Translational control by maternal Nanog promotes oogenesis and early embryonic development

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MS TITLE: Translation control by maternal Nanog promotes oocyte maturation and early embryonic development

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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.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision. If it would be helpful, you are welcome to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating your plans for addressing the referee's comments, and we will look over this and provide further guidance.

Please attend to all of the reviewers' comments and 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. 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

Using zebrafish as the model organism, He et al. studied the role of maternal Nanog in the regulation of oocyte maturation and early embryogenesis. The authors demonstrated that loss of

maternal nanog leads to elevated translational activity, increased endoplasmic reticulum stress, and an activated unfold protein response. The authors further discovered that maternal Nanog inhibits the transcription of eukaryotic elongation factor 1 alpha 1, like 2 (eEF1a1l2).

Further, depletion of eef1a1l2 in nanog mutant females effectively rescued the elevated translational activity and embryonic defects of Mnanog embryos suggesting that Nanog regulates the global translational activity in the maturing oocytes and early-stage embryos.

While the data is compelling, there are several major issues which needs to be addressed.

Comments for the author

Major comments:

1. Oocyte maturation in zebrafish, as well as other nonmammalian vertebrates requires new protein synthesis (PMID: 18482399, PMID: 35908193). In zebrafish previous studies have shown that synthesis of cyclin B is essential for oocyte maturation (PMID: 9331337, PMID: 11150242), while translational inhibition blocks oocyte maturation (PMID: 23623869). The authors need to address this discrepancy.
The authors might check the level of cyclin B protein in the nanog mutants. Hence the idea of "regulation of global translation level" may not be accurate.

2. The abundance of Nanog protein (Figure S1C) is detected mostly in the primary and early-vitellogenic follicles and NOT in the fully-grown oocytes. Expression of eef1a1l2 in nanog mutant ovary is primarily found in the early stages of oogenesis (Figure 3C). Further, loss of nanog results in apoptosis in these early follicles (Figure S1A). Moreover, the defects detected in ER, Golgi bodies and mitochondria, start early in the developing follicles (Figure 5G). Together these data hint toward the possibility that the oocyte maturation defect is an outcome of these early defects. It is possible that these oocytes never attended the maturational competence.

3. It is well established in majority of the vertebrate and invertebrate species that translation in immature oocyte is regulated by the size of the poly A tail of the mRNAs (PMID: 2568313, PMID: 27474798, PMID: 9606198, PMID: 26596258). How does loss of nanog trigger translation by regulating an elongation factor if the poly A tail is shorter in those mRNAs?

4. Since the authors used whole follicles (oocyte + theca-granulosa cells) for all their analysis, it is essential to describe and call as "follicle-enclosed oocyte" rather than "oocyte". This should be corrected throughout, especially in the method section.

5. Figure 1: Zebrafish follicle-enclosed oocytes mature spontaneously (~15-25% in 4-6 h culture) without any treatment (PMID: 26853486). Thus, vehicle control is essential in panel H and I.
The follicle showed in panel J seems to contain dead oocyte, as the morphology of the follicle is severely deformed, a sign of dead oocyte. It is essential that the authors confirm whether the oocyte is alive, which can be easily checked by Trypan blue staining.
The data presented in panel K is not convincing. In fishes, the yolk protein vitellogenin (Vtg) has several isoforms and often forms homo or heterodimers of very high molecular weight proteins. Thus, showing two Coomassie bands without any specific antibody marker and assuming the second band as the cleaved product of the first one is quite a stretch.
L137- lower GSI does not suggest defects in oocyte maturation. This could be the result of either defective vitellogenesis or lower number of follicles or more apoptosis of the follicles (which is detected by the authors) or all of them.
Thus, it is recommended to call it "defects in oocyte development"
Figure S1D missing the control: Tg(CMV:nanog-myc) with WT background

6. Figure 2: Is the GAPDH blot same in both E and J? Why?
Panel L should include WT group as well.

7. Figure S2E: As mentioned earlier, inhibition of translation blocks zebrafish oocyte maturation. Thus, experimental control group should include eif4a inhibitor-treated and untreated WT follicles

with or without DHP. Similarly eif4a inhibitor-treated and untreated nanog mutant follicles with or without DHP.

8. Figure 4 and 5: It is evident from the present data that eef1a1l2 functions downstream of nanog and loss of eef1a1l2 rescued the oogenic defects of nanog mutants. However, in each one of the panels eef1a1l2 single mutant should be presented as a control. The authors mentioned (L275-276) "We then generated homozygous mutant of eef1a1l2 and two types of eef1a1l2 mutants were obtained and neither of them showed obvious defect (Fig. S4)". However, Figure S4 shows "Generation of the eef1a1l2 mutant allele using CRISPR/Cas9" and no data for any phenotypic analysis.
Figure 4 H: Please see comment for figure 1 K.
Figure 4 I: needs vehicle control groups without DHP as mentioned in comment for figure 1 H.

9. L415-418: Please see comment 2.

10. Figure S6: How did the authors check 'fertilization rate' for good or poor quality of eggs as they mentioned in the legend (?Fertilization rate >90% was defined as good-quality egg, and Fertilization rate <10% was defined as poor-quality egg?)? There is no method section for this. It is important to discuss this experiment properly, especially because the authors found that nanog expression is lower in these "bad" eggs. Based on which the authors suggested ?
Nanog might be a new factor for oocyte quality assessment? However, Nanog was not detected in the fully-grown oocytes even in the overexpressed strain [Tg(CMV:nanog-myc)]. Thus, relative expression of nanog mRNA is not enough for this argument.

Minor comments:

L48: needs citation(s).
L130: should be "during oocyte development" not "maturation"
L184: "Western blot"
L244: "supported" sound better than "proved"
L288: "(compare Movie 1 and Movie 3)"
L417-418: "Impaired oocyte maturation correspondingly tends to produce poor-quality egg". The data is quite prominent (discussed in comment 2) that Nanog expression in the early stage of oogenesis possibly regulate oocyte quality.
L441-443: The sentence is unclear.
L446: "The genes, which are"
L452-453: Figure S4 does not show anything mentioned in the statement.
Figure 6C: In the model, name of the gene is written "eef1a1b". Is it a mistake or this is the same as "eef1a1l2"? Please check and keep it consistent.

Reviewer 2

Advance summary and potential significance to field

Zebrafish provides an exciting model to investigate maternal contribution to early embryogenesis. During oogenesis and early embryogenesis, translation of maternal mRNA is tightly controlled in a spatio-temporal manner. A mechanism of repression/activation of translation must occur to avoid a defective embryogenesis. However, a global regulator of translation during oogenesis and its mechanism has not been elucidated yet. This manuscript contributes to this field through the findings that Nanog acts as a repressor and targets eef1a1l2 to globally control translation of maternal mRNA before maternal to zygotic transition.

Strengths of the manuscript include the extensive and detailed study of the loss of nanog during oogenesis using various molecular and cellular tools and the finding that Nanog can act as a repressor on a translational machinery gene eef1a1l2. Most importantly, the partial rescue of Mnanog phenotype in Mnanog;eef1a1l2 double mutant is striking and reinforce the idea of a global translational mechanism. The examination of the ER and UPR pathway is nicely analyzed.
The weaknesses in the manuscript are largely presentational in nature and could be rather easily fixed by providing clarification and textual modifications. Neither does the manuscript clarify the

underlying biochemical mechanisms by which Nanog act as repressor nor does it address the complex relationship between RNA binding proteins and translation mechanism during oocyte maturation. These are clearly complex issues of high interest beyond the scope of the present study, which does provide useful data for the field. The manuscript is overall well written, well referenced and figures well presented. The authors should consider the following points.

Comments for the author

Minor points:

Fig2A: The picture of Mnanog in the mCherry channel have almost no signal compared to the WT. This disagrees with Fig.2B, 2C and 2D and the main text. The Supp Fig S5 is in adequation with the main findings. Please clarify because this figure is at the base of all the subsequent analyses and findings.

Fig2D: Rrelative should be read Relative Fig4D: Phalloidine should be read Phalloidin Legend Fig S1: DAPI was co-stained for 'nucleus' should be read DAPI was co-stained for 'DNA'
S1 Table: Please add a description for each protein. Only accession number is given.
l.39: Please remove the comma to smooth the reading.
l.366: Please revise the sentence.

First revision

Author response to reviewers' comments

Point-by-Point Response to Reviewers

1. Summary of major comments from the reviewers.

Based on the requests and comments from the reviewers, we have performed further analyses and additional experiments. Our major revision and new supporting data are summarized and listed in the following Table 1.

Table 1. Revision summary and new supporting data to address major comments from the reviewers.

	Questions	Reviewers	Clarification on the original submission data and New data supporting
1	Oocyte maturation in zebrafish, as well as other nonmammalian vertebrates, requires new protein synthesis (PMID: 18482399, PMID: 35908193). In zebrafish, previous studies have shown that synthesis of cyclin B is essential for oocyte maturation (PMID: 9331337, PMID: 11150242), while translational inhibition blocks oocyte maturation (PMID: 23623869). The authors need to address this discrepancy. The authors might check the level of cyclin B protein	1#: Q1	We totally agree with the reviewer's comment that new protein synthesis is required for oocyte maturation, while maternal mRNA translation is temporally and spatially controlled during oocyte development and maturation. Taking cyclin B1 as an example, the translation of cyclin B1 should be repressed in immature oocyte (early oocyte stage), and activated during oocyte maturation (late oocyte stage). Under the suggestion of the reviewer, we detected the translation of cyclin B1 in immature follicles, and the result showed that the translation level of cyclin B1 was significantly increased in *nanog* mutant follicles, and restored in *nanog* and *eef1a1l2* double mutant follicles (Response new Figure 1, also see revised Figure S5A, B). Another maternal mRNA *zp3b* also showed the same translation pattern with cyclin B1 in *nanog* mutant and double mutant follicles (Response new Figure 1, also see revised Figure S5C, D).

	in the nanog mutants. Hence the idea of "regulation of global translation level" may not be accurate.		
2	The abundance of Nanog protein (Figure S1C) is detected mostly in the primary and early-vitellogenic follicles and NOT in the fully-grown oocytes. Expression of eef1a1l2 in nanog mutant ovary is primarily found in the early stages of oogenesis (Figure 3C). Further, loss of nanog results in apoptosis in these early follicles (Figure S1A). Moreover, the defects detected in ER, Golgi bodies and mitochondria, start early in the developing follicles (Figure 5G). Together these data hint toward the possibility that the oocyte maturation defect is an outcome of these early defects. It is possible that these oocytes never attended the maturational competence.	1#: Q2	Based on the expression profile of Nanog and *eef1a1l2*, we agree with the reviewer that Nanog mainly regulates transcription of *eef1a1l2* in early-stage follicles. However, not all the early-stage follicles without *nanog* expression show defects related to ER stress and UPR, and undergo apoptosis (Figure S1A and Figure 5G). In contrast, the *nanog* mutant follicles that could develop to late stage show defects in oocyte maturation (Figure 1H, I), and the defects could be rescued by inhibiting translational activity using inhibitors (Figure S2E and F, Figure 2L and M). Together with these data, we concluded that Nanog regulates the translational level of maternal mRNA during oocyte development and maturation. Therefore, we revised the manuscript title to "Translational control by maternal Nanog promotes oogenesis and early embryonic development", and changed "oocyte maturation" to "oogenesis" in the revised manuscript.

3	It is well established in majority of the vertebrate and invertebrate species that translation in immature oocyte is regulated by the size of the poly A tail of the mRNAs (PMID: 2568313, PMID: 27474798, PMID: 9606198, PMID: 26596258). How does loss of nanog trigger translation by regulating an elongation factor if the poly A tail is shorter in those mRNAs?	1#: Q3	We agree with the reviewer that translation in immature oocyte is regulated by the size of the poly A tail of the mRNAs, however, poly A tail is not the only way to regulate translation level in oocyte. For example, the interaction of mRNA transcripts with RNA-binding proteins in a nonspecific or sequence-specific manner is also an important regulatory mechanism of translational control in oocyte development (Nakamura et al., 2001; Richter and Lasko, 2011; Tanaka et al., 2014). Some miRNA or long non-coding RNA has also been shown to regulate the translation of specific mRNA by interfering with the formation of translational complex or cooperating with RNA-binding proteins during oocyte development (Valencia-Sanchez et al., 2006; Bushati and Cohen, 2007; Aleshkina et al., 2021). In this study, we have demonstrated that translational activation in *nanog* mutant immature oocytes is mainly dependent on the upregulation of *eef1a1l2*. According to the previously established theory of translational control based on the poly A tail length, the poly A tails of *cyclin B1* and *zp3b* should be relatively short in early oocytes, because they are translationally silent in early oocytes. However, these two mRNAs were still translationally activated in *nanog* mutant immature oocytes, whereas they were translationally silent in *nanog* and *eef1a1l2* double mutant immature oocytes (Response new Figure 1, revised Figure S5A-D). Therefore, we assumed that the changes in the translation level of maternal mRNA in *nanog*-null oocytes are mainly dependent on the transcriptional activation of *eef1a1l2* but not on the size of the poly A tail of mRNAs.
4	The data presented in panel K is not convincing. In fishes, the yolk protein, vitellogenin (Vtg) has several isoforms and often forms homo or heterodimers of very high molecular weight proteins. Thus, showing two Coomassie bands without any specific antibody marker and assuming the second band as the cleaved product of the first one is quite a stretch.	1#: Q5-3	During the oocyte maturation from stage III to V, the major yolk proteins undergo cleavage, which leads to the translucency of oocyte. Comparing the intensity of these two bands using Coomassie staining has been widely applied in previous studies (Selman et al., 1993; Dosch et al., 2004; Sun et al., 2018). The yolk proteins are the most abundant proteins (almost 90% of the total proteins) in oocytes, thus when we performed SDS-PAGE analysis with low amount of total proteins (see Method section, P28-29, L592-597), Coomassie staining only visualized these two bands (Response new Figure 2A), which is identical to the Western blot analysis (Response new Figure 2B). However, since the transfer efficiency of the high molecular weight band is lower than that of the low molecular weight band (Response new Figure 2B), using Western blot to evaluate the ratio of high yolk protein to low yolk protein is somehow inappropriate.

5	Figure 4 and 5: It is evident from the present data that eef1a1l2 functions downstream of nanog and loss of eef1a1l2 rescued the oogenic defects of nanog mutants. However, in each one of the panels eef1a1l2 single mutant should be presented as a control. The authors mentioned (L275-276) "We then generated homozygous mutant of eef1a1l2 and two types of eef1a1l2 mutants were obtained and neither of them showed obvious defect (Fig. S4)". However, Figure S4 shows "Generation of the eef1a1l2 mutant allele using CRISPR/Cas9" and no data for any phenotypic analysis.	1#: Q8-1	We have observed the embryonic phenotype of *eel1a1l2* mutant, and maternal-zygotic mutant of *eef1a1l2* (MZ*eef1a1l2*) showed no developmental defects (see revised Figure S4B, see also revised manuscript P14L283). We also compared the oocyte diameter, GSI, oocyte transparency, and GVBD ratio of the *eef1a1l2* mutant with WT. All these aspects showed no difference between *eef1a1l2*$^{-/-}$ and WT (see revised Figure 4A, B, C, E, F, G, I and J, see also revised manuscript P15L307-310), indicating the depletion of *eef1a1l2* does not lead to defects in oocyte maturation and embryonic development. Thus, we did not examine the *eef1a1l2* mutant in the subsequent studies.
6	Figure 4 H: Please see comment for figure 1 K.	1#: Q8-2	As we mentioned in Response 5-3, comparing the intensity of two Vtg bands between 95 KD and 130 KD using Coomassie staining has been widely used to evaluate the cleavage efficiency of major yolk protein. Thus, we performed SDS-PAGE analysis with a small amount from total proteins of WT, *nanog* mutant, *nanog* and *eef1a1l2* double mutant follicles. The original picture of panel K in Figure 1 also showed only two Vtg bands by Coomassie staining (Response new Figure 3A), which is identical to the Western blot analysis (Response new Figure 3B).
7	10. Figure S6: How did the authors check 'fertilization rate' for good or poor quality of eggs as they mentioned in the legend (?Fertilization rate >90% was defined as good-quality egg, and Fertilization rate <10% was defined as poor-quality egg?)? There is no method section for this. It is important to discuss this experiment properly, especially because the authors found that nanog expression is lower in these "bad" eggs. Based on which the authors suggested ? Nanog might be a new	1#: Q10	After thoroughly revised the manuscript, we agree with the reviewer that definition of Nanog as a new factor for assessment of oocyte quality based on the data of this study alone is not sufficient. Therefore, we deleted the previous Figure S6 and revised the manuscript accordingly.

	factor for oocyte quality assessment? However, Nanog was not detected in the fully-grown oocytes even in the overexpressed strain [Tg(CMV:nanog-myc)]. Thus, relative expression of nanog mRNA is not enough for this argument.		

2. Point-by-point responses to reviewers

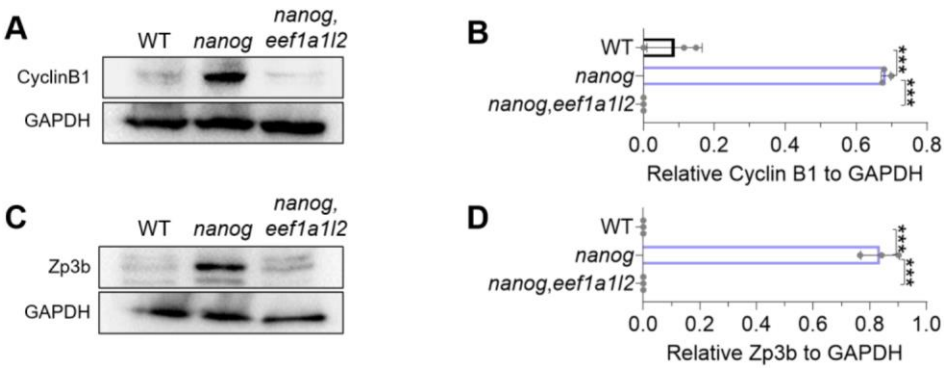
Reviewer #1:

Advance Summary and Potential Significance to Field:

Using zebrafish as the model organism, He et al. studied the role of maternal Nanog in the regulation of oocyte maturation and early embryogenesis. The authors demonstrated that loss of maternal nanog leads to elevated translational activity, increased endoplasmic reticulum stress, and an activated unfold protein response. The authors further discovered that maternal Nanog inhibits the transcription of eukaryotic elongation factor 1 alpha 1, like 2 (eEF1a1l2). Further, depletion of eef1a1l2 in nanog mutant females effectively rescued the elevated translational activity and embryonic defects of Mnanog embryos, suggesting that Nanog regulates the global translational activity in the maturing oocytes and early-stage embryos.
While the data is compelling, there are several major issues which needs to be addressed.
-Thank you very much for your appreciation and critical comments on our work.

Major comments:
1. Oocyte maturation in zebrafish, as well as other nonmammalian vertebrates, requires new protein synthesis (PMID: 18482399, PMID: 35908193). In zebrafish, previous studies have shown that synthesis of cyclin B is essential for oocyte maturation (PMID: 9331337, PMID: 11150242), while translational inhibition blocks oocyte maturation (PMID: 23623869). The authors need to address this discrepancy.
The authors might check the level of cyclin B protein in the nanog mutants. Hence the idea of "regulation of global translation level" may not be accurate.
Response 1: Thank you for the comment. We totally agree with you.
The translation of many maternal mRNAs is inhibited to safeguard oocyte growth at early oocyte development stage (Richter and Lasko, 2011; Takahashi et al., 2014; Miao et al., 2017), and the translation of mRNAs related to meiosis and fertilization is activated to prepare for early embryogenesis at late oocyte stage (Kotani et al., 2013; Martins et al., 2016). Thus, maternal mRNA translation is temporally and spatially controlled during oocyte development and maturation. In this study, we found Nanog regulates the translation level of maternal mRNA by repressing the activity of translation machine during oocyte development, which is different from the mechanism previously reported that sequence-specific regulators shape translation levels of mRNAs containing targeted cis-regulatory element (Chen et al., 2013; Sha et al., 2017).

According to your suggestion, we detected the translation level of Cyclin B1and Zp3b, which is also translationally repressed during oogenesis (Miao et al., 2017), in WT, *nanog* mutant, and *nanog* and *eef1a1l2* double mutant immature follicles (stage I/II). The result showed that the translation of Cyclin B1 and Zp3b were silent in WT immature oocyte, but the protein level of Cyclin B1 and Zp3b were significantly increased in *nanog* mutant immature oocyte, indicating that the translation level is elevated in *nanog* mutant. While this elevated translation level could be restored by deletion of *eef1a1l2* in *nanog* mutant (Response new Figure 1, revised Figure S5A-D). This result confirmed the conclusion that Nanog controls the translation of maternal mRNAs at a relatively global level, through inhibiting the transcription of *eef1a1l2*, which encodes a translational elongation factor. We have added this result in the revised manuscript (P15-16, L312-324).



Response new Figure 1. Western blot of cyclin B1 and Zp3b in WT, *nanog* mutant, and *nanog* and *eef1a1l2* double mutant immature follicles. Follicles at stage I/II of indicated genotype were collected and used for western blot. GAPDH was used as an internal control.

2. The abundance of Nanog protein (Figure S1C) is detected mostly in the primary and early-vitellogenic follicles and NOT in the fully-grown oocytes. Expression of eef1a1l2 in nanog mutant ovary is primarily found in the early stages of oogenesis (Figure 3C). Further, loss of nanog results in apoptosis in these early follicles (Figure S1A). Moreover, the defects detected in ER, Golgi bodies and mitochondria, start early in the developing follicles (Figure 5G). Together these data hint toward the possibility that the oocyte maturation defect is an outcome of these early defects. It is possible that these oocytes never attended the maturational competence.

Response 2: Thank you for the comment. Based on the expression profile of Nanog and *eef1a1l2*, we agree with the you that Nanog mainly regulates transcription of *eef1a1l2* in early-stage follicles. However, not all the early-stage follicles without *nanog* expression show defects related to ER stress and UPR, and undergo apoptosis (Figure S1A and Figure 5G). In contrast, the *nanog* mutant follicles that could develop to late stage show defects in oocyte maturation (Figure 1H, I), and the defects could be still rescued by inhibiting translational activity using inhibitors (Figure S2E and F, Figure 2L and M), indicating that the translational level of *nanog* mutant mature oocytes is still higher than WT mature oocytes. Together with these data, we concluded that Nanog regulates the translational level of maternal mRNA during oocyte development and maturation. Anyway, we revised the manuscript title to "Translational control by maternal Nanog promotes oogenesis and early embryonic development", and changed "oocyte maturation" to "oogenesis" in the revised manuscript.

3. It is well established in majority of the vertebrate and invertebrate species that translation in immature oocyte is regulated by the size of the poly A tail of the mRNAs (PMID: 2568313, PMID: 27474798, PMID: 9606198, PMID: 26596258). How does loss of nanog trigger translation by regulating an elongation factor if the poly A tail is shorter in those mRNAs?

Response 3: Thank you for the comment. We agree with you that translation in immature oocyte is regulated by the size of the poly A tail of the mRNAs, however, the size of poly A tail is not the only way to regulate translation level in oocyte. For example, the interaction of mRNA transcripts with RNA-binding proteins in a nonspecific or sequence-specific manner is also an important regulatory mechanism of translational control in oocyte development (Nakamura et al., 2001; Richter and Lasko, 2011; Tanaka et al., 2014). Some miRNA or long non-coding RNA has also been shown to regulate the translation of specific mRNA by interfering with the formation of translational complex or cooperating with RNA-binding proteins during oocyte development (Aleshkina et al., 2021; Bushati and Cohen, 2007; Valencia-Sanchez et al., 2006).

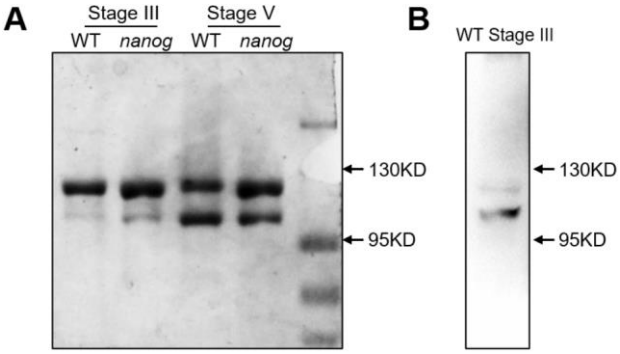
In this study, we have demonstrated that translational activation in *nanog* mutant immature oocytes is mainly dependent on the upregulation of *eef1a1l2*. According to the previously established theory of translational control based on the poly A tail length, the poly A tails of *cyclin B1* and *zp3b* mRNA should be relatively short in early oocytes, because they are translationally silent in early oocytes. However, these two mRNAs were still translationally activated in *nanog* mutant immature oocytes, whereas they were translationally silent in *nanog* and *eef1a1l2* double mutant immature oocytes (Response new Figure 1, revised Figure S5A-D). Therefore, we assumed that the changes in the translation level of maternal mRNA in *nanog*-null oocytes are mainly dependent on the transcriptional activation of *eef1a1l2* but not on the size of the poly A tail of mRNAs. We have discussed this point in the revised manuscript (P21-22, L451-467).

4. Since the authors used whole follicles (oocyte + theca-granulosa cells) for all their analysis, it is essential to describe and call as "follicle-enclosed oocyte" rather than "oocyte". This should be corrected throughout, especially in the method section.
Response 4: We agree with you. We have corrected the “oocyte” related to analyzed in our experiments to “follicles” (follicle-enclosed oocyte) in the revised manuscript, and we have noted this in the method section (P27-28, L565-572).

5. Figure 1: Zebrafish follicle-enclosed oocytes mature spontaneously (~15-25% in 4-6 h culture) without any treatment (PMID: 26853486). Thus, vehicle control is essential in panel H and I.
Response 5-1: Thanks for your suggestion. In fact, we performed the in vitro culture of WT and *nanog* mutant follicle-enclosed oocytes without DHP treatment but we did not show these results in the original submission. Now we have added these controls to the revised Figure 1 panel H and I (see revised Figure 1H and 1I).

The follicle showed in panel J seems to contain dead oocyte, as the morphology of the follicle is severely deformed, a sign of dead oocyte. It is essential that the authors confirm whether the oocyte is alive, which can be easily checked by Trypan blue staining.
Response 5-2: Sorry for the mistake. The follicles we showed in original panel J were indeed near-death follicles. In the revision, we have changed the photos of follicles in revised Figure 1 panel J according to your suggestion (see revised Figure 1J).

The data presented in panel K is not convincing. In fishes, the yolk protein, vitellogenin (Vtg) has several isoforms and often forms homo or heterodimers of very high molecular weight proteins. Thus, showing two Coomassie bands without any specific antibody marker and assuming the second band as the cleaved product of the first one is quite a stretch.
Response 5-3: Thanks for your comment. During the oocyte maturation from stage III to V, the major yolk proteins undergo cleavage, which leads to the translucency of oocyte. Comparing the intensity of these two bands using Coomassie staining has been widely applied in previous studies (Dosch et al., 2004; Selman et al., 1993; Sun et al., 2018). The yolk proteins are the most abundant proteins (almost 90% of the total proteins) in oocytes, thus when we performed SDS-PAGE analysis with low amount of total proteins (see Method section, P28-29, L592-597), Coomassie staining only visualized these two bands (Response new Figure 2A), which is identical to the Western blot analysis (Response new Figure 2B). However, since the transfer efficiency of the high molecular weight band is lower than that of the low molecular weight band (Response new Figure 2B), using Western blot to evaluate the ratio of high yolk protein to low yolk protein is somehow inappropriate.



Response new Figure 2. Coomassie staining of major yolk protein and western blot of Vtg antibody. (A) Original picture of Figure 1K (Coomassie staining) only showed two bands which were between 95 KD-130 KD. (B) Western blot of Vtg antibody using stage III follicles of WT, and two bands between 95 KD-130 KD were observed, which is identical to the Coomassie staining result.

L137- lower GSI does not suggest defects in oocyte maturation. This could be the result of either defective vitellogenesis or lower number of follicles or more apoptosis of the follicles (which is detected by the authors) or all of them. Thus, it is recommended to call it "defects in oocyte development"

Response 5-4: Thanks for your suggestion. It is indeed that lower GSI suggests defects of oocyte development but not oocyte maturation, and we have corrected description to "indicating defects of oocyte development in *nanog* mutants" (P7, L137).

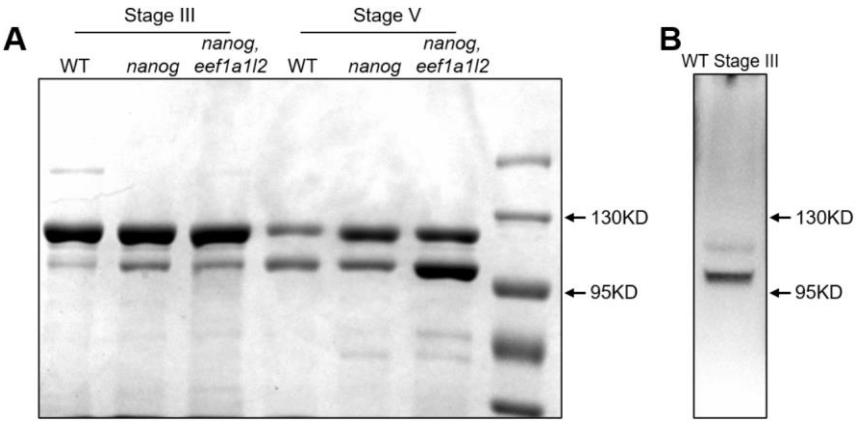
Figure S1D missing the control: Tg(CMV:nanog-myc) with WT background
Response 5-5: Thanks for your suggestion. In revised Supplementary Figures, we have added the WT control and control Tg(CMV:nanog-myc) with WT background (see revised Figure S1D).

6. Figure 2: Is the GAPDH blot same in both E and J? Why?
Response 6: Thank you for your question. The GAPDH blot in E and J is the same, because the western blot of Hsp5a, Ddit3, S6, pS6 and GAPDH were carried out in the same batch. We combined the GAPDH result of previous Figure E and J and pointed out this in Figure 2J legend (see revised Figure 2E and J).

7. Figure S2E: As mentioned earlier, inhibition of translation blocks zebrafish oocyte maturation. Thus, experimental control group should include eif4a inhibitor-treated and untreated WT follicles with or without DHP. Similarly, eif4a inhibitor-treated and untreated nanog mutant follicles with or without DHP.
Response 7: Thanks for your suggestion. We have added the control groups of WT follicles treated with 4EGI-1 (interaction inhibitor of eIF4E and eIF4G, which was incorrectly described as eif4a inhibitor previously, and we have corrected the description in the revised manuscript (P10, L189-195)). Moreover, we have also added the groups of WT and *nanog* mutant follicles without treated with DHP (see revised Figure S2E).

8. Figure 4 and 5: It is evident from the present data that eef1a1l2 functions downstream of nanog and loss of eef1a1l2 rescued the oogenic defects of nanog mutants. However, in each one of the panels eef1a1l2 single mutant should be presented as a control. The authors mentioned (L275-276) "We then generated homozygous mutant of eef1a1l2 and two types of eef1a1l2 mutants were obtained and neither of them showed obvious defect (Fig. S4)". However, Figure S4 shows "Generation of the eef1a1l2 mutant allele using CRISPR/Cas9" and no data for any phenotypic analysis.
Response 8-1: Thanks for your comment. We have observed the embryonic phenotype of *eel1a1l2* mutant, and maternal-zygotic mutant of *eef1a1l2* (MZ*eef1a1l2*) showed no developmental defects (see revised Figure S4B, see also revised manuscript P14L283). We also compared the oocyte diameter, GSI, oocyte transparency, and GVBD ratio of the *eef1a1l2* mutant with WT. All these aspects showed no difference between *eef1a1l2*$^{-/-}$ and WT (see revised Figure 4A, B, C, E, F, G, I and J, see also revised manuscript P15L307-310), indicating the depletion of *eef1a1l2* does not lead to defects in oocyte maturation and embryonic development. Thus, we did not examine the *eef1a1l2* mutant in the subsequent studies.

Figure 4 H: Please see comment for figure 1 K.
Response 8-2: Thanks for your question. As we mentioned in **Response 5-3**, comparing the intensity of two Vtg bands between 95 KD and 130 KD using Coomassie staining has been widely used to evaluate the cleavage efficiency of major yolk protein. Thus, we performed SDS-PAGE analysis with low amount of total proteins of WT, *nanog* mutant, *nanog* and *eef1a1l2* double mutant follicles (see Method section, P28-29, L592-597), the original picture of panel K in Figure 1 also only visualized two vtg bands by Coomassie staining (Response new Figure 3A), which is identical with the Western blot analysis (Response new Figure 3B).



Response new Figure 3. Original picture of Figure 4H and Western blot of Vtg antibody. (A) Original picture of Figure 4H (Coomassie staining) showed two yolk protein bands between 95 KD-130KD. (B) Western blot of Vtg antibody using stage III follicles of WT, and two bands between 95 KD-130KD were observed, which is identical to the Coomassie staining result.

Figure 4 I: needs vehicle control groups without DHP as mentioned in comment for figure 1 H.
Response 8-3: Thanks for your suggestion. We have added the GVBD results of each group without DHP treated, and we also added the GVBD result of *eef1a1l2* mutant follicles treated with or without DHP (see revised Figure 4I).

9. L415-418: Please see comment 2.
Response 9: Thanks for your comment. After thoroughly revised the manuscript, we agree with you that definition of Nanog as a new factor for assessment of oocyte quality based on the data of this study alone is not sufficient. Therefore, we deleted the original point of L415-L418 and revised the manuscript accordingly.

10. Figure S6: How did the authors check 'fertilization rate' for good or poor quality of eggs as they mentioned in the legend (?Fertilization rate >90% was defined as good-quality egg, and Fertilization rate <10% was defined as poor-quality egg?)? There is no method section for this. It is important to discuss this experiment properly, especially because the authors found that nanog expression is lower in these "bad" eggs. Based on which the authors suggested ?
Nanog might be a new factor for oocyte quality assessment? However, Nanog was not detected in the fully-grown oocytes even in the overexpressed strain [Tg(CMV:nanog-myc)]. Thus, relative expression of nanog mRNA is not enough for this argument.
Response 10: Thanks for your comment. After thoroughly revised the manuscript, we agree with you that definition of Nanog as a new factor for assessment of oocyte quality based on the data of this study alone is not sufficient. Therefore, we deleted the previous Figure S6 and revised the manuscript accordingly.

Minor comments:
L48: needs citation(s).
Response 11: Thank you for pointing out. We have cited 4 relevant literatures (P3, L47-48).

L130: should be "during oocyte development" not "maturation"
Response 12: Thank you for pointing out. We have corrected the sentence to “during oocyte development” (P7, L130).

L184: "Western blot"
Response 13: Sorry for the mistake. We have corrected it to “western blot” (P9, L186).

L244: "supported" sound better than "proved"
Response 14: Thanks for your suggestion. We have changed “proved” to “supported” (P12, L250).

L288: "(compare Movie 1 and Movie 3)"
Response 15: Thanks for your suggestion. We have corrected the "(Movie1)" to "(comparing Movie1 and Movie3) (P14, L297).

L417-418: "Impaired oocyte maturation correspondingly tends to produce poor-quality egg". The data is quite prominent (discussed in comment 2) that Nanog expression in the early stage of oogenesis possibly regulate oocyte quality.
Response 16: Thanks for your suggestion. As we mentioned in Response 9 and Response 10, we have deleted the point of Nanog regulating oocyte quality in the revised manuscript.

L441-443: The sentence is unclear.
Response 17: Thanks for your comment. According to your suggestion, we have totally revised this paragraph, and revised the original sentence to "However, different from these mechanisms, in this study we demonstrated a novel translational control mechanism mediated by Nanog, which transcriptionally inhibits the expression of a translational elongation factor, eEF1A1l2, and controls the maternal mRNA translational activity during oogenesis. The translational control mediated by Nanog is relatively a global one, and does not depend on the specificity of mRNA sequence and on the size of the poly(A) tail of mRNAs." (P22-23, L474-480), which is more clearly to clarify the unique of Nanog regulatory mechanism.

L446: "The genes, which are"
Response 18: Thanks for your suggestion. We have corrected the previous "The genes who are" to "The genes, which are" (P23, L483) in the revised manuscript.

L452-453: Figure S4 does not show anything mentioned in the statement.
Response 19: Thanks for the comment. This sentence should be referred to Figure S3. We have corrected to "(Figure 3B and 3C, S3)" (P23, L490) in the revised manuscript.

Figure 6C: In the model, name of the gene is written "eef1a1b". Is it a mistake or this is the same as "eef1a1l2"? Please check and keep it consistent.
Response 20: We are sorry for the mistake. *eef1a1b* is the previous name of *eef1a1l2*, and we have corrected the gene name to *eef1a1l2* in the revised model (see revised Figure 6C).

Reviewer #2:

Advance Summary and Potential Significance to Field:

Zebrafish provides an exciting model to investigate maternal contribution to early embryogenesis. During oogenesis and early embryogenesis, translation of maternal mRNA is tightly controlled in a spatio-temporal manner. A mechanism of repression/activation of translation must occur to avoid a defective embryogenesis. However, a global regulator of translation during oogenesis and its mechanism has not been elucidated yet. This manuscript contributes to this field through the findings that Nanog acts as a repressor and targets eef1a1l2 to globally control translation of maternal mRNA before maternal to zygotic transition.
Strengths of the manuscript include the extensive and detailed study of the loss of nanog during oogenesis using various molecular and cellular tools and the finding that Nanog can act as a repressor on a translational machinery gene, eef1a1l2. Most importantly, the partial rescue of Mnanog phenotype in Mnanog;eef1a1l2 double mutant is striking and reinforce the idea of a global translational mechanism. The examination of the ER and UPR pathway is nicely analyzed.
The weaknesses in the manuscript are largely presentational in nature and could be rather easily fixed by providing clarification and textual modifications. Neither does the manuscript clarify the underlying biochemical mechanisms by which Nanog act as repressor nor does it address the complex relationship between RNA binding proteins and translation mechanism during oocyte maturation. These are clearly complex issues of high interest beyond the scope of the present study, which does provide useful data for the field. The manuscript is overall well written, well referenced and figures well presented. The authors should consider the following points.
-Thank you very much for your appreciation of our work.

Fig2A: The picture of Mnanog in the mCherry channel have almost no signal compared to the WT. This disagrees with Fig.2B, 2C and 2D and the main text. The Supp Fig S5 is in adequation with the main findings. Please clarify because this figure is at the base of all the subsequent analyses and findings.
Response 21: We are sorry for the mistake of reversing WT and M*nanog* mCherry channel. We have corrected and rearranged the pictures (revised Figure 2A).

Fig2D: Rrelative should be read Relative
Response 22: We are sorry for the mistake, and we have corrected it to "Relative" (see revised Figure 2D).

Fig4D: Phalloidine should be read Phalloidin
Response 23: We are sorry for the mistake, and we have corrected it to "Phalloidin" (see revised Figure 4D).

Legend Fig S1: DAPI was co-stained for 'nucleus' should be read DAPI was co-stained for 'DNA'
Response 24: Thank you. We have changed "nucleus" to "DNA" in the revised Figure S1 legend.

S1 Table: Please add a description for each protein. Only accession number is given.
Response 25: Thanks for your suggestion. We have added a description and the gene name for each protein in the revised S1 Table.

l.39: Please remove the comma to smooth the reading.
Response 25: Thanks for your suggestion. We have removed the comma in P3L38.

l.366: Please revise the sentence.
Response 25: Thanks for pointing out. We have corrected the writing error in P19L390.

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Second decision letter

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MS TITLE: Translational control by maternal Nanog promotes oogenesis and early embryonic development

AUTHORS: Mudan He, Shengbo Jiao, Ru Zhang, Ding Ye, Houpeng Wang, and Yonghua Sun
ARTICLE TYPE: Research Article

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

Reviewer 1

Advance summary and potential significance to field

This reviewer is satisfied with the additional experiments performed and current textual changes made by the authors. Thank you.

Comments for the author

The revised manuscript is suitable for publication.

Reviewer 2

Advance summary and potential significance to field

The authors have nicely revised the manuscript and addressed all my concerns.

Comments for the author

Well done!