further address the difference in splice variants of Mad2 mRNA, we have examined the expression of long version of Mad2 mRNA by FISH analysis. This splicing variant was not detected in oocytes by FISH analysis (New Fig. S1A). Based on the results of RT-PCR, quantitative PCR and in situ hybridization with alkali phosphatase or fluorescence probes, we added the sentence "These results suggest that short Mad2 mRNA is crucial for the synthesis of protein in oocytes" (p.5, lines 25-26). As in the case of distribution of Mad2 and Cyclin B1 RNA granules, we have deleted the sentence, since this statement is not important for remaining experiments.

More detailed description is needed about the regions selected for FRAP experiments. The panels in Fig 4B and 5B appear to show regions with diffuse, non-granular signal. When looking at Pum1 in immature oocytes, are granules of similar size selected or are regions of more diffuse signal included in the ROIs? Scale bars are needed in the panels (Fig 4B and 5B). Also, the recovery rates (Fig 4C and 5C) are derived by fitting the FRAP data to a double exponential, however only the raw data are shown in Figs 4B and 5B. The actual fit and quality of the fit should be shown.

> Since the confocal microscope used in this experiment could not distinguish GFP-Pum1 in aggregates from that diffused in the cytoplasm, we performed similar experiments using a high-resolution confocal microscope. The intensity of GFP-Pum1 in aggregates recovered slowly and only partially (New Fig. 4D), supporting the notion that Pum1 aggregates exhibit an immobile state. In addition, we have added the scale bars in Fig. 4B and 5B and the graphs showing the actual fittings and quality of the fittings to single and double exponential model (New Fig. S3).

Fig S1: Figure should state and show which loading control was used to normalize the Mad2 protein signal. Values from both stated replicates should be shown in the graph.

> We have added the immunoblot of gamma-tubulin protein (New Fig. 1B) and stated that the intensities of Mad2 were normalized by that of gamma-tubulin. We have changed the graph for that showing the average amount with standard deviation.

Page 9 (lines 6-9): the statement "Taken together, the results indicate that Pum1 aggregates dissolve during oocyte maturation and suggest that the change in the property of Pum1 from insoluble and immobile to soluble and mobile is crucial for temporal regulation of target mRNA translation" should be toned down. The results show a correlation between Pum1 aggregation and RNA translation. Whether the change in Pum1 status is crucial cannot be inferred.

> According to the reviewer's comments, we have changed the sentence as follows. "Taken together, the results indicate that Pum1 aggregates dissolve during oocyte maturation and suggest the relationship between changes in the property of Pum1 and temporal regulation of target mRNA translation." (p.9, lines 27-29)

Fig 4A, 4E, 6E: statistical analysis should be included.

> We have performed statistical analysis in the experiments shown in New Fig. 4A, 4F and 7A.

Fig 7B: It is stated that arrowheads indicate non-specific bands. It is not clear what is the basis for this conclusion. It rather seems that these bands correspond to the phosphorylated-Pum1 detected in the panels of Fig 7A.

> We have performed the same experiments again and added a new picture (New Fig. 8B, top), which does not show non-specific bands.

Reviewer 2
1- Many RNA-binding proteins non-specifically aggregate when mutated or truncated, especially in the RNA-binding domain. Therefore, the increase in the number of stable large aggregates following GFP-PUM1-dC may represent non-specific aggregation. If they are RNA-free non-specific aggregates, it is difficult to establish a correlation between the presence or not of these aggregates and translation. What is the level of expression of the mutants compared to endogenous PUM1? Do they contain (colocalize) with MAD2 or Cyclin B1 RNAs?

> According to the reviewer's comments, we analyzed expression levels of GFP-Pum1 and mutant forms of Pum1 by immunoblotting. The amounts of GFP-Pum1 and mutant forms of Pum1 were 1.6-1.8-fold larger than that of endogenous Pum1 (New Fig. S4D). In addition, we analyzed distribution of GFP-Pum1deltaC with that of Cyclin B1 mRNA by FISH analysis followed by immunostaining. We found that the aggregates of GFP-Pum1ΔC, but not GFP alone, surrounded Cyclin B1 RNA granules (New Fig. S4C). The results strengthened the conclusion that GFP-Pum1deltaC stabilized Cyclin B1 and Mad2 RNA granules and inhibited translational activation of these mRNAs.

2- Fig 2. The authors speculated that mRNAs coding for cyclin B1 and MAD2 should be assembled into the same granules and that it is intriguing that they are not. It was previously shown that RNA-granules contain in average only few RNA molecules and that each granule contains different RNAs (Mikl et al., EMBO Reports 12, 1077. 2011).

> We agree with the reviewer's comment and have added the sentence as follows. "Formation of distinct granules of Mad2 and Cyclin B1 mRNAs resembles formation of Map2, CaMKIIα and β-actin RNA granules in neurons, in which distinct mRNAs were assembled into different granules (Mikl et al., 2011)." (p. 6, lines 20-23)

3- Fig 7. The phosphorylation experiments are interesting but seem preliminary. Especially, the western blots with subtle differences between phosphorylated and non-phosphorylated proteins are difficult to interpret. Other approaches should be used to strengthen their conclusions. Many additional questions remain: Is PUM1 a known target of Plk4? Does inhibition of other kinases involved in meiosis (Cdk1, MAPK…) generate a different output?

> To improve the results showing Pum1 phosphorylation, we performed immunoblot analysis by extending the running time of SDS-PAGE. New pictures (New Fig. 8B) show the shift of Pum1 band more clearly than previous pictures. In addition, we have performed additional experiments supporting the link between Pum1 phosphorylation and translation of Pum1-target mRNAs (New Fig. 8E-G). Moreover, we have analyzed the effects of inhibition of MAPK and MPF (Cdk1 kinase) on Pum1 aggregates by observing GFP-Pum1. No effect on the dissolution of Pum1 was observed when activation of MAPK was inhibited, while aggregate disolution was delayed but not prevented when the activity of MPF was inhibited (New Fig. S6A), suggesting that MPF, which consists of pre-existing Cyclin B2 and Cdc2 kinase (Daldello et al., 2019), is partially involved in Pum1 aggregate dissolution. In discussion section, we have added the sentence describing the kinase known to

phosphorylate Pum1 as follows. “To date, only Nemo-like kinase 1 (Nlk1) has been shown to phosphorylate Pum1 (Ota et al., 2011b). Our results suggest the participation of MPF in dissolution of Pum1 aggregates (Fig. S6A). Involvement of Nlk1, MPF and other kinases in phosphorylation of Pum1 and dissolution of aggregates remains to be investigated.” (p. 15, lines 7-11)

Reviewer 3
Minor revisions:-
-Discussion section
The meaning and potential impact of the results should be better discussed. In particular the authors should consider that mRNA localization is a conserved post-transcriptional process crucial for a variety of systems not only for oocytes; for example, in somatic cells, the asymmetrical distribution of mRNAs in the cytoplasm may facilitate protein import into organelles and several mechanisms for mRNA localization have been identified. These comments could be beneficial for the discussion. There are related papers that might be mentioned/discussed too:
-Russo A et al. cis-acting sequences and trans-acting factors in the localization of mRNA for mitochondrial ribosomal proteins Biochimica et Biophysica Acta (2008)

> We agree with the reviewer’s comments and have added the sentence in Discussion section as follows. “In various cells besides oocytes, many mRNAs are known to be transported and localized at subcellular regions through binding of RNA-binding proteins to mainly 3’UTRs (Martin and Ephrussi, 2009; Milli and Macara, 2009; Russo et al., 2008). Recent studies demonstrated the accumulation of translationally repressed mRNAs at protrusions of fibroblast cells and synapses of neuronal cells in a static state (Buxbaum et al., 2014; Moissoglu et al., 2019).” (p.16, line 30-p.17, line 2)

Second decision letter

MS ID#: JOCES/2020/249128

MS TITLE: Changes in subcellular structures and states of Pumilio1 regulate the translation of target *Mad2* and *Cyclin B1* mRNAs

AUTHORS: Natsumi Takei, Yuki Takada, Shohei Kawamura, Keisuke Sato, Atsushi Saitoh, Jenny Bormann, Wai Shan Yuen, John Carroll, and Tomoya Kotani
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.

Reviewer 1

Advance summary and potential significance to field

The work proposes a model whereby changes in the aggregation state of an RNA-binding protein control the translation of its target mRNAs and should be of interest to the field.

Comments for the author

In the revised version of their manuscript the authors have included several additional experiments that have strengthened the conclusions and have addressed all of my concerns. I think the work is now suitable for publication in the Journal of Cell Science.

Reviewer 2

Advance summary and potential significance to field

In this paper, Takei et al reported the role of the RNA-binding protein PUM1 on translation of MAD2 and cyclin B1 mRNAs during meiosis. They propose an aggregation-dissolution model for the spatial and temporal activation of translation by PUM1. This paper is interesting and should be of broad interest for the community.

Comments for the author

Authors responded adequately to my previous comments and submitted an improved manuscript. I support its publication in JCS.

Reviewer 3

Advance summary and potential significance to field

I have no further comments

Comments for the author

I have no further comments