Meiosis requires a translational positive loop where CPEB1 ensues its replacement by CPEB4

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Review timeline:

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 15 March 2010

We have now eventually received two of the solicited three sets of reports on your recent submission, EMBOJ-2010-73890. Given the substantial delay in its evaluation and the fact that both these referees are more or less in agreement regarding their overall assessment, I have chosen to contact you with an editorial decision at this point, in order to avoid any unnecessary further delays. As you will see, the two reviewers both consider your study interesting and potentially suited for The EMBO Journal, but also raise a number of substantive points that would need to be clarified and/or experimentally addressed before publication may be warranted. These concerns pertain both to experimental issues as well as to interpretation and conclusiveness of the data. Should you be able to adequately address these various points, we should be happy to consider a revised manuscript further for publication. Please be however reminded that it is EMBO Journal policy to allow a single round of major revision only, and that it will thus be important to diligently answer to all the various experimental and editorial points raised at this stage. When preparing your letter of response, please also bear in mind that this will form part of the Peer Review Process File, and will therefore be available online to the community in the case of publication (for more details on our Transparent Editorial Process initiative, please visit our website: http://www.nature.com/emboj/about/process.html). In any case, please do not hesitate to get back to us should you need feedback on any issue regarding your revision.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal
REFEREE REPORTS:

Referee #1 (Remarks to the Author):

Sequential cytoplasmic polyadenylation of key mRNAs is important to guide Xenopus oocytes through the different maturation steps. A key player is CPEB1, which drives the first two waves of polyadenylation. It remains open, instead, how the third wave of polyadenylation - and with it the second meiosis - is regulated. In this manuscript, the authors show that the CPEB1 homologue, CPEB4, is necessary for the MI-MII transition. Its mRNA is activated by CPEB1-mediated polyadenylation during the first meiotic phase, thus creating a second phase of CPE mediated polyadenylation activity that comes up when CPEB1 is degraded.

The story is not yet complete (what makes cyclin E1 mRNA resistant to CPEB1-mediated polyadenylation, but accessible for CPEB4? What kinases regulate CPEB4?), but I think it is an important step ahead. I would therefore recommend publication in the EMBO Journal, if the authors can address the points below, especially point 7.

1) Fig. 1B is cited as showing that CPEB4 mRNA is polyadenylated in MI and partially deadenylated in MII. This is more what the reporter assay in 1C shows. The endogenous mRNA, instead, is well polyadenylated in MI (presumably the signal is very weak) and completely deadenylated in MII. Reporters are convenient tools, but I think it is important to show - in a better panel - that the endogenous mRNA behaves likewise since the endogenous the authors detected does not show a smear but quite a sharp band.

2) It perplexes me to see that CPEB4 protein really accumulates only late in MII - when polyadenylation and hence translation of the mRNA is already greatly reduced. Can the authors discuss this delay and apparent conflicting results?

3) The authors should definitively show the length of the poly(A) tail.

4) Fig. 2B, right panel. Stimulations are much more informative when calculated as an x-fold increase over the same mRNA before stimulation. The authors should graph the data in this way and see if the outcome is the same.

5) At the end page 9, it is stated that "MPF was activated normally". Shown where? The authors have to show that or eliminate the statement.

6) Fig 5B, The authors show that expressing a not degradable CPEB1 during MII does not alleviate CPEB4 absence, but aggravate it. Is this an effect due to apoptosis?

7) Fig 5D shows how cyclin E1 mRNA is not polyadenylated in the absence of CPEB4. But from the compacted panel, polyadenylation cannot be judged that well. This selective polyadenylation by CPEB4 is crucial for the story - why didn't the authors use a gene-specific primer that is much closer to the poly(A) tail, as in the other panels? This needs to be confirmed with a different primer.

8) Fig 6: the authors state that they observe a morphological rescue by CPEB1-CAD mutant after KO CPEB4. To me the morphology of WT and rescue looks quite different. How can state that the sharp focus in the rescued oocyte is really the polar body?

9) Page 13 concludes that CPEB4 is required for deadenylation of "early" and "late" mRNAs in general. But in fact, they show only one example for the two classes, ccnB1. There is no example of a "late" mRNA. Option one is to show a "late" mRNA, option two is to tune down this statement and make it more direct towards ccnB1 mRNA regulation.

10) Page14, lane 7, the authors hypothesize that CPEB1 assembles inhibitory complexes after MI. It should be noted that CPEB1 is degraded at that point. How can they reconcile these discrepancies with the published literature.

Referee #2 (Remarks to the Author):

The sequential translational activation of maternal mRNAs is essential for progression of meiotic cycles in animal oocytes. This activation mainly depends on cytoplasmic polyadenylation of maternal mRNAs, but it remains unclear how the polyadenylation occurs sequentially. In the present study, the authors demonstrated a new translational circuit, in which CPEB1 activates the synthesis of CPEB4 that, in turn, compensates for the inactivation and degradation of CPEB1 due to
differential regulation by phase-specific kinases. Although this is a very interesting and beautiful scenario, I think several issues indicated below should be clarified.

1. The authors argue that CPEB4 is required for three stages, i.e., exit from meiosis I (establishment of meta-I, meta-I/ana-I transition, the first polar body emission), progression through interkinesis period (suppression of S-phase), and meiosis II (entry into meiosis II and meta-II arrest). However, protein levels of CPEB4 until the end of interkinesis period are almost the same as those in prophase I-arrested oocytes (Fig. 1A), excluding possible roles of CPEB4 in the first two stages. Although the authors intended to justify this (p.10-line 8~), the explanation looks like somewhat biased. Could show more convincing data. In relation to the above, why was CPEB4 almost undetectable until interkinesis period, even though its mRNA was already fully polyadenylated at GVBD (Fig. 1C, upper left)? Meiotic cycle progression in Fig. 2A appears to be unusual (for example, compare with Fig. 1A).

2. Even if CPEB4 would actually be required for the exit from meiosis I, it is not easy to imagine what proteins should be translated via CPEB4 for normal chromosome alignment in the meta-I plate, chromosome segregation at meta-I/ana-I transition and the first polar body formation (Fig. 3B). Could comment on possible targets of CPEB4.

3. Compensation by CPEB1-CAD for CPEB4 depletion was shown only by chromosome behavior in exit from meiosis I (Fig. 6B). To confirm the compensation in the third "late-late" stage, could show polyadenylation status of cyclin E1 and particularly Emi2, as like in Fig. 5D.

1st Revision - authors' response 19 April 2010

EMBOJ-2010-73890

Referee #1:

We thank the reviewer for his/her comments on our work and for his/her valuable constructive suggestions to strengthen the experiments supporting the conclusions presented in our manuscript and to clarify some conceptual issues. Accordingly, we have performed the required experiments, shown in five new panels, and modified the text following his/her suggestions.

1) Fig. 1B "...it is important to show - in a better panel - that the endogenous mRNA behaves likewise ..."

We have now repeated the measurements of the polyadenylation status of CPEB4 mRNA (New Figure 1C), to show that the endogenous mRNA is polyadenylated in MI and (only) partially deadenylated in MII, thus behaving like the reporters.

2) "It perplexes me to see that CPEB4 protein really accumulates only late in MII - when polyadenylation and hence translation of the mRNA is already greatly reduced. Can the authors discuss this delay and apparent conflicting results?"

Although CPEB4 accumulates during the whole length of meiotic resumption, to reach its maximal levels at MII, CPEB4 protein starts to be synthesized in response to the stimulation by progesterone and an increase of CPEB4 levels is already appreciable in MI (GVBD), as shown in new figure 1B. Thus, consistently with the polyadenylation of the mRNA, CPEB4 starts to be synthesized in the PI-MI transition and continues to accumulate until MII, even if at later times the mRNA is partially deadenylated. Although the late deadenylation shortens the poly(A) from 200 residues to around 80 residues, this mRNA should still be translated and therefore, even if at different rates, translational activation caused by progesterone stimulation continues for the whole meiotic cycle resulting in gradual accumulation of CPEB4.
3) "The authors should definitively show the length of the poly(A) tail.

We have modified the figures accordingly.

4) "Fig. 2B, right panel. Stimulations are much more informative when calculated as an x-fold increase over the same mRNA before stimulation. The authors should graph the data in this way and see if the outcome is the same."

We have added a new panel to New figure 2B graphing the data as fold increase over the same mRNA before stimulation. Given that the overall stimulation is a factor of two independent events (De-repression and translation stimulation by polyadenylation) we also show repression and stimulation referred to the standard cyclin B1 3' UTR. The fold increase, shown in the new panel, is an addition of the de-repression and the stimulation, shown in the original panels, and therefore the conclusions derived are the same.

5) "At the end page 9, it is stated that "MPF was activated normally. Shown where? The authors have to show that or eliminate the statement."

MPF activity is shown (as H1 kinase activity) in the lower panels of figure 3B. We have now better clarified this point in the text.

6) "Fig 5B, The authors show that expressing a not degradable CPEB1 during MII does not alleviate CPEB4 absence, but aggravate it. Is this an effect due to apoptosis?"

The phenotypes shown in figure 5B (expressing a not degradable CPEB1 in the absence of CPEB4) precede the morphological changes characteristic of apoptosis. Thus, most likely the effect of overexpressing an inactive non-degradable CPEB1 in chromosome dynamics is cause rather than consequence of apoptosis. We interpret the aggravation of these meiotic defects as originated from the fact that CPEB4 depletion prevents the third wave of cytoplasmic polyadenylation whereas substitution by not degradable CPEB1 and not-constitutively active CPEB1, in addition to the polyadenylation defects caused by the absence of CPEB4, causes the deadenylation during interkinesis of the previously (PI and MI) polyadenylated mRNAs (Fig 5D).

7) "Fig 5D shows how cyclin E1 mRNA is not polyadenylated in the absence of CPEB4. But from the compacted panel, polyadenylation cannot be judged that well. This selective polyadenylation by CPEB4 is crucial for the story - why didn't the authors use a gene-specific primer that is much closer to the poly(A) tail, as in the other panels? This needs to be confirmed with a different primer."

Following the suggestion by the reviewer, we have repeated this experiment, Shown in new Figure 5D. Now, the lack of cyclin E mRNA polyadenylation is more clearly shown.

8) "Fig 6: the authors state that they observe a morphological rescue by CPEB1-CAD mutant after KO CPEB4. To me the morphology of WT and rescue looks quite different. How can state that the sharp focus in the rescued oocyte is really the polar body?"

The focus of the polar body changes according to whether the extruded polar body and the metaphasic plate are in the same (and different) planes, therefore the polar body sometimes looks more focused (when they are, by chance, in the same plane) and other times more diffuse (when they are in different planes, more frequent). We have included an additional picture (New figure 6B, with the polar body and the metaphasic plate in different planes).

9) "Page 13 concludes that CPEB4 is required for deadenylation of "early" and "late" mRNAs in general. But in fact, they show only one example for the two classes, ccnB1. There is no example of a "late" mRNA. Option one is to show a "late" mRNA, option two is to tune down this statement and make it more direct towards ccnB1 mRNA regulation."

We have modified the text accordingly, substituting the previous statement by the following one:

"Surprisingly, substitution of CPEB4 by CPEB1 resulted in a shortened poly(A) tail for cyclin B1
Because this is an mRNA polyadenylated by CPEB1 in the PI-MI transition and this polyadenylation was not affected by overexpressing CPEB1-CA (Figure 5C) in the presence of CPEB4, this result indicates that replacement of CPEB1 by CPEB4 after anaphase I is not only required to sustain the polyadenylation of the "late-late" mRNAs, but also to prevent the deadenylation of the previously CPEB1-polyadenylated mRNAs. Therefore, degradation of CPEB1 and new synthesis of CPEB4 in late meiosis seems to be required to prevent deadenylation during interkinesis of the mRNAs polyadenylated by CPEB1 during PI-MI, while maintaining the oocyte capability to generate the third wave of "late-late" polyadenylation. Considering that both CPEB1 and CPEB4 share multiple "early" and "late" mRNA targets (Figure 4B), the interkinesis deadenylation inhibition should not be limited to cyclin B1 mRNA, but, most likely, would be a general effect to all CPEB1/CPEB4 regulated mRNAs.

Therefore, we hypothesized that, if CPEB1 would remain present after MI, it may result inactivated during interkinesis and reassemble the repression/deadenylation complexes. Thus, overexpression of non-degradable CPEB1, rather than being able to rescue for the lack of CPEB4, will cause the deadenylation of CPE-containing mRNAs polyadenylated in PI and early-MI. This hypothesis would explain not only the lack of rescue, but also the observed deadenylation of cyclin B1 mRNA in oocytes depleted of CPEB4 and injected with the CPEB1 stable mutant.

This observation is fully consistent with both previously published works on CPEB1 and our work on CPEB4.

We have modified the manuscript accordingly.

Referee #2:

We thank the reviewer for his/her comments on our work and for his/her valuable constructive suggestions to strengthen the experiments supporting the conclusions presented in our manuscript. Accordingly, we have performed the required experiments, shown in four new panels and incorporated the requested clarifications in the text.

1. "The authors argue that CPEB4 is required for three stages, i.e., exit from meiosis I (establishment of meta-I, meta-I/ana-I transition, the first polar body emission), progression through interkinesis period (suppression of S-phase), and meiosis II (entry into meiosis II and meta-II arrest). However, protein levels of CPEB4 until the end of interkinesis period are almost the same as those in prophase I-arrested oocytes (Fig. 1A), excluding possible roles of CPEB4 in the first two stages. Although the authors intended to justify this (p.10-line 8-), the explanation looks like somewhat biased. Could show more convincing data.

In relation to the above, why was CPEB4 almost undetectable until interkinesis period, even though its mRNA was already fully polyadenylated at GVBD (Fig. 1C, upper left)?"

We have now included a new western (with a larger number of oocytes) that shows clearly the increased levels of CPEB4 from stage VI arrested oocytes to MI-oocytes. Although CPEB4 accumulates during the whole length of meiotic resumption, to reach its maximal levels at MII, CPEB4 protein starts to be synthesized in response to the stimulation by progesterone and an increase of CPEB4 levels is already appreciable in MI (GVBD), as shown in new figure 1B. Thus, consistently with the polyadenylation of the mRNA, CPEB4 starts to be synthesized in the PI-MI transition and continues to accumulate until MII, even if at later times the mRNA is partially deadenylated. Although the late deadenylation shortens the poly(A) from 200 residues to around 80 residues, this mRNA should still be translated and therefore, even if at different rates, translational activation caused by progesterone stimulation continues for the whole meiotic cycle resulting in gradual accumulation of CPEB4.

This expression of CPEB4 in MI is consistent with the reported phenotype for CPEB4 depletion.
"Meiotic cycle progression in Fig. 2A appears to be unusual (for example, compare with Fig. 1A)."

The maturation kinetics for oocytes extracted from unprimed frogs is quite variable between frogs and a range of 3 to 5 hours, after progesterone-addition, to reach 80-100% GVBD is within normal maturation times.

2. "Even if CPEB4 would actually be required for the exit from meiosis I, it is not easy to imagine what proteins should be translated via CPEB4 for normal chromosome alignment in the meta-I plate, chromosome segregation at meta-I/ana-I transition and the first polar body formation (Fig. 3B). Could comment on possible targets of CPEB4."

Our results indicate that the targets of CPEB4 and CPEB1 are the same (one CPEB would be gradually replaced by the other starting at MI), therefore the described CPE-regulated mRNAs whose translation is required for MI-MII transition and chromosome segregation would be possible candidates (XKid, TPX2, Cyclin E, emi2, Cyclin B4 etc. Eliscovich et al 2008, Belloc and Mendez 2008, Hochegger et al 2001). It is noteworthy that the phenotype generated by depleting CPEB4 is very similar to the one caused by XKid mRNA depletion (Perez et al. 2002).

This comment has been included in the discussion. To provide experimental support for this hypothesis, we have now included XKid and cyclin B4 mRNAs as targets for CPEB4 in the IP-qRTPCR experiment (New figure 4B).

3. "Compensation by CPEB1-CAD for CPEB4 depletion was shown only by chromosome behavior in exit from meiosis I (Fig. 6B). To confirm the compensation in the third "late-late" stage, could show polyadenylation status of cyclin E1 and particularly Emi2, as like in Fig. 5D."

The rescue of cyclin E1 and Emi2 polyadenylation by overexpressing CPEB1-CAD to compensate for CPEB4 depletion is shown in New Figure 6C. This experiment confirms the rescue phenotype observed at the chromosomal behavior level (Fig 6B).

Acceptance letter 10 May 2010

Thank you for submitting your revised manuscript for our consideration. It has now been seen once more by one of the original referees (see comments below), and I am happy to inform you that there are no further objections towards publication in The EMBO Journal.

You shall receive a formal letter of acceptance shortly.

Yours sincerely,

Editor
The EMBO Journal

Referee 1 (comments to authors):

The authors have addressed all the major points raised by the two reviewers. I think this work is an important contribution to the understanding of the cytoplasmic polyadenylation mechanism occurring during the meiotic phases.