EMBO reports

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R-loops cause replication impairment and genome instability during meiosis

Maikel Castellano-Pozo, Tatiana Garcia-Muse and Andres Aguilera

Corresponding author: Andres Aguilera, University of Sevilla

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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 10 April 2012

Thank you for the submission of your manuscript to EMBO reports. We have now received the enclosed reports on it. Referee 1 has taken part in a structured referee report trial, which is why this report is in a different format.

As you will see, while the referees agree that the study is potentially interesting, both referees 1 and 2 point out that it needs to be shown that RNase H rescues yeast meiosis and DNA replication in order to support the hypothesis that the observed meiotic defects are due to R-loop formation. Given that RNase H is expressed from a GAL gene promoter, a different rescue strategy may need to be followed. Referees 2 and 3 further indicate that it needs to be shown whether C. elegans thoc-2 mutants enter meiosis at all. Referees 1 and 2 also point out that significant more details on the methods and figures need to be provided.

Given these evaluations, I would like to invite you to revise your manuscript, with the understanding that the referee concerns must be fully addressed and their suggestions (as detailed above and in their reports) taken on board. Acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports 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.

I look forward to seeing a revised version of your manuscript when it is ready.

Yours sincerely,

Editor
EMBO Reports

REFEREE REPORTS:

Referee #1:

1. Do the contents of this manuscript report a single key finding? YES

2. Is the main message supported by compelling experimental evidence? YES , mostly

The overall scientific conclusions appear sound, and very intriguing. There needs to be some improvement of the quality of data, and some discussion of differences between systems.

1) Particularly, it would be important to know whether the yeast meiosis is rescued by RNAse H. The data they present simply shows that the checkpoint is not activated. One would expect that RNAse H should allow the cells to improve meiotic progression.
2) Do yeast cells have increased Rad51 foci consistent with their conclusion (rad51 and H2AX are not the same thing).
3) Legends need more detail and methods need to be evident to the non-specialist. E.g., define the arrows in Fig 1B, both above the diagram and on the PFGE gel.; explain how the DSBs were quantified.
Fig 3B: germline staining with Rad51. Are the data in the charts summed across all 10 animals? How much variation is there per animal?
Fig 4A and B: how many animals were observed in total for the graphs?

And there appears to be less defect in replication in the yeast meiosis while quite a serious one in the worm. This deserves some conversation!

3. Have similar findings been reported elsewhere (e.g. on a closely related protein; in another organism or context)? NO

4. Is the main finding of general interest to molecular biologists? YES

Please justify:
The role of R-loops in generating damage has only recently become known; extending that to meiosis is therefore extremely interesting. Two systems a particular plus

5. After appropriate revision, would a resubmitted manuscript be most suited for publication:

[a] in EMBO reports

6. Please add any further comments you consider relevant:

Authors need to fix some English (several problems noticed on P 6 in particular) Fig 2: actin, not actine

Referee #2:

R loops, caused by transcription-linked RNA/DNA hybrids, have been shown to be a source of DNA damage and genome instability during the mitotic cell cycle, especially in mutants lacking protein complexes that process primary transcripts and escort them away from the site of transcription. The paper under consideration examines the meiotic phenotypes of budding yeast and nematode mutants defective in this process. Mutants lacking THO complex components show meiotic defects, in particular delayed DNA replication and signs of unscheduled DNA damage. Expression or introduction of RNAse H partially suppresses some of these phenotypes, suggesting that the defects may be due to the presence of R loops.

The paper, in its current form, clearly shows that loss of the THO complex can cause problems during meiosis. However, the demonstration that these defects are due to R loops requires that they be suppressed by RNase H. In the yeast studies, unfortunately, RNase H is expressed from a GAL gene promoter, and requires addition of galactose to sporulation medium. It is likely that this addition of galactose (the amount of galactose added is never mentioned) causes cells to exit meiosis, since meiosis is suppressed by fermentable carbon sources. Thus, it is not surprising that only suppression of Rad53 phosphorylation is clearly documented, and it appears that RNase H expression (if it is being expressed--expression of RNase H is never documented) does not suppress the DNA replication defect to any significant extent. Furthermore, suppression of the other hpr1 meiotic defects, such as spore inviability and changes in DSB patterns, are not documented at all. Therefore, the statement in the discussion, that replication impairment and DNA damage accumulation in yeast hpr1 mutants are suppressed by RNase H expression, cannot be considered to have been documented. Similarly, in the nematode studies, it is not at all clear whether the thoc-2 mutants cause a delay in meiotic replication or simply abolish it, perhaps because of earlier defects in germline cell proliferation, since only a very small fraction of thoc-2 meiotic cells appear to be incorporating Cy3-dUTP. Furthermore, the restoration of Cy3-dUTP incorporation upon RNase H co-injection is limited, indeed--only 1/5 of cells show any signs of Cy3-dUPT incorporation, as opposed to 60% in wild-type.

Thus, while the current paper clearly shows that loss of the THO complex causes meiotic defects in both yeast and worms, it has not really shown that these defects are caused by R-loops. One could imagine, for example, that defects in meiotic gene expression could be causing the progression defects that are seen on both yeast and worms.

Other comments:

1. There are several places where non-standard English are used; some are mentioned below, but the use of a good copy-editor is recommended.

2. p3 "a number of mutations that cause genome INSTABILITY linked to R-loop formation"

3. p6 "Finally, to PROVE THAT R-loops were responsible..."

4. p6 "are responsible FOR pre-meiotic replication..."

5. p9 "repair functions to ENSURE genome integrity..."

6. Fig2 ACTIN

7. Fig4A--the use of a log scale for the Y axis makes it difficult to compare wild type and thoc-2 mutants. While some detail will be lost, a linear scale would be preferable.

8. FigS1B--it looks as if RNase H expression makes absolutely no difference to DNA replication in the hpr1 mutant. In fact, the - and + panels look to be identical. Perhaps the authors duplicated the same panel by mistake? Certainly the current data do not show a suppression of the replication defect.

9. FigS2, S3--some parts of these figures cannot be clearly read, should be enlarged.

Referee #3:

In this manuscript by Castellano-Pozo et al., the authors investigate connections between RNA transcription and R-loop formation, and meiosis progression. The authors show that R-loop formation affect premeiotic DNA replication and induce DNA damage and sterility both in yeast and in the metazoan model C. elegans. They show that S. cerevisiae hpr1Δ mutant shows a decrease in sporulation proficiency and spore viability. The timing of DSBs appearance is not altered, but resolution appears delayed. hpr1Δ cells show spo-11 independent Rad53 phosphorylation suggesting genome instability and activation of the DNA damage checkpoint. Finally RNase H overexpression abolishes Rad53 phosphorylation in hpr1Δ cells. In C. elegans similar findings were obtained: thoc-2 mutants are sterile, spo-11 independent RAD-51 foci appear early and persist in the gonad, a net increase in germ-line apoptosis is observed, premeiotic replication is impaired, however most defects are abolished by injection of RNase H in the gonad.
The experiments seem well done, the manuscript is well written, and the results are very interesting and novel. I believe this manuscript merits publication in EMBO Report, provided the authors can address my concerns listed below.

I wonder whether C. elegans oocytes precursors enter and proceed in meiotic prophase at all, since no diakinesis chromosomes are observed. I tried to look at nuclei shape along the thoc-2 mutant gonad in figure 3 however resolution was poor and magnification too low to visualize the actual shape of the chromatin, I strongly suggest to show representative images of the nuclei at the relevant stages at a higher magnification instead that the entire gonads. Furthermore, it would be very informative to add an immunostaining with an antibody against a structural component of the synaptonemal complex, such as syp-1, in order to understand whether or not nuclei progress along meiotic prophase.

Below, I also add few minor suggestions that might help improve the understanding of the paper.

Introduction:
Pag. 3 "Interestingly they found a number of mutations that cause genome linked to R-loop formation", not clear, please re-phrase.
Pag.4 "chromosomes align and synapse, double-strand breaks (DSBs) are generated by SPO-11 and recombination ..." please change the order as follow: "double-strand breaks (DSBs) are generated by SPO-11, chromosomes align and synapse, and recombination...". There are no evidences that synapses occur before DSBs in C. elegans!!!

Results:
Pag. 5 the sentence "The timing of meiotic DSB generation was not altered in hpr1Δ cells but its processing was delayed" is redundant and can be omitted.
Pag. 6 "Finally, to proof whether R-loops were responsible..." change in "Finally, to investigate whether R-loops were responsible..."
Pag. 7 "indicating that thoc-2 is essential for C. elegans viability." change in "indicating that thoc-2 is essential for C. elegans fertility."

Fig.3 Please make changes as suggested above taking also into account that a relative region of the gonad can be described as pachytene only if the shape of the chromosome shows indeed that they are properly paired and aligned. Otherwise just refer as zone 1,2,3, etc.

1st Revision - authors' response 02 July 2012

Referee #1:

1. Do the contents of this manuscript report a single key finding? YES

2. Is the main message supported by compelling experimental evidence? YES, mostly

The overall scientific conclusions appear sound, and very intriguing. There needs to be some improvement of the quality of data, and some discussion of differences between systems.

Thank you for the constructive comments

1) Particularly, it would be important to know whether the yeast meiosis is rescued by RNAse H. The data they present simply shows that the checkpoint is not activated. One would expect that RNAse H should allow the cells to improve meiotic progression.

As suggested, we extended the analysis with new experiments made with RNase H1 overexpression. We analyzed MI and MII progression, sporulation frequency and spore viability. The new data are shown in new Fig. 3C and Table S2. RNase H1 has a toxic effect in spore viability, which is more dramatic in worms limiting our capacity to make conclusions. However, the results are consistent with a partial suppression of the phenotype. Results have been included in the text in page 7, which now reads:

"Lastly, we analyzed the kinetics of meiotic nuclear divisions (MI and MII) with and without RNase H1 overexpression. In wild-type cells MI and MII show a similar profile under both conditions (98% of cells have completed the two divisions after 10 hr) (Fig. 3C, S2C and Table S2). MI and MII nuclear divisions in *hpr1Δ* cells only showed a slight improvement upon overexpression of RNase H1, with 79.7% of cells completing meiosis after 25 hr in sporulation media versus 74.5% without RNase H1, whereas this value was 98.5% in WT cells. Therefore, RNase H1 overexpression rescues the defect in meiosis progression only slightly in *hpr1Δ* cells. Nevertheless, this rescue may be significant because, as shown in Table S2, RNase H1 overexpression reduces spore viability even in the WT."

2) Do yeast cells have increased Rad51 foci consistent with their conclusion (rad51 and H2AX are not the same thing).

We determined Rad52 foci and the results are consistent with our conclusions. The new data are included in new Fig. 3B and results are discussed in page 6, where it now reads:

"To correlate Rad53 phosphorylation with DSB formation we monitored DSB levels by Rad52 foci accumulation during meiosis (Fig 3B). The levels of Rad52 foci were clearly higher in meiotic *hpr1Δ* cells respect to the wild type, and were reduced to WT levels upon RNase H1 overexpression"

As the referee suggested we first tried Rad51 foci, but the epitope generates a non-functional protein as previously reported (Lisby et al., 2004). For this reason we decided to measure Rad52.

3) Legends need more detail and methods need to be evident to the non-specialist. E.g., define the arrows in Fig 1B, both above the diagram and on the PFGE gel.; explain how the DSBs were quantified.

As suggested, we included more details in the legends, and extended material and methods in the main manuscript (Page 12) and in Supplementary Information.

Fig 3B: germline staining with Rad51. Are the data in the charts summed across all 10 animals?

We count 10 nuclei per region of 10 worms per experiment, and we always perform a minimal of three independent experiments. Therefore data are the average of 30 worms.

How much variation is there per animal?

There is not much variation per animal as shown in the Fig. R1 (attached to this rebuttal), which shows the average and SD of nuclei per animal.

Fig 4A and B: how many animals were observed in total for the graphs?

We included this data in the now Figure 5 legend. In Fig.5A we used 30 worms, in 5B 15 worms for each strain.

And there appears to be less defect in replication in the yeast meiosis while quite a serious one in the worm. This deserves some conversation!

We believe that the FACS analysis is less sensitive than the Cy3-dUTP incorporation technique. For this reason, we observe a partial suppression of the meiotic replication defect in the worm that cannot be detected to the same levels in yeast. This has been discussed in the text in page 6, which now reads:

"Furthermore FACS analysis showed that *hpr1Δ* cells pass though S phase slightly faster under RNase H1 overexpression (Fig. S1B and S2B)."

Authors need to fix some English (several problems noticed on P 6 in particular) Fig 2: actin, not actine
Changed as requested.

Referee #2:

The paper, in its current form, clearly shows that loss of the THO complex can cause problems during meiosis. However, the demonstration that these defects are due to R loops requires that they be suppressed by RNase H. In the yeast studies, unfortunately, RNase H is expressed from a GAL gene promoter, and requires addition of galactose to sporulation medium. It is likely that this addition of galactose (the amount of galactose added is never mentioned) causes cells to exit meiosis, since meiosis is suppressed by fermentable carbon sources. Thus, it is not surprising that only suppression of Rad53 phosphorylation is clearly documented, and it appears that RNase H expression (if it is being expressed--expression of RNase H is never documented) does not suppress the DNA replication defect to any significant extent. Furthermore, suppression of the other hpr1 meiotic defects, such as spore inviability and changes in DSB patterns, are not documented at all. Therefore, the statement in the discussion, that replication impairment and DNA damage accumulation in yeast hpr1 mutants are suppressed by RNase H expression, cannot be considered to have been documented.

As indicated to referee #1, we extended the analysis with RNase H1 overexpression. We analyzed MI and MII progression and sporulation frequency and spore viability. The new data are shown in new Fig. 3C and Table S2. RNase H1 has a toxic effect in spore viability, which is more dramatic in worms, limiting our capacity to make conclusions. However, the results are consistent with a partial suppression of the phenotype. Results have been included in the text in page 7, which now reads: "Lastly, we analyzed the kinetics of meiotic nuclear divisions (MI and MII) with and without RNase H1 overexpression. In wild-type cells MI and MII show a similar profile under both conditions (98% of cells have completed the two divisions after 10 h) (Fig. 3C, S2C and Table S2). MI and MII nuclear divisions in *hpr1Δ* cells only showed a slight improvement upon overexpression of RNase H1, with 79.7% of cells completing meiosis after 25 hr in sporulation media versus 74.5% without RNase H1, whereas this value was 98.5% in WT cells. Therefore, RNase H1 overexpression rescues the defect in meiosis progression only slightly in *hpr1Δ* cells. Nevertheless, this rescue may be significant because, as shown in Table S2, RNase H1 overexpression reduces spore viability even in the WT."

We also took into account the referee's concerns about the carbon source and repeated the overexpression of RNase H1 experiments using a TET promoter (see Fig. S2). As can be observed the new data recapitulate our previous results. Results are discussed in page 6, which now reads:

"but to avoid carbon source issues we also confirmed the results using RNase H1 under the *tet* promoter (Fig. S2)."

As suggested, to further validate our previous analysis of RNase H1 overexpression under the GAL promoter, we examined WT meiosis progression in the presence of 0.5% galactose. The sporulation frequency and spore viability are summarized in Table S2. We believe that the conditions used do not interfere with meiosis progression. Results are discussed in page 6. Now it reads:

"We used the *GAL1* promoter to overexpress RNase H1 (Fig. 3). As shown in Table S2 galactose addition to the sporulation media not interfere with meiosis progression"

Similarly, in the nematode studies, it is not at all clear whether the thoc-2 mutants cause a delay in meiotic replication or simply abolish it, perhaps because of earlier defects in germline cell proliferation, since only a very small fraction of thoc-2 meiotic cells appear to be incorporating Cy3-dUTP. Furthermore, the restoration of Cy3-dUTP incorporation upon RNase H co-injection is limited, indeed--only 1/5 of cells show any signs of Cy3-dUPT incorporation, as opposed to 60% in wild-type.

Thank you for raising this interesting point. We have included Fig S4A and Fig S5 to support that *thoc-2* nuclei enter meiosis as they show synaptonemal complex protein SYP-1 assembly. Results are discussed in page 7. Now it reads: "Immunostaining with antibody against the SYP-1 core SC component (MacQueen et al, 2002), revealed that the SC assembled in meiotic prophase nuclei (Fig. S4A)."

Concerning the second point about Cy3-dUTP incorporation, we agree with the referee, the suppression is only partial as we mentioned in the manuscript, and probably this could be due to

technical limitations or that RNase H is toxic as shown by the reduced lifespan of microinjected worms, also observed in yeast (Table S2).

Thus, while the current paper clearly shows that loss of the THO complex causes meiotic defects in both yeast and worms, it has not really shown that these defects are caused by R-loops. One could imagine, for example, that defects in meiotic gene expression could be causing the progression defects thatare seen on both yeast and worms.

Other comments:

1. There are several places where non-standard English are used; some are mentioned below, but the use of a good copy-editor is recommended.

2. p3 "a number of mutations that cause genome INSTABILITY linked to R-loop formation"
Changed as requested.

3. p6 "Finally, to PROVE THAT R-loops were responsible..."
Changed, new sentence reads:
"Finally, we took advantage of RNase H1 overexpression to specifically degrade DNA::RNA hybrids and partially suppress R-loop-dependent phenotypes as previously shown in mitotic yeast cells (Huertas & Aguilera, 2003) as a way to investigate whether R-loops were responsible for the observed DNA-damage checkpoint activation."

4. p6 "are responsible FOR pre-meiotic replication..."
Changed as requested.

5. p9 "repair functions to ENSURE genome integrity..."
Changed as requested.

6. Fig2 ACTIN
Changed as requested.

7. Fig4A--the use of a log scale for the Y axis makes it difficult to compare wild type and thoc-2 mutants. While some detail will be lost, a linear scale would be preferable.
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8. FigS1B--it looks as if RNase H expression makes absolutely no difference to DNA replication in the hpr1 mutant. In fact, the - and + panels look to be identical. Perhaps the authors duplicated the same panel by mistake? Certainly the current data do not show a suppression of the replication defect.

As suggested we have revised the data and included a new FACS analysis (Fig S1B), in which a slightly replication improvement after RNase H1 overexpression can be observed. This is also supported with the FACS from RNase overexpression using the *tet* promoter (Fig S3), which also shows a slightly replication improvement in the presence of RNase H1. Results are discussed in page 6. Now it reads:

"Furthermore, FACS analyses showed that *hpr1Δ* cells pass through S phase slightly faster under RNase H1 overexpression (Fig. S1B and S2B)."

9. FigS2, S3--some parts of these figures cannot be clearly read, should be enlarged.
We enlarged the Table in FigS2, and the gene scheme of FigS3, as suggested.

Referee #3:

…The experiments seem well done, the manuscript is well written, and the results are very interesting and novel. I believe this manuscript merits publication in EMBO Report, provided the authors can address my concerns listed below.

I wonder whether C. elegans oocytes precursors enter and proceed in meiotic prophase at all, since no diakinesis chromosomes are observed. I tried to look at nuclei shape along the thoc-2 mutant gonad in

figure 3 however resolution was poor and magnification too low to visualize the actual shape of the chromatin, I strongly suggest to show representative images of the nuclei at the relevant stages at a higher magnification instead that the entire gonads. Furthermore, it would be very informative to add an immunostaining with an antibody against a structural component of the synaptonemal complex, such as syp-1, in order to understand whether or not nuclei progress along meiotic prophase.

Thank you for the comment. As answered to referee #2 we have included Fig S4A and Fig S5 to support that *thoc-2* nuclei enter meiosis as they show synaptonemal complex protein SYP-1 assembly. Results are discussed in page 7. Now it reads:

"Immunostaining with antibody against the SYP-1 core SC component (MacQueen et al, 2002), revealed that the SC assembled in meiotic prophase nuclei (Fig. S4B)."

As suggested, in order to improve the data of Fig 3, we have included Fig S5 that shows in higher magnification nuclei from the different germline regions stained for both RAD-51 and SYP-1.

Below, I also add few minor suggestions that might help improve the understanding of the paper.

Introduction:
Pag. 3 "Interestingly they found a number of mutations that cause genome linked to R-loop formation", not clear, please re-phrase.
Changed to "Interestingly they found a number of mutations that cause genome instability linked to R-loop formation".

Pag.4 "chromosomes align and synapse, double-strand breaks (DSBs) are generated by SPO-11 and recombination ..." please change the order as follow: "double-strand breaks (DSBs) are generated by SPO-11, chromosomes align and synapse, and recombination...". There are no evidences that synapses occur before DSBs in C. elegans!!!
Changed as requested

Results:
Pag. 5 the sentence "The timing of meiotic DSB generation was not altered in hpr1Δ; cells but its processing was delayed" is redundant and can be omitted.
Removed as suggested

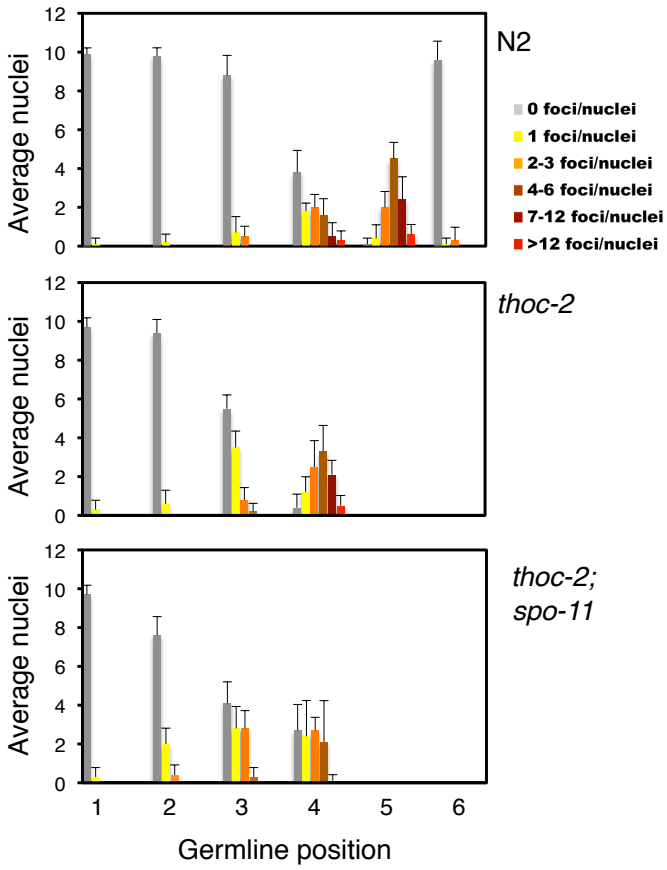
Pag. 6 "Finally, to proof whether R-loops were responsible..." change in "Finally, to investigate whether R-loops were responsible..."
Changed as requested

Pag. 7 "indicating that thoc-2 is essential for C. elegans viability." change in "indicating that thoc-2 is essential for C. elegans fertility."
Changed as requested

Fig.3 Please make changes as suggested above taking also into account that a relative region of the gonad can be described as pachytene only if the shape of the chromosome shows indeed that they are properly paired and aligned. Otherwise just refer as zone 1,2,3, etc.

This is a nice possibility. However, as Fig S5 shows a magnification of nuclei from the different germline regions stained for both SYP-1 and RAD-51, we believe that the regions can be identified and we would like to keep them as they are.

Fig. R1


Average nuclei
12
10
8
6
4
2
0
N2
0 foci/nuclei
1 foci/nuclei
2-3 foci/nuclei
4-6 foci/nuclei
7-12 foci/nuclei
>12 foci/nuclei
thoc-2
thoc-2;
spo-11
1
2
3
4
5
6
Germline position

2nd Editorial Decision 13 July 2012

Thank you for the submission of your revised manuscript. We have now received the enclosed reports from the referees. Referee 3 has only a minor suggestion for a subtitle change that I would like you to incorporate before we can proceed with the official acceptance of your manuscript. I also noticed that the explanation for the error bars is missing in the legends of figure 3B, C and figure S2. You can just upload the modified text file and the supplementary pdf file on the EMBO reports webpage as V3, and we will upload the remaining files for you.

I look forward to seeing a new revised version of your manuscript as soon as possible.

Yours sincerely

Editor
EMBO Reports

REFEREE REPORTS:

Referee #1:

The authors have improved the Manuscript and have answered my concerns. This is an interesting paper that will be of wide interest.

Referee #2:

Castellano-Pozo et al. have addressed a major concern with the previous version by showing that a Tet promoter-driven RNaseH also appears to suppress the meiotic S-phase damage phenotype of hpr1-del, and by showing that galactose (at the concentration used to induce pGAL-RNaseH expression) does not impair normal meiosis.

The current findings, with regards to RNaseH expression, can be summarized as follows:

1. Premeiotic S-phase DNA damage in yeast hpr1-del mutants can be suppressed by RNaseH expression, as judged by loss of Rad53 phosphorylation and a reduction in Rad52 foci.

2. Meiotic progression defects in yeast hpr1-del mutants are not well suppressed by RNaseH expression.

3. Reduced premeiotic DNA synthesis in worm thoc-2 mutants is partially restored by RNaseH injection. Suppression of other defects (spo-11-independent DNA damage, progression defects, apoptosis) is not addressed.

In summary, this paper documents multiple meiotic defects in hpr1/thoc-2 mutants, but only a subset of these, most directly related to DNA replication, are shown to be due to RNA-DNA hybrids. Therefore, it remains possible that the other meiotic defects are due to other mechanisms, such as altered mRNA processing/export leading to altered gene expression. Therefore, the novelty of the yeast portion of the paper is somewhat limited. However, because they have not been described before, the description and characterization of cognate C. elegans mutants is indeed novel, although the nature of the meiotic defects remains uncertain.

Referee #3:

In this manuscript by Castellano-Pozo et al., the authors investigate connections between RNA transcription and R-loop formation, and meiosis progression. The authors show that R-loop

formation affect premeiotic DNA replication and induce DNA damage and sterility both in yeast and in the metazoan model C. elegans. They show that S. cerevisiae hpr1 deletion mutant shows a decrease in sporulation proficiency and spore viability. The timing of DSBs appearance is not altered, but resolution appears delayed. hpr1Δ cells show spo-11 independent Rad53 phosphorylation suggesting genome instability and activation of the DNA damage checkpoint. Finally RNase H overexpression abolishes Rad53 phosphorylation in hpr1 deleted cells. In C. elegans similar findings were obtained: thoc-2 mutants are sterile, although synaptonemal complex seems to form properly spo-11 independent RAD-51 foci appear early and persist in the gonad, a net increase in germ-line apoptosis is observed, premeiotic replication is impaired, however some although not all the defects are abolished by injection of RNase H in the gonad.

I am fully satisfied with the additions, changes and improvement of the manuscript. If possible I would only change the title of the third paragraph (page 7) from "C. elegans THOC-2 is essential for viability" in "C. elegans THOC-2 is essential for fertility": viability in fact could not be assayed since, as the authors clearly explain, the first generation homozygous nematodes have a normal development due to maternal effect but they are completely sterile and therefore it is not possible to look at embryo viability in the second generation.

I strongly recommend a fast publication in EMBO Report

3rd Editorial Decision 16 July 2012

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports.

Thank you for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,

Editor
EMBO Reports