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Importance of polyadenylation to the selective elimination of meiotic mRNAs in growing *S. pombe* cells

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(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 23 February 2010

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has now been seen by three referees whose comments are shown below. As you will see from their comments the referees provide mixed recommendations regarding publication in the EMBO Journal.

While they find the role of the polyadenylation machinery in degradation of DSR-containing transcripts via the exosome to be potentially interesting, they disagree regarding the level of mechanistic insight required for publication. Given the interest in the study we would like to invite you to submit a revised version of the manuscript, but we would like you to provide some further insight into how polyadenylation targets the transcripts to the exosome, as outlined in point 2 raised by referee #3. I believe that his would significantly strengthen and improve study and it would make a good contribution to the EMBO Journal. Should you be able to address these issues, we would be wiling to consider a revised manuscript.

I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. When you submit a revised version to the EMBO Journal, please make sure you upload a letter of response to the referees' comments. Please note that when preparing your letter of response to the referees' comments that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website: http://www.nature.com/emboj/about/process.html

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

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1 (Remarks to the Author):

This is a very interesting paper in which Yamananka and colleagues analyze the effect of polyadenylation on the degradation of DSR-containing meiotic transcripts. Degradation of these RNAs during mitotic growth depends on a factor that recognizes the DSR, Mmi1. The Authors isolated Rna15p and Pab2p, involved in 3'-end processing, as Mmi1p interacting partners in a two-hybrid screen. They also identified the gene encoding the poly(A) polymerase (pla1) in a screen for suppressors of a mutant defective for degradation of these meiotic transcripts. In follow up experiments they show that mutation of these factors stabilizes the meiotic transcripts during vegetative growth in a DSR-dependent manner, suggesting that polyadenylation is, for these transcripts, a degradation mark. Consistently, they show that these transcripts are hyperadenylated when stabilized by degradation (exosome) mutants. Moreover, addition of a poly(A) tail encoded in the DNA promotes DSR-dependent degradation in transcripts that are 3'-processed by a different pathway. These observations unveil a novel function of the polyadenylation machinery in the degradation of specific transcripts that is distinct from the pathway depending on degradative polyadenylation by the TRAMP complex.
The evidence presented is convincing and the results fully support the conclusions. The novelty of this work and the quality of the data largely justify publication in EMBO Journal. I have only a few, minor comments:
- Fig. 3E: the T50 construct behaves differently from the other constructs, the Authors should comment on this.
- Same figure: it is unclear if the best way to quantify the poly(A) destabilization effect is to compare DSR to DSR-M10 constructs. This is because stabilization of the latter constructs might be an independent event occurring after the nuclear event that destabilizes the DSR constructs. I suggest that the authors show both the absolute quantification of each series of constructs (i.e. the DSR and DSR(M10) series alone) and the ratio DSR/DSR(M10) (already shown in fig 3F).
- In figs. 2A/C the transcripts stabilized in the Mmi1 mutant have a shorter poly(A) tail than transcripts stabilized in the rrp6 strain. This suggests that the function of Mmi1p is required for hyperadenylation of these RNAs, which might even be the major role of the protein in selective degradation. The authors should describe this important result and discuss its possible implications.
- Have the Authors verified by some other technique (e.g. co-IP or GST-pull down) the physical interactions of Mmi1p with Rna15p, Pab1p or poly(A) polymerase ?

Referee #2 (Remarks to the Author):

The authors report that the efficiency of degradation of meiotic transcripts in mitotic cells is reduced in strains with defects in polyadenylation factors, Pab2 or the exosome. These and other data support a model for poly(A) and Pab2 mediated recruitment of the exosome to DSR-containing RNAs.

The work is technically good, the conclusions drawn are sound and the MS well written, other than some minor problems with English usage. However, the actual links involved are unclear. As the authors note, the mechanism does not appear to resemble TRAMP-mediated degradation or the role of oligo(A) addition in Bacteria. The localization data is interesting but, again, it is unclear what conclusions should be drawn.

Overall, I regret to write that while this is good work, without clearer mechanistic data it would probably be better suited to a more specialized journal.

Referee #3 (Remarks to the Author):

Echoing the role of polyadenylation in bacteria, earlier work in fission yeast and other eukaryotes has identified a role for polyadenylation in the turnover of short-lived nuclear RNAs. While earlier studies have highlighted the role of the non-canonical poly(A) polymerase activity of the TRAMP complex in nuclear RNA turnover, this manuscript provides clear evidence that the canonical nuclear poly(A) polymerase can also contribute to such processes. In both cases nuclear polyadenylation appears necessary for the efficient targeting of RNA substrates to the exosome. The data are of a high overall quality and the manuscript is generally well written. In my view the authors should be encouraged to address the following points before publication:

1 The data presented suggest a model in which somehow abnormal polyadenylation of meiotic transcripts (for example in a pla1-ts37 strain) leads to their accumulation in vegetatively growing cells (Fig. 1A). The meiotic mRNAs in pla1-ts37 cells are, however, no shorter (as judged by northern blot) than those in mmi1-ts3 cells, where polyadenylation is presumably not impaired. If anything, the mei4 mRNAs in the pla1-ts37 strain look slightly longer, and the partial suppression of the meiotic defect of the sme2 deletion further suggests that they are translationally competent. It would appear that something other than a simple lack of polyadenylation leads to meiotic mRNA accumulation under these circumstances. Given that polyadenylation is generally considered to be important for nuclear export of mRNAs, it would be informative to determine whether the mRNAs that accumulate cytoplasmic or nuclear, or at least to measure poly(A) tail lengths of meiotic mRNAs in pla1-ts37 and mmi1-ts3.

2 The experiments involving the use of reporter RNAs with poly(A) or poly(U) tracts of defined lengths (Fig. 3) are very elegant, but these tracts are internal, being followed by the snu2 terminator sequence, rather than terminal, as would be the case with the poly(A) tail of authentic meiotic mRNAs. Assuming the snu2 terminator leaves a significant number of nucleotides beyond the tract (and this should be stated in the text), the observed influence of the poly(A) tract seems unlikely to involve the generation of a 'preferred' 3' end for the exosome, as seems to be the case in the TRAMP-exosome pathway. In light of this, the authors could usefully include a diagram of their preferred model to explain the observed data.

3 Methodology: on p4 the authors should clarify whether the yeast two-hybrid screening was genome-wide, or directed at a subset of genes. In the Materials & Methods section, the description of the construction of ts alleles seems unnecessarily brief, and quite puzzling; without positive selection for transformants, how were they identified?

4 Minor points: on p4 "RNA15, which is a subunit of CF1A composing the polyadenylation complex" and "Consequently" should be re-worded to avoid potential confusion. On p6 "in the absence active" should read "in the absence of active".

1st Revision - authors' response 21 April 2010

We greatly appreciate the constructive comments raised by the referees and the thoughtful decision by the editor. We have treated each comment of the referees as described below. In addition, the manuscript has been thoroughly reviewed by a native English speaker, and now harbors a number of minor changes in wording from the original. However, no significant change has been made in the arrangement of the text.

Response to the referees:

Referee #1

1. Fig. 3E: the T50 construct behaves differently from the other constructs, the Authors should comment on this.

The T50 construct was apparently more stable than the T10 and T100 constructs, whether it harbored functional DSR or not, but the reason for this is unclear. We have described this in the text, as in the following: the T50 constructs exhibited abundance nearly twice as much as the others for reasons unknown (p.9, l.18-19)

2. Same figure: it is unclear if the best way to quantify the poly(A) destabilization effect is to compare DSR to DSR-M10 constructs. This is because stabilization of the latter constructs might be an independent event occurring after the nuclear event that destabilizes the DSR constructs. I suggest that the authors show both the absolute quantification of each series of constructs (i.e. the DSR and DSR(M10) series alone) and the ratio DSR/DSR(M10) (already shown in fig 3F).

We now show the absolute quantification of each series of constructs underneath the Northern blot panel in Figure 3E. The ratio DSR/DSR(M10) remains to be shown in Figure 3F.

3. In figs. 2A/C the transcripts stabilized in the Mmi1 mutant have a shorter poly(A) tail than transcripts stabilized in the rrp6 strain. This suggests that the function of Mmi1p is required for hyperadenylation of these RNAs, which might even be the major role of the protein in selective degradation. The authors should describe this important result and discuss its possible implications.

We appreciate this comment very much. The inference of this referee indeed points a likely function of Mmi1p. It is now even strengthened because we have seen that the hyperadenylation of meiosis-specific mRNAs does not occur in the mmi1 rrp6 double mutant. We have presented this observation as Figure 2D in the revised manuscript and given relevant explanations in the text (p.7, l.18 – l.4 from the bottom; p.14, l.10-12) .

4. Have the Authors verified by some other technique (e.g. co-IP or GST-pull down) the physical interactions of Mmi1p with Rna15p, Pab1p or poly(A) polymerase ?

Yes, we have. Both IP and pull-down assays were done. This is mentioned in the text (p.5, l.7-8), and the data are given in the supplemental Figure S3. (Original Figure S3 has been re-numbered S4.)

Referee #2

Overall, I regret to write that while this is good work, without clearer mechanistic data it would probably be better suited to a more specialized journal.

Although this referee gave no specific comments, we hope that his/her concern has been alleviated by the improvement of the manuscript in response to the comments of Referees #1 and #3.

Referee #3

1 The data presented suggest a model in which somehow abnormal polyadenylation of meiotic transcripts (for example in a pla1-ts37 strain) leads to their accumulation in vegetatively growing cells (Fig. 1A). The meiotic mRNAs in pla1-ts37 cells are, however, no shorter (as judged by northern blot) than those in mmi1-ts3 cells, where polyadenylation is presumably not impaired. If anything, the mei4 mRNAs in the pla1-ts37 strain look slightly longer, and the partial suppression of the meiotic defect of the sme2 deletion further suggests that they are translationally competent. It would appear that something other than a simple lack of polyadenylation leads to meiotic mRNA accumulation under these circumstances. Given that polyadenylation is generally considered to be important for nuclear export of mRNAs, it would be informative to determine whether the mRNAs that accumulate cytoplasmic or nuclear, or at least to measure poly(A) tail lengths of meiotic mRNAs in pla1-ts37 and mmi1-ts3.

This referee is right in pointing that the way in which Pla1 is involved in the selective elimination is not simple, as it plays a role in the production of both functional mRNAs and mRNAs targeted to destruction. However, it is evident at least that transcripts produced in pla1-ts37cells at the restrictive temperature (as shown in Figure 1A) have a shorter poly(A) tail: To address this referee's

comment, we measured poly(A) tail lengths of mei4 mRNA in pla1-ts37 and mmi1-ts3 cells. A fraction of mei4 transcripts in pla1-ts37 was indeed longer than the control. However, we found that this was not because it underwent hyperpolyadenylation but because it gained a longer 3' UTR stretch, apparently due to a failure in cleavage at the proper polyadenylation site. We have stated this observation in the text as data not shown (p.7, l.3 from the bottom – p.8, l.3).

2 The experiments involving the use of reporter RNAs with poly(A) or poly(U) tracts of defined lengths (Fig. 3) are very elegant, but these tracts are internal, being followed by the snu2 terminator sequence, rather than terminal, as would be the case with the poly(A) tail of authentic meiotic mRNAs. Assuming the snu2 terminator leaves a significant number of nucleotides beyond the tract (and this should be stated in the text), the observed influence of the poly(A) tract seems unlikely to involve the generation of a 'preferred' 3' end for the exosome, as seems to be the case in the TRAMP-exosome pathway. In light of this, the authors could usefully include a diagram of their preferred model to explain the observed data.

We have stated that the transcripts produced in these experiments do not carry a poly(A) tail at their very 3' end (p.9, l.6-7).
According to this referee's comment and the suggestion by the editor, we present a possible scheme for the activation of selective elimination as Figure 6 in the revised manuscript. The scheme consists of three steps: Step1, stimulation of polyadenylation by Mmi1p; Step 2, recruitment of Pab2p by the poly(A) tail and Mmi1p; and Step 3, recruitment of exosome by Pab2p and Mmi1p. Detailed explanations of the model are given in the text (p.14, l.6-14) and the figure legend.

3 Methodology: on p4 the authors should clarify whether the yeast two-hybrid screening was genome-wide, or directed at a subset of genes. In the Materials & Methods section, the description of the construction of ts alleles seems unnecessarily brief, and quite puzzling; without positive selection for transformants, how were they identified?

The yeast two-hybrid screening was genome-wide. This has been indicated in the text (p.4, l.13).
We have described how we constructed ts mutants used in this study in the Materials and methods section (p.15, l.16 – p.16, l.4)

4 Minor points: on p4 "RNA15, which is a subunit of CF1A composing the polyadenylation complex" and "Consequently" should be re-worded to avoid potential confusion. On p6 "in the absence active" should read "in the absence of active".

The first sentence has been replaced by the following: RNA15, which is a subunit of the multi-subunit cleavage factor CF1A, a component of the polyadenylation complex (p.4, l.16-17).
"Consequently" has been replaced by "In this screen" (p.5, l.2).
The missing "of" has been supplied (p.7, l.2)

2nd Editorial Decision 30 April 2010

Your revised manuscript has been reviewed once more by two of the original referees. I am happy to inform you that both find that you have satisfactorily addressed the issues raised and recommend publication of the manuscript in the EMBO Journal. I enclose their comments below. Your study has been accepted and you will receive the official acceptance letter in the next couple of days.

Sincerely

Editor
EMBO Journal

REFEREE COMMENTS

Referee #1
The authors have answered appropriately to the (minor) concerns raised.

Referee #3
The authors have satisfactorily addressed each of the points raised in my review of the earlier version, with the result that the manuscript is now, in my opinion, ready for publication. The observation that polyadenylation by the canonical nuclear poly(A) polymerase can, in some circumstances, be involved in targeting RNAs for degradation by the exosome is likely to be of considerable general interest, even in the absence of detailed delineation of the pathway.

