Aurora kinase B inhibits aurora kinase A to control maternal mRNA translation in mouse oocytes

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AUTHORS: Mansour Aboelenain and Karen Schindler

I have now received all of the reviewers' comments and have reached a decision. 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. Specifically, reviewers 1 and 2 point out that one of the major claims in the study that there is mechanistic interplay between Aurora A and B has not been experimentally addressed, in that the manuscript does not test how Aurora B regulates Aurora A; reviewer 1 suggests experiments that would need to be performed to address this central claim. Being able to experimentally address this central claim will be critical for the revised manuscript. Both reviewers also point out control experiments that will need to be performed to add to the rigor of the analysis, for example, reviewer 2 points out control experiments with 3' UTR that do not contain the CPEB binding sites. In addition, the experimental verification of CPEB with poly(A) tail lengths would be a nice experimental advance as suggested by reviewer 2. Reviewer 1 also suggests adding a time course analysis, for example, to the luciferase experiment. This would further help cement some of the conclusions in the manuscript and add rigor to the analysis. 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.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. 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 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

In this paper, the authors investigated how the kinase Aurora B impacts mRNA translation during the meiotic maturation of mouse oocytes. Using conditional knock out mice for Aurora B and/or Aurora A and pharmacological tools, they propose that Aurora B negatively regulates mRNA translation through an indirect mechanism involving another Aurora A, which controls the RNA binding protein CPEB1. From their results, they conclude that Aurora A and Aurora B are functionally but antagonistically interconnected to RNA translation in order to regulate the meiotic cell division.

Deciphering the specific functions of two Aurora isoforms is an attractive question to understand the regulation of meiotic cell divisions. Furthermore, this question is of general interest for readers in the field of cell cycle. However, the authors propose that the regulation of RNA translation by Aurora B is mediated by Aurora A, which in turn acts on CPEB1. This later point has been extensively investigated over the past years in several experimental models (mouse porcine xenopus) and Aurora A function on CPEB1 has been well documented in mouse oocytes. Hence, the novelty of the manuscript mostly relies on the antagonistic interplay between Aurora B and Aurora A. This original question is however not addressed in the paper, which makes it very descriptive. In particular, the functional consequences of this new Aurora B/ Aurora A network on the meiotic maturation process are not investigated. Finally, it is difficult to agree with some of the conclusions because of the lack of control experiments. Hence, many experiments and major revisions will be mandatory to improve the quality of the manuscript and I do not recommend its publication in Development.

Comments for the author

To the authors,
In this paper, the authors investigated how the kinase Aurora B impacts mRNA translation during the meiotic maturation of mouse oocytes. Using conditional knock out mouse for Aurora B and/or Aurora A and pharmacological tools, they propose that Aurora B negatively regulates mRNA translation through an indirect mechanism involving another Aurora A, which controls the RNA binding protein CPEB1. From their results, they conclude that Aurora A and Aurora B are functionally but antagonistically interconnected to RNA translation in order to regulate the meiotic cell division.

Deciphering the specific functions of two Aurora isoforms is an attractive question to understand the regulation of meiotic cell divisions. Furthermore, this question is of general interest for readers in the field of cell cycle. However, the authors propose that the regulation of RNA translation by Aurora B is mediated by Aurora A, which in turn acts on CPEB1. This later point has been extensively investigated over the past years in several experimental models (mouse porcine xenopus) and Aurora A function on CPEB1 has been well documented in mouse oocytes. Hence, the novelty of the manuscript mostly relies on the antagonistic interplay between Aurora B and Aurora A. This original question is however not addressed in the paper. Moreover, the functional consequences of this new Aurora B/ Aurora A network on the meiotic maturation process are not investigated. Finally, it is difficult to agree with some of the conclusions because of the lack of control experiments. Hence, many experiments and major revisions will be mandatory to improve the quality of the manuscript.

Major points:
The authors claim that Aurora B regulates the activity of Aurora A based on results previously published by the team. To investigate this regulation, the authors use mouse oocytes obtained from

either a single or a double Aurora B / Aurora A knock-out or treated with pharmacological inhibitors. They analysed the overall RNA translation or the one driven by the 3' UTR of Aurora C using a reporter gene, known to be controlled by CPEB1. The experiments done with the single/double KO, which demonstrate that the loss of Aurora A expression/activity suppresses the effects of Aurora B on translation, are very convincing. However, many experiments are further required to improve the quality and the biological significance of the manuscript.

1 – In all the experiments analysing HPG incorporation, CPEB1 expression levels or luciferase intensity, important controls are missing. These processes can be already deregulated in prophase oocytes coming from KO mice or treated with pharmacological tools. Thus, important controls must be provided to fully validate the conclusions as illustrated in Figure 2D: prophase oocytes and MI and/or MII oocytes from any described conditions (WT, KO or treated with pharmacological inhibitors). Moreover, the luciferase intensity must not be quantified as a ratio but instead illustrated for each condition (WT pro, KO pro, WT MI/MII and KO MI/MII).

2 - There is no experimental data showing that the increase of RNA translation observed in Aurora B KO mouse has a biological significance, i.e. can regulate the meiotic maturation of oocytes. The functional consequences of this module on both the timing and the activity of well-known cell cycle regulators, such as Cdk1 or the Mos/MAPK pathway, must be investigated.

3 - An in-depth analysis of how Aurora B controls Aurora A must be investigated in order to clarify this mechanism. The expression levels, the post-translational modifications, especially the phosphorylation, and the overall activity of Aurora A must be precisely determined during the meiotic maturation in Aurora B KO oocytes. Also, one hypothesis to explain the increase of Aurora A activity in these cells could originate from the fact that the other kinases, which are activated during meiotic maturation, do not find their endogenous substrate in the absence of Aurora B. The function of these kinases (Cdk1, MAPK, see also point 2) could be tackled using pharmacological tools. Altogether, these experiments would help a lot to decipher whether the mechanism regulating Aurora A is direct or indirect.

4 - A major conclusion of the paper is that the protein CPEB1 mediates the inhibition of RNA translation promoted by Aurora B. Whether this process involves CPEB1 turn-over or stability must be thoroughly analysed. Indeed, depending on the Figures, oocytes are collected at different stages of meiotic maturation, either in MII or in MI. The time of oocyte collection must be homogenised.

5 - A critical point is to determine whether this CPEB1-mediated mechanism regulates the translation and/or the accumulation of endogenous proteins besides Aurora C. Indeed, several endogenous proteins are known to be translated under the control of CPEB1 during mouse meiotic maturation, including Cyclins B. The behaviour of both endogenous Aurora C and other proteins must be monitored during the course of meiotic maturation. This will allow to determine whether Aurora B/Aurora A targets either specifically Aurora C or other proteins whose translation depends on CPEB1.

6 – The authors concluded that Aurora B regulates the stability of CPEB1 using western blot. I agree with the author that CPEB1 activity is difficult to follow as this protein is regulated by its hyperphosphorylation, which in turn promotes its degradation during the first meiotic division. The experiments designed to analyse CPEB1 turn-over/activity is very puzzling for the following reasons:

- Following the overexpression of Aurora B, the expression levels of CPEB1 increase but the protein is not phosphorylated (Figure 2). Does it mean that the protein remains stable because it cannot be phosphorylated ? On the other hand, it is also possible that increasing the expression level of CPEB1 expression locks RNA translation.
- In Aurora A KO oocytes, CPEB1 expression levels also increase (Figure 3). Accordingly, the CPEB1-induced translation monitored with the Aurora C reporter gene is strongly inhibited and the overall RNA translation is reduced (Figure 3). As for Aurora B KO oocytes, loss of Aurora A expression seems to lock CPEB1 under its inactive state that cannot be degraded. This result suggests that Aurora A and Aurora B have redundant effects on CPEB1 regulation.
- In Figure 1, some CPEB1 is still expressed in MII control oocytes while it fully disappears in oocytes collected at the same stage in Figure S1. It is therefore very difficult to conclude that Aurora B has an effect on CPEB1 stability.

In order to get a better vision on CPEB1 regulation, the expression levels as well as the phosphorylation of CPEB1 must be analysed by western blot in a time course way during meiotic maturation for the KO oocytes. Moreover, the use of pharmacological inhibitors of protein translation (cycloheximide) and proteasome may help to decipher the precise role of Aurora A and Aurora B on CPEB1.

7 - The activity of CPEB1 is monitored by a luciferase assay using a reporter gene containing the 3' UTR of Aurora C. As mentioned above, the accumulation of endogenous proteins is missing and the functional consequences of Aurora A and/or Aurora B depletion are not investigated. As the authors analysed CPEB1 activation using an exogenous RNA, any change in the timing of meiotic maturation could affect the expression level of the reporter gene. It will be therefore important to perform this experiment in a time course-way and to use a control reporter gene deleted for the CPEB1 binding site. As mentioned before, western blot analysis of Aurora C to monitor its expression would strengthen the data.

Minor points:
- The sequence of the gene reporter used in the luciferase is missing in the material and methods
- I wonder why the overexpression of Aurora B has only been done in WT oocytes. Would it be possible to overexpress Aurora B in Aurora B KO oocytes to determine this restore both RNA translation and CPEB1 expression levels ?

Reviewer 2

Advance summary and potential significance to field

The Aurora serine/threonine protein kinases are key regulators of meiosis and mitosis. Notably, they play key roles in regulating microtubule dynamics and spindle function. Two key Aurora kinase paralogs, Aurora A and Aurora B, mediate all known essential mitotic functions of these kinases. Interestingly, mammalian germ cells also express a third Aurora kinase paralog, Aurora C. This is of particular interest with regard to female meiosis in which chromosome segregation depends on the formation and function of acentriolar spindles. In very nice and comprehensive work, the Schindler lab has extensively studied the Aurora kinases in female meiosis—Aurora B and Aurora C are largely dispensable (double null mutant oocytes exhibit high fertility; Nguyen et al., 2018); whereas Aurora A is essential (meiosis I arrest, Blengini et al. 2021 preprint).

In this new work, the authors use genetic analysis in mice to provide evidence for a regulatory network of Aurora kinases in female meiosis. Their data support a model in which Aurora B negatively regulates Aurora A, which in turn promotes CPEB1-dependent translation of Aurora C. This model explains why it is that Aurora C activity is elevated when Aurora B is depleted. The study's key advance is the establishment of the regulatory interplay between the Aurora kinases, but the nature of the mechanism by which Aurora A activity might be increased upon Aurora B depletion is not addressed in this work. On the whole, the work is nicely done—the authors extensively utilize strong genetic tools for inactivating the Aurora kinases. Certainly, one can think of a great many experiments that the authors might do to further support their model (e.g., more direct measures of protein translation, RNA stability, and polyadenylation). One frequently sees these types of analyses in large multi-author investigations (which this is not). I am reluctant to request these extensive analyses in this initial report because I doubt the message will change. The authors should consider the following specific points in the box below.

Comments for the author

1. The authors' analysis of protein translation largely hinges on fluorescence detection of L-homopropargylglycine (HPG, a methionine analog) incorporation. It is a bit unsettling that a key conclusion of the study relies on a single method of looking at protein translation (and a largely global non-specific one). From the images shown in Figure 1, it appears that the background levels of oocyte autofluorescence might vary from experiment to experiment. It would have been nice if cycloheximide controls were used. Can the authors address this concern?

From the data shown by Rong et al (2019) in which this method was extensively utilized, it seems that timing may also be important. Do Aurora B knockout oocytes exhibit similar timing of meiotic maturation events as wild-type controls?

2. A potentially confusing aspect of the presentation is that the authors use the inverse of CPEB1 levels as a surrogate for CPEB1 activity, the idea being that when CPEB1 is activated by phosphorylation, it turns over more rapidly. An improvement would be adding some direct measures of poly(A) length.

3. Figure 2, panels G and H, show a nice experiment in which an Aurora C 3'UTR reporter construct was utilized. An improvement in the experimental setup would be to add 3'UTR reporters that do not contain CPEB1 binding sites.

4. The legend to Figure 4 does not match what is shown in the figure (the text refers also to panels 4E-F. The authors should fix this.

5. It would be helpful if the manuscript had page numbers.

Reviewer 3

Advance summary and potential significance to field

In manuscript by Aboelenain and Schindler authors investigated a role of Aurora kinase B in regulation of translation in mouse oocytes. Their aim was to elucidate the previous observation that the absence of Aurora B causes overexpression of Aurora C kinase with subsequent increase of aneuploidy. Since the oocytes are transcriptionally silent, the regulated translation is essentially important for successful progression of meiosis, and therefore this work is of high importance. In this study authors discovered a new meiosis I specific regulatory circuit, which involves Aurora kinase A and B in control of translation via CPEB1.

Comments for the author

In this study the authors used a combination of genetically modified mouse lines with depleted Aurora kinases and also techniques, allowing to quantify translation in general or translation of specific proteins, such as CPEB1. They established that the depletion of Aurora B, but not the Aurora C, increases overall rate of translation in meiosis I and negatively affects the stability of the CPEB1 protein, as well as CPEB1-dependent translation. Importantly, they discovered that the simultaneous depletion of Aurora B and pharmacological inhibition of Aurora A leads to reduced translation. And this was also recapitulated in oocytes depleted of Aurora A.

This work is important for our understanding of the control mechanisms of meiotic division, as well as origin of aneuploidy in oocytes. Therefore, I recommend this work for publication, after bellow mentioned problems will be resolved:

Major points:
It seems that the figure 4 contains only repeated panels from figure 3.

Minor points:
1. Can authors explain the difference between Figure S1, panel A, in which the CPEB1 protein is gone by 16 hours and Figure 2, panel C, where in ovulated eggs this protein is still expressed?
2. In the figure legends of Figure 2 panel C and D, prophase I cells are not mentioned.

First revision

Author response to reviewers' comments

Enclosed is the revision of our manuscript in the style of a short Research Report entitled “Aurora kinase B negatively regulates Aurora kinase A to control maternal mRNA translation in mouse oocytes” for consideration for publication in Development. We are grateful for the reviewers’ expert insight and have made all modifications possible. The most significant changes you will find are: 1) including proteins in addition to AURKC that can be regulated by AURKB/CPEB1, 2) evaluation of the biological significance of the coordination between AURKB and AURKA of translation regulation in the production of euploid eggs, 3) control experiments to assess proteins at prophase I and luciferase assays with CPE mutation, and 4) rescue experiments in the 2 KO strains. We find that these modifications and adjustments to the text greatly improve our manuscript.

Below, reviewers will find a point-by-point response to their queries and suggestions. We have copied their points and italicize them; our responses are below their points.

Reviewer 1:
Deciphering the specific functions of two Aurora isoforms is an attractive question to understand the regulation of meiotic cell divisions. Furthermore, this question is of general interest for readers in the field of cell cycle. However, the authors propose that the regulation of RNA translation by Aurora B is mediated by Aurora A, which in turn acts on CPEB1. This later point has been extensively investigated over the past years in several experimental models (mouse porcine xenopus) and Aurora A function on CPEB1 has been well documented in mouse oocytes. Hence, the novelty of the manuscript mostly relies on the antagonistic interplay between Aurora B and Aurora A.
Response: Although we agree that the interplay between Aurora B and Aurora A is an important finding that we report, we respectively disagree that the connection between Aurora A and CPEB1 has been extensively investigated in mouse as it has in Xenopus. In 2001, Hodgman et al showed that AURKA is expressed in mouse oocytes. They examined AURK phosphorylation sites by mixing purified Xenopus CPEB protein (mouse CPEB was reportedly not soluble) with γ32P-ATP and mouse oocyte extracts and find phosphorylation at Ser174. They blocked AURKA activity by injecting a peptide that works in Xenopus into mouse oocytes and find lack of meiotic progression. However, an activity change on CPEB was not examined. The data are consistent with Xenopus findings and suggest a function for AURKA, but do not explore it in more detail. To our knowledge, this function was not explored again until Han et al in 2017, where they evaluated the electrophoretic mobility of CPEB1 when AURKA was inhibited with MLN. In opposition to Hodgman’s conclusion, they find no role for AURKA in phosphorylating CPEB. This is like what is observed in porcine oocytes- inhibition of AURKA with MLN does not affect CPEB phosphorylation (Komrskova et al 2014). In our hands, MLN easily precipitates from solution, and we speculate that this disparity in result could be due to issues with using MLN. Regardless, we strongly believe that our report is the first to take a comprehensive genetic and pharmacological approach to clarify this function in mouse oocytes.

This original question is however not addressed in the paper. Moreover, the functional consequences of this new Aurora B/ Aurora A network on the meiotic maturation process are not investigated. Finally, it is difficult to agree with some of the conclusions because of the lack of control experiments. Hence, many experiments and major revisions will be mandatory to improve the quality of the manuscript.

Response: Based on the experiments suggested by the reviewer, we believe that our data now more strongly support the model that we put forward. We thank the reviewer for their assessment and suggestions. We note that this manuscript is submitted as a Research report, which are short, high-impact papers. The inquiries and suggestions by this reviewer are important and are of high interest, but we find that some go beyond the scope of the Research report purpose. We focused our revision efforts on including controls and expanding beyond AURKC. Our rationale for the exclusions are included in the point-by-point responses below. We hope that the reviewer will agree with our strategy.

Comments:

1. In all the experiments analysing HPG incorporation, CPEB1 expression levels or luciferase intensity, important controls are missing. These processes can be already deregulated in prophase oocytes coming from KO mice or treated with pharmacological tools. Thus, important controls must be provided to fully validate the conclusions as illustrated in Figure 2D: prophase oocytes and MI and/or MII oocytes from any described conditions (WT, KO or treated with pharmacological inhibitors).

Response: The reviewer raises an important point that we overlooked. We have now carefully evaluated protein levels in Prophase I oocytes and find that these processes are not deregulated in prophase oocytes. These additions are described in detail below.
HPG incorporation: We added assessment of HPG levels in prophase oocytes for all the mouse genotypes used in this manuscript (new Figure S1 C-D). Importantly there was no significant difference between WT, B cKO and B cKO hetA oocytes, indicating that translation levels start the same, regardless of genotype and that they only differ upon meiotic maturation to Metaphase I in B cKO oocytes (Figure 1 A-B) and after AURKA inhibition (Figure 3).

CPEB1 expression levels: The control in the original submission (Figure 2C-D) showd that CPEB1 levels in prophase I oocytes from WT and B cKO mice by western blotting were not different. We note that this was also demonstrated when WT prophase I oocytes were incubated in the AURKA inhibitor (new Figure S4E-F), the 0h time point showed similar CPEB1 protein levels by western.
Recruitment of RNAs other than Aurkc: Because we added evaluation of other proteins to our study (from point 5 below), we also confirmed that the expression of these proteins (CyclinB1, PRC1, MOS and Hec1) did not differ in prophase I WT and B cKO oocytes (new Figure S3 B-K).
Luciferase intensity: In the revision, we re-graphed the luciferase assay to show the Prophase I levels (Figure 2N-O). These levels did not differ between WT and B cKO for either Aurkc or Ccnb1. Moreover, the luciferase intensity must not be quantified as a ratio but instead illustrated for each condition (WT pro, KO pro, WT MI/MII and KO MI/MII).
Response: We revised this figure as suggested and include it in the revised Figure 2N-O. We also note that we did not evaluate luciferase at MII because Aurora A cKO oocytes arrest at Metaphase I (Blengini et al 2021) and would therefore not be a equal comparison.

2. There is no experimental data showing that the increase of RNA translation observed in Aurora B KO mouse has a biological significance, i.e. can regulate the meiotic maturation of oocytes.
Response: This was a great suggestion by the reviewer. In Figure 3A-B, we showed that the increased translation level in B cKO oocytes is likely caused by an excess of AURKA activity because HPG levels significantly decreased when we inhibited AURKA activity in B cKO oocytes and when 1 copy of Aurka is removed from the Aurkb KO strain background (new Figure 4E-F). Moreover, CPEB1 degradation in B cKO oocytes was rescued when we inhibited AURKA activity or when we reduced Aurka to one copy (i.e. B cKO Het A) in the genome (Figure 4A-D). Based on our published study (Nguyen et al 2018) B cKO eggs have high levels of aneuploidy and prematurely separated sister chromatids (PSSC). To connect our findings of translation changes with the aneuploidy phenotypes, we evaluated aneuploidy and PSSC in eggs from B cKO (high translation) and B cKO hetA mice (low translation). Importantly, we found a significant reduction of aneuploidy and PSSC in B cKO het A eggs (new Figure 4G-I). This finding suggests a biological significance of the elevated translation in B cKO oocytes. We conclude that AURKB and AURKA regulation of translation is required for production of euploid eggs.

The functional consequences of this module on both the timing and the activity of well-known cell cycle regulators, such as Cdk1 or the Mos/MAPK pathway, must be investigated.
Response: We conducted a cell-cycle time course experiment as suggested and we show that there are no significant changes meiotic maturation timing (via time of polar body extrusion) between WT and B cKO oocytes. This is included in Figure S1, panel E. Therefore, we think it is unlikely that cell cycle regulator changes could account for this recruitment difference since there is not a significant indication that cell cycle kinetics are altered.

3. An in-depth analysis of how Aurora B controls Aurora A must be investigated in order to clarify this mechanism. The expression levels, the post-translational modifications, especially the phosphorylation, and the overall activity of Aurora A must be precisely determined during the meiotic maturation in Aurora B KO oocytes.

Response: Some of these mechanisms have been published by us previously (Nguyen et al 2018). There we showed that activated AURKA levels increased in B cKO oocytes while total AURKA remained the same. Based on the reviewer's inquiry, to show that this increased activation leads to increased activity, we assessed phosphorylation of an AURKA substrate, CDC25B. As shown in the new Figure S4A-B, pCDC25B intensity is increased in B cKO eggs, thereby supporting our model that excess AURKA is driving excess CPEB1 activity. The mechanism of how AURKB controls AURKA is of interest but is outside of the scope of this report (which was to understand how AURKB controls AURKC).

Also, one hypothesis to explain the increase of Aurora A activity in these cells could originate from the fact that the other kinases, which are activated during meiotic maturation, do not find their endogenous substrate in the absence of Aurora B. The function of these kinases (Cdk1, MAPK, see also point 2) could be tackled using pharmacological tools. Altogether, these experiments would help a lot to decipher whether the mechanism regulating Aurora A is direct or indirect.
Response: We appreciate the suggestions. Determining the exact mechanism of regulation will take extensive analysis and is outside of the scope of the report which was to determine why AURKC was upregulated in B cKO oocytes. We instead chose to take a comprehensive genetic and pharmacological approach to clarify AURKA/CPEB1 function in mouse oocytes.

4. A major conclusion of the paper is that the protein CPEB1 mediates the inhibition of RNA translation promoted by Aurora B. Whether this process involves CPEB1 turn-over or stability must be thoroughly analysed.
Response: The conclusion that we make is that AURKB is required to maintain a certain reduced level of AURKA activity which, in turn, controls the level of CPEB1 mediated RNA-translation. We view this as a fine-tuning mechanism to promote high egg quality. The conclusion summarized by the reviewer, while it could be correct, is too strong for the data presented here, and not in line the desired scope of this manuscript which is a Research report (not a full article). Determining these types of mechanisms would be a stand-alone paper or would require changing the manuscript into a full article.

Indeed, depending on the Figures, oocytes are collected at different stages of meiotic maturation, either in MII or in MI. The time of oocyte collection must be homogenised.
Response: As shown by others, and by us here in Figure S2, MI is the peak time point for translation and the starting point for a significant CPEB1 degradation. We therefore evaluated CPEB1 and HPG at this stage throughout the manuscript. We now also include evaluation of prophase I oocytes. Occasionally, we do have Met II included for analysis of B cKO eggs because the differences in protein abundance is more clearly visualized (Fig. 2C and Fig. S3H). We cannot do Met II analysis in A cKO oocytes because these oocytes have a complete arrest in MI (Blengini et al 2021).

5. A critical point is to determine whether this CPEB1-mediated mechanism regulates the translation and/or the accumulation of endogenous proteins besides Aurora C. Indeed, several endogenous proteins are known to be translated under the control of CPEB1 during mouse meiotic maturation, including Cyclins B. The behavior of both endogenous Aurora C and other proteins must be monitored during the course of meiotic maturation. This will allow to determine whether Aurora B/Aurora A targets either specifically Aurora C or other proteins whose translation depends on CPEB1.

Response: Thank you for this great suggestion. We evaluated other CPE-containing candidates: Cyclin B1, PRC1, MOS and HEC1. These candidates were selected based on established recruitment profiles in the published literature and which had reagents readily available for our use. We include a new schematic diagram for these candidates' UTRs showing the CPE sequences and the specific hexamer nucleotide sequence for polyadenylation (PAS) in a new Figure S3 A.
Cyclin B1: CCNB1 was chosen because it is well reported to be translated in a CPE-dependent manner (Tay et al., 2000; Han et al 2017). Using the luciferase assay, we assessed Ccnb1-UTR enrichment in WT and B cKO oocytes. The constructs were the same as reported in Murai et al., 2010. We show a significant increase of the Ccnb1-UTR enrichment in B cKO MI (8-fold) oocytes compared to WT MI oocytes (4-fold). In contrast, when we mutated the CPE sites in the Ccnb1-UTR, luciferase did not accumulate in either WT or B cKO oocytes (new figure—Fig. 2O). To confirm that the oocytes start with the same amount of Cyclin B1 protein, we evaluated CCNB1 by western blotting, comparing WT and B cKO oocytes. We found similar levels of CCNB1 in prophase I oocytes

(new Figure S3B-C) and a significant increase of CCNB1 in B cKO MI oocytes compared to WT oocytes was observed (new Fig. 2G-H).
PRC1: We chose PRC1 because it has a CPE and we happened to have reagents in the lab. PRC1 levels are significantly higher in B cKO MI oocytes compared to WT MI oocytes (new Figure 2I-J). Also, the prophase I level of PRC1 in WT and B cKO was not different (Figure S3 D-E).
HEC1: We chose HEC1 because it has a CPE and we had reagents in the lab. By immunostaining Met II eggs, we find significantly higher HEC1 at kinetochores in B cKOs compared to WT (Figure S3 H-I). The levels at prophase I were not different between WT and B cKO oocytes (Figure S3 F-G).
MOS: We chose MOS because it is well documented to undergo recruitment in a CPE-dependent manner. Using immunostaining, we evaluated MOS level in WT and B cKO oocytes and find a significant enrichment in B cKO MI oocytes compared to WT (Figure 3 N-O). Levels of MOS at prophase I were not different between WT and B cKO (Figure S3 J-K).
Addition of these genes strongly suggest that AURKA/B can regulate the levels of many proteins in a CPEB-dependent mechanism during meiotic maturation.

6. The authors concluded that Aurora B regulates the stability of CPEB1 using western blot. I agree with the author that CPEB1 activity is difficult to follow as this protein is regulated by its hyperphosphorylation, which in turn promotes its degradation during the first meiotic division. The experiments designed to analyse CPEB1 turn-over/activity is very puzzling for the following reasons:
Following the overexpression of Aurora B, the expression levels of CPEB1 increase but the protein is not phosphorylated (Figure 2). Does it mean that the protein remains stable because it cannot be phosphorylated? On the other hand, it is also possible that increasing the expression level of CPEB1 expression locks RNA translation.

In Aurora A KO oocytes, CPEB1 expression levels also increase (Figure 3). Accordingly, the CPEB1-induced translation monitored with the Aurora C reporter gene is strongly inhibited and the overall RNA translation is reduced (Figure 3).

As for Aurora B KO oocytes, loss of Aurora A expression seems to lock CPEB1 under its inactive state that cannot be degraded. This result suggests that Aurora A and Aurora B have redundant effects on CPEB1 regulation.
Response: We are not entirely certain what the reviewer means by “locks translation.” However, because the reviewer suggested rescue experiments in the KO oocytes (new Figures 2D-E and 3E-F), these new data are stronger and more convincing than the overexpression approach in the original submission. We therefore, have simplified this revision by removing the overexpression in WT oocyte panels (old Figure 1F/G; Figure 2E/F). We also removed old Figure 4A/B because the genetic data with reducing Aurka copy number and new translation data (new Figure 4E/F) with this strain is a more precise and direct approach. Because of the opposite CPEB stability phenotypes between the B cKO and A cKOs, and because inhibition of AURKB with ZM and inhibition of AURKA with MLN have different effects on translation (Figure S4C-D), the data indicates different effects (not redundant) on CPEB1 for these kinases.

In Figure 1, some CPEB1 is still expressed in MII control oocytes while it fully disappears in oocytes collected at the same stage in Figure S1. It is therefore very difficult to conclude that Aurora B has an effect on CPEB1 stability.
Response: This difference was due to using different imaging systems, one of which was more sensitive. We therefore re-did the blot using the same detection system as in Figure 2 (the more sensitive system) and show more similar representative images (new Figure S2A-B).

In order to get a better vision on CPEB1 regulation, the expression levels as well as the phosphorylation of CPEB1 must be analysed by western blot in a time course way during meiotic maturation for the KO oocytes. Moreover, the use of pharmacological inhibitors of protein translation (cycloheximide) and proteasome may help to decipher the precise role of Aurora A and Aurora B on CPEB1.
Response: Although we formally did not conduct a time course expression experiment, collectively this information is present. That is, in B cKO oocytes, CPEB1 expression is evaluated at time points of meiosis: Prophase I (Figure 2C) MI (Figure 4A), and MII (Figure 2C). A cKO oocytes arrest at MI (Blengini et al 2021), precluding an MII evaluation (Figure 3). It is an interesting idea to use

cycloheximide for future studies, but an in-depth dissection of AURKA/B role on CPEB1 is outside of the scope of this manuscript.

7. The activity of CPEB1 is monitored by a luciferase assay using a reporter gene containing the 3' UTR of Aurora C. As mentioned above, the accumulation of endogenous proteins is missing…
Response: This documentation was previously published in Nguyen et al 2018. When we added new genes, as suggested by the reviewer, we documented their increase in B cKO oocytes by either IF or Western, depending on the antibody (Figure 2 J-O; Figure S3 B-K).
… and the functional consequences of Aurora A and/or Aurora B depletion are not investigated.
Response: The functional consequences of AURKA deletion was published previously (Blengini et al 2021) and the consequences of AURKB deletion was published previously (Nguyen et al. 2018) and is expanded upon in another manuscript that is under review (Blengini et al, pending review of revision at Aging Cell).

As the authors analysed CPEB1 activation using an exogenous RNA, any change in the timing of meiotic maturation could affect the expression level of the reporter gene. It will be therefore important to perform this experiment in a time course-way and to use a control reporter gene deleted for the CPEB1 binding site.
Response: As we shared above in point 2, the kinetics of B cKO oocytes undergoing meiotic maturation are similar to WT. Therefore, analysis of the peak time point (Met I) provides the most accurate picture of the difference in recruitment. A time course could show that recruitment starts earlier, but the information we would glean from this experiment would be incremental to the manuscript. We instead chose to focus our efforts (and mice) to essential control experiments where we mutated the CPEB1 binding site and show that recruitment of Aurkc and Ccnb1 driven luciferase is abolished in both WT and B cKO oocytes.

As mentioned before, western blot analysis of Aurora C to monitor its expression would strengthen the data.
Response: This documentation was previously published in Nguyen et al 2018. Unfortunately, despite many attempts over the years, we cannot detect total AURKC in oocytes by western blot.

Minor points:

-The sequence of the gene reporter used in the luciferase is missing in the material and methods
Response: We now provide the sequence of the luciferase reporters that we used in the assay in a supplemental file.

-I wonder why the overexpression of Aurora B has only been done in WT oocytes. Would it be possible to overexpress Aurora B in Aurora B KO oocytes to determine this restore both RNA translation and CPEB1 expression levels?

Response: Thank you for the great suggestion. We performed this rescue experiment where we overexpressed Aurkb in B cKO oocytes and found restoration of the translation levels to that of WT (new Figure 1D-E). In addition, we performed the same rescue experiment in A cKO oocytes and found that expression of Aurka restores translation levels to that of WT oocytes (new Figure 3 E-F). These results strengthen our interpretation of the data that AURKB and AURKA regulate translation in negative and positive ways, respectively.

Reviewer 2
1. …From the images shown in Figure 1, it appears that the background levels of oocyte autofluorescence might vary from experiment to experiment. It would have been nice if cycloheximide controls were used. Can the authors address this concern?
Response: According to this recommendation, we conducted control experiments with cycloheximide and show specificity for HPG; treatment lowers HPG levels and is now in revised Figure S1A-B. In Figure 1, the levels of HPG may appear to vary, but this is due to our imaging settings for each experiment. Specifically, in 1B, the laser was set to the level of HPG in the BKO so that the signal wasn't saturated and therefore the signal in WT is comparatively lower than in 1D where the laser was set to the signal in WT because the Aurkb injected oocyte signal was lower.

From the data shown by Rong et al (2019) in which this method was extensively utilized, it seems that timing may also be important. Do Aurora B knockout oocytes exhibit similar timing of meiotic maturation events as wild-type
controls?
Response: We conducted a cell cycle time course experiment as suggested and we show that there are no significant changes meiotic maturation timing (via time of polar body extrusion) between WT and B cKO oocytes. This is included in Figure S1.

2. A potentially confusing aspect of the presentation is that the authors use the inverse of CPEB1 levels as a surrogate for CPEB1 activity, the idea being that when CPEB1 is activated by phosphorylation, it turns over more rapidly. An improvement would be adding some direct measures of poly(A) length.

Response: We agree with the reviewer that a direct measure of poly(A) length would be more straight forward. However, this is not an assay that our team is set up to do, and given the Covid-restrictions still in place, we elected instead to broaden our analysis beyond Aurkc. Based on Reviewer 1's suggestion, we extended our analysis of other CPE-containing genes and find that they too are recruited to a higher level in BKO oocytes (Figure 2G-O). The consistent difference in recruitment that we observe in 5 CPE-containing genes strengthens our interpretation.

3. Figure 2, panels G and H, show a nice experiment in which an Aurora C 3'UTR reporter construct was utilized. An improvement in the experimental setup would be to add 3'UTR reporters that do not contain CPEB1 binding sites.
Response: We agree with the reviewer and now include a luciferase assay for Aurkc-UTR where we mutated the CPE-binding site as described before (Schindler et al., 2012). Unlike with the WT UTR, there was no enrichment in either in WT or B cKO oocytes with the mutant Aurkc-3'UTR. This assay was also performed in another UTR (Ccnb1) which is known to be translated in a CPEB1-mediated mechanism (Murai et al., 2010; Yang et al.,2017). We found the same pattern as Aurkc: the Ccnb1-UTR is recruited in WT and the recruitment was significantly higher in B cKO oocytes. The mutated Ccnb1-3'UTR prevented the enrichment in both WT and B cKO oocytes. These results confirm the AURKB-regulation of CPEB1-dependent proteins.

4. The legend to Figure 4 does not match what is shown in the figure (the text refers also to panels 4E-F. The authors should fix this.
Response: We greatly thank the reviewer for catching this mistake. We regrettably uploaded the wrong Figure 4 in the original submission and have corrected this mistake here.

5. It would be helpful if the manuscript had page numbers.
Response: We added page numbers to the revision.

Reviewer 3:
1. It seems that the figure 4 contains only repeated panels from figure 3.
Response: We greatly thank the reviewer for catching this mistake. We regrettably uploaded the wrong Figure 4 in the original submission and have corrected this mistake here.

2. Can the authors explain the difference between Figure S1, panel A, in which the CPEB1 protein is gone by 16 hours and Figure 2, panel C, where in ovulated eggs this protein is still expressed?
Response: We apologize for this confusing presentation. This difference was due to using different imaging systems, one of which was more sensitive. We therefore redid the blot in Figure S2 using the same detection system as in Figure 2 (the more sensitive one) and show more similar representative images (new Figure S2 A-B).

3. The figure legends of Figure 2 panel C and D, prophase I cells are not mentioned.
Response: We fixed these labels.

Second decision letter

MS ID#: DEVELOP/2021/199560

MS TITLE: Aurora kinase B negatively regulates Aurora kinase A to control maternal mRNA translation in mouse oocytes

AUTHORS: Mansour Aboelenain and Karen Schindler

I have now received the referees reports on the above manuscript, and have reached a decision. The overall evaluation is positive and Development remains interested in publishing this work, provided that Reviewer 1's comments are satisfactorily addressed. Reviewer 1 points out some important details and potential reorganization which would need to be addressed. I invite you to address Reviewer 1's points in a revised manuscript and detail them in your point-by-point response. In addition to the points raised by Reviewer 1, one important point that remains to be addressed is that the title states "Aurora kinase B negatively regulates Aurora Kinase A" which leads the reader to believe that a mechanism underlying this negative regulation will be determined in the study. I encourage you to reconsider this title and maybe change it to something like "Aurora Kinase B inhibits Aurora Kinase A" since that that would be in line with the genetic and pharmacologic analysis presented in the study without eluding to mechanisms.

Reviewer 1

Advance summary and potential significance to field

This work is important as it discovers a new regulatory mechanisms controlled by Aurora B which targets RNA translation through Aurora A/CPEB1 during the meiotic cell division in mouse oocytes.

Comments for the author

To the authors,
The authors did an extensive work to answer my comments and these new data greatly improve the quality. In particular, they include new control experiments and many results regarding the translation of other proteins than CPEB1 including Cyclin B1, PRC1 and Mos as sugegsted. However, I still have few comments that must be addressed before its final acceptance.

1) My major comment concerns mainly how the statistical analysis are illustrated in the manuscript. All the quantifications (HPG incorporation, protein expression level) should have been compared and/or normalized to prophase oocytes (using the same set ups) and not only on “WT” oocytes collected at the same stage of meiosis. An example can be found in a recent paper published in 2021 in NAR (https://doi.org/10.1093/nar/gkab431). This would allow to better visualize the stimulation of translation from one condition to another and as such, strengthen their conclusion that Aurora B upregulates this process during meiosis resumption.

2) Regarding the kinase Mos, I am very surprised that the authors are able to detect this protein in prophase arrested oocytes (Fig. S3J). From the literature, it is well known that this protein is not expressed in prophase oocytes, neither in Xenopus nor in mouse. Its expression level must be therefore compared to a positive control as it was done for the protein HEC1 and the Metaphase II arrested oocytes (Fig. S3F-I).

3) As deregulating protein translation in Aurora B KO mice has no impact on meiosis progression, some hypothesis on how the regulation of translation by Aurora B through Aurora A is connected to aneuploidy should be added in the final paragraph of the “results and discussion” part in order to reinforce the biological significance of the paper.

4) Given the major role of Aurora A played during meiosis in mouse oocyte (Results that are now published by the team), western blots monitoring Aurora A expression levels and phosphorylation in “B cKO” and “B cKO het A” would be nice controls to include in the supplementary figures. Also, I am wondering whether the meiotic progression of “B cKO het A”

oocytes is changed compared to “WT” or “A KO” oocytes, the latter ones being arrested in MI as published in Blengini 2021.

5) The text still needs to be proofread to improve its quality as errors in figure references are still present. For example, “message” should be replaced by “messagers” in the introduction, Fig. S2A-B does not correspond to the described figure in the results and discussion part (Line 150). In the method section and in the figure, the phosphorylation site of Cdc25 by Aurora A must be included. In figure legends, “alpha-tubulin” or “α-tubulin” should be homogenized between the main text and the supplementary information. Also, it would help readers to select either “Cyclin B1” or “Ccnb1” for Cyclin B1. In Figure 2, panel E: CPEB1 and DNA are missing. The condition “B cKO HetA” illustrated in Figures S1C and S1D regarding the incorporation of HPG is never described or referred in the text.

6) The organization of panels in Figures 2 and 4 is difficult to follow. It might be better to put the quantification panels next to the ones that have been quantified and not below.

Reviewer 3

Advance summary and potential significance to field

This manuscript describes a new role of Aurora B kinases in regulation of translation during mammalian meiosis. Since the regulated translation is essentially important for completion of meiosis, this manuscript brings important information.

Comments for the author

In this version authors addressed all comments raised in my previous review and therefore I recommend to accept this manuscript for publication in Development journal.

Second revision

Author response to reviewers' comments

Enclosed is the revision of our manuscript in the style of a short Research Report entitled “Aurora kinase B inhibits Aurora kinase A to control maternal mRNA translation in mouse oocytes” for consideration for publication in Development. We are grateful for the reviewer’s expert insight and have made all modifications possible.
Below, reviewer will find a point-by-point response to their queries and suggestions. We have copied their points and italicize them; our responses are below their points.

1. My major comment concerns mainly how the statistical analysis are illustrated in the manuscript. All the quantifications (HPG incorporation, protein expression level) should have been compared and/or normalized to prophase oocytes (using the same set ups) and not only on “WT” oocytes collected at the same stage of meiosis. An example can be found in a recent paper published in 2021 in NAR (https://doi.org/10.1093/nar/gkab431). This would allow to better visualize the stimulation of translation from one condition to another and as such, strengthen their conclusion that Aurora B upregulates this process during meiosis resumption.

We acknowledge that comparing prophase to metaphase would be visually appealing to the reader. However, in the last review, the reviewer asked us to demonstrate that prophase I KO oocytes were similar to WT. In doing so, except for the Luciferase assays, we only conducted experiments on prophase I oocytes and did not repeat the metaphase experiments. Therefore, because the images were obtained at different settings and at different times, we cannot combine these experiments to express the data as fold change from prophase I.

The point of the suggested control was to demonstrate that the starting point (prophase I) was not different and that the difference arises later at metaphase. Our separate experiments show this important point. If we were to repeat each experiment again, with 3 replicates, we would need at least 6 mice per genotype per figure panel (for at least 7 different experiments). Because the result and interpretation would not change, we believe that this would be wasteful of our precious animals and we elect to keep the data presentation as is.

2. Regarding the kinase Mos, I am very surprised that the authors are able to detect this protein in prophase arrested oocytes (Fig. S3J). From the literature, it is well known that this protein is not expressed in prophase oocytes, neither in Xenopus nor in mouse. Its expression level must be therefore compared to a positive control as it was done for the protein HEC1 and the Metaphase II arrested oocytes (Fig. S3F-I).
This result is because we imaged the prophase I oocytes separate from metaphase oocytes in response to the reviewer's suggestion to include prophase I as a baseline. If they were imaged together, the laser power would have been set for levels in metaphase, and therefore no signal would have been observed in prophase I. To make this more obvious and consistent with the literature, we moved the Mos result from Figure 2 into the supplemental figure (S3J-N) next to the prophase I result.

3. As deregulating protein translation in Aurora B KO mice has no impact on meiosis progression, some hypothesis on how the regulation of translation by Aurora B through Aurora A is connected to aneuploidy should be added in the final paragraph of the "results and discussion" part in order to reinforce the biological significance of the paper.
We now include a line stating: Abnormal translation could affect the expression levels of important meiotic proteins resulting in aneuploid eggs.

4. Given the major role of Aurora A played during meiosis in mouse oocyte (Results that are now published by the team), western blots monitoring Aurora A expression levels and phosphorylation in "B cKO" and "B cKO het A" would be nice controls to include in the supplementary figures. Also, I am wondering whether the meiotic progression of "B cKO het A" oocytes is changed compared to "WT" or "A KO" oocytes, the latter ones being arrested in MI as published in Blengini 2021.

To assess Aurora A or its phosphorylation level, we need many mice per genotype and we do not currently have an abundance of B cKO het A animals in the colony. To make best use of our animals, we instead looked at Aurora A activity by staining single oocytes with the antibody to detect an Aurora A substrate- phosphorylated CDC25B. We also monitored meiotic progression kinetics of these oocytes live before their fixation, staining and imaging. These data are included in revised supplemental figures S1E and S4A-B. We find that meiotic progression was normal and pCDC25B levels were reduced to WT levels.

5. The text still needs to be proofread to improve its quality as errors in figure references are still present.

We have correct these.
a. For example, "message" should be replaced by "messagers" in the introduction.
We respectfully disagree with this suggestion. "Messagers" is not an English word. Messengers would not be appropriates in this context either unless we changed the phrase to messenger RNAs.
b. Fig. S2A-B does not correspond to the described figure in the results and discussion part (Line 150).
In this context reference to Figure S2A-B refers back to why we chose Met I for this experiment and is therefore correct as is.
c. In the method section and in the figure, the phosphorylation site of Cdc25 by Aurora A must be included.
This is now included.
d. In figure legends, "alpha-tubulin" or "α-tubulin" should be homogenized between the main text and the supplementary information.
This is now standardized.
e. Also, it would help readers to select either "Cyclin B1" or "Ccnb1" for Cyclin B1.
This is now standardized to Ccnb1.

f. In Figure 2, panel E: CPEB1 and DNA are missing.
This is now corrected
g. The condition “B cKO HetA” illustrated in Figures S1C and S1D regarding the incorporation of HPG is never described or referred in the text.
We now reference these figures on page 10.

6. The organization of panels in Figures 2 and 4 is difficult to follow. It might be better to put the quantification panels next to the ones that have been quantified and not below.
We have edited these figures as recommended.

Third decision letter

MS ID#: DEVELOP/2021/199560

MS TITLE: Aurora kinase B inhibits Aurora kinase A to control maternal mRNA translation in mouse oocytes

AUTHORS: Mansour Aboelenain and Karen Schindler
ARTICLE TYPE: Research Report

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 work is important because it highlights new regulatory mechanisms controlled by the Aurora B kinase, which targets the translation of mRNAs that are critical for meiotic divisions in oocyte.

Comments for the author

In this new version of the manucrit, the authors have addressed all my previous comments. I therefore recommend its publication in the journal "Development".