Translesion synthesis polymerases contribute to meiotic chromosome segregation and cohesin dynamics in *Schizosaccharomyces pombe*

Tara L. Mastro, Vishnu P. Tripathi and Susan L. Forsburg
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First decision letter

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MS TITLE: Translesion synthesis polymerases contribute to meiotic chromosome segregation and cohesin dynamics in *S. pombe*
AUTHORS: Tara L. Mastro and Susan Forsburg
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

Please 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. Please attend to all of the reviewers' comments. 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

Mastro et al report a thorough characterization of the requirement of trans-lesion synthesis in fission yeast meiosis. Their work is consistent with a number of other studies is other organisms

suggesting a role for TLS polymerases in meiosis. Furthermore, it extends previous observations by carefully describing the requirements for TLS in a series of specific meiotic functions. As such, it contributes to a growing body of work and provides an important foundation for future mechanistic studies to investigate this intriguing observation.

Comments for the author

One point that deserves to be discussed more explicitly is the surprising coincidence that pol eta is fused to Eco1 and TLS seems to affect Rec8 stability, a known function of Eco1. Given the rest of the genetic data presented, in particular the redundant nature of the TLS polymerases in supporting spore viability, I have no concern that TLS is not involved in meiosis. However, it does seem possible that the pol eta allele used, which deletes the c-terminal polymerase domain of Eco1, could contribute to the observed Rec8 phenotypes via modification of Eco1 function instead of, or in addition to, loss of pol eta function. Ideally, the question would be addressed experimentally, perhaps with a catalytically inactive, but otherwise intact Eco1-pol eta, or at least a characterization of Rec8 dynamics in the Eco1Δeta allele alone. However, I would be satisfied with a robust treatment of the caveat in the Discussion.

Although the paper is generally well-written, it is missing important details, and would be improved by the following revisions.

Strains should be referred to by strain numbers, in addition to partial genotype: ie rev1Δ (5259) or rev1 (5401).

A description of how new alleles were made, along with the sequences for the primers and a reference for the integration cassettes used, should be included

The designation YW, used with kan in the strains table, should be explained or referenced.

Minor points:

Figure 1C: The TBZ panel is not labeled.

Reviewer 2

Advance summary and potential significance to field

This paper by Mastro and Forsburg describes intriguing phenotypes in meiosis associated with deletion of all four translesion polymerases in fission yeast. Deletion of all four TLSPs results in poor spore viability. The authors methodically examine each stage of meiosis and show defects in chromosome segregation in meiosis II. This is an exciting finding, not a phenotype one would expect to see in translesion polymerase mutants and thus an important contribution to the field. In an attempt to identify the mechanism responsible they show that chromatin association of Rec8 is not normal; first there is a delay in the removal of Rec8 from chromosome arms during MI, then it is removed prematurely from the centromeric region in meiosis II. They suggest that this is associated with reduced phosphorylation and higher turnover.

Comments for the author

Major comments: Each of the single mutants shows a somewhat reduced spore viability, but the quadruple mutant is more severely affected. This suggests that the reason for the reduced spore viability is different in the individual mutants. Consistently, there is no sign of redundancy in the drug-sensitivity assays except for MMS. The authors put the reduced spore viability down to the chromosome-segregation defects, on the basis that nothing else seems to be affected apart from a small reduction in recombination in kpa1Δ. This might well be so, but it would be interesting to know which of the single mutants is/are responsible for the chromosome segregation defects and the premature release of Rec8 from centromeric chromatin. Are all the TLS proteins involved in chromosome segregation? On a similar note, I am a bit puzzled by the mis-segregated chromosome fragments in meiosis I. Are the authors suggesting that they are a result of the delayed removal of

Rec8 from chromosome arms during meiosis I, or are there additional defects impinging on meiosis I?
I wonder whether/to what extent the poor spore viability of the quadruple mutant would be rescued by mutations that only allow meiosis I but inhibit meiosis II.

There was no significant difference in the chromatin association of Rec8 at act1, but there was a delay in the removal of Rec8 from chromosome arms during MI observed by live-cell imaging. It would be interesting to hear the authors' view on these apparently contradicting observations.

I don't think that the qPCR results showing rec8 levels add anything. The experiment was done twice, yet error bars are shown and they are huge. If the authors wish to analyze transcript levels, the experiment should be repeated so that statistical analysis can be performed. Having said that, I think the protein levels would be more informative. However, the western showing Rec8 levels is not all that convincing especially the quantification. Why does it show quantification of one blot when the blot itself is "representative of three biological replicates"? Were these samples run on the same gel? On my screen the background for tubulin in the wt looks very different, as if a different panel had been pasted in or the brightness/contrast had been adjusted for that panel only. Since the Rec8 bands on the quadΔ blot are weaker it is difficult to say whether the phosphorylation is affected, since the shifted band is so much weaker even in the wt, it would not be visible in the mutant on these westerns, so one cannot conclude whether Rec8 phosphorylation is affected in the mutant.

Minor comments

There are some typoes and inaccuracies that should be corrected.

Fig1 Explain why rad3Δ is shown.

Fig 2 why "DAPI focus" in the title? Number of nuclei would be more accurate, would it not?

P2 line 24 "TSLP"
P3 line 44 "base dimmers"
P4 Line 71 "it is a gene fusion of two proteins"
P4 line 90 "In humans, pol h has enriched expression in the mouse testis"
P5 lines 102, 108 TSLP P5 line 112 reductions in viability compared to wild-type of 64% and 61% should be corrected to reductions TO 64% and 61%
P5 Line 114 modest reduction of 81% should read reduction TO 81%
Fig 1c label missing. 17 µg/ml of what? Guess TBZ...
P6 line 131 the statement that "the quadΔ shows a less severe phenotype when challenged with all these drugs with the exception of MMS. It appears that in some situations the lack of all four of the polymerases is less deleterious than the absence of one."
should be rephrased. This is only obvious for rev3Δ versus quadΔ in CPT and maybe rev1Δ and quadΔ in TBZ.
P11 line 283 I don't think it is correct to say that "in mitosis, the same trend is not observed…", when one compares sensitivity to various treatments. There is no obvious difference on the control plate, which is the only one providing information about phenotypes in the mitotic cycle - which is not even the point here.

First revision

Author response to reviewers' comments

We are grateful to the editor for patience in allowing us to revise this manuscript. We have added an additional author who helped with the new experiments.

We thank the reviewers for their helpful remarks. Reviewer 1 comments that our work 'is consistent with a number of other studies in other organisms suggesting a role for TLS polymerases in meiosis. Furthermore, it extends previous observations by carefully describing the requirements for TLS in a series of specific meiotic functions. As such, it contributes to a growing body of work and provides an important foundation for future mechanistic studies to investigate this intriguing observation.' Reviewer 2 also calls our work intriguing, saying 'This is an exciting finding, not a phenotype one would expect to see in translesion polymerase mutants and thus an important contribution to the field.' We are confident we have addressed their specific concerns.

Reviewer 1
1) One point that deserves to be discussed more explicitly is the surprising coincidence that pol eta is fused to Eco1 and TLS seems to affect Rec8 stability, a known function of Eco1. Given the rest of the genetic data presented, in particular the redundant nature of the TLS polymerases in supporting spore viability, I have no concern that TLS is not involved in meiosis. However, it does seem possible that the pol eta allele used, which deletes the c-terminal polymerase domain of Eco1, could contribute to the observed Rec8 phenotypes via modification of Eco1 function instead of, or in addition to, loss of pol eta function. Ideally, the question would be addressed experimentally, perhaps with a catalytically inactive, but otherwise intact Eco1-pol eta, or at least a characterization of Rec8 dynamics in the Eco1Δeta allele alone. However, I would be satisfied with a robust treatment of the caveat in the Discussion.
Response: We now addressed this in lines 315-327 in the discussion.

2) Strains should be referred to by strain numbers, in addition to partial genotype: ie rev1Δ (5259) or rev1 (5401). Response: These are provided in the figure legends

3) A description of how new alleles were made, along with the sequences for the primers and a reference for the integration cassettes used, should be included.
Response: No new alleles were created in this paper, but rather multiple mutant strains were created by genetic crosses. We have provided an explicit pathway for sources and constructs in a new Supplemental table 2.

4) The designation YW, used with kan in the strains table, should be explained or referenced.
Response: The YW designation indicates the Rec8-GFP allele origination from Y. Watanabe (PY204). This has now been clarified in Supplemental table 2.

Minor points:

Figure 1C: The TBZ panel is not labeled.
Response: Fixed

Reviewer 2
1) It would be interesting to know which of the single mutants is/are responsible for the chromosome segregation defects and the premature release of Rec8 from centromeric chromatin. Are all the TLS proteins involved in chromosome segregation? On a similar note, I am a bit puzzled by the mis-segregated chromosome fragments in meiosis I. Are the authors suggesting that they are a result of the delayed removal of Rec8 from chromosome arms during meiosis I, or are there additional defects impinging on meiosis I? I wonder whether/to what extent the poor spore viability of the quadruple mutant would be rescued by mutations that only allow meiosis I but inhibit meiosis II.
Response: These are very intriguing questions but beyond the scope of this initial study.

2) There was no significant difference in the chromatin association of Rec8 at act1, but there was a delay in the removal of Rec8 from chromosome arms during MI observed by live-cell imaging. It would be interesting to hear the authors' view on these apparently contradicting observations.
Response: Please see lines 257-268.

3) I don't think that the qPCR results showing rec8 levels add anything.
Response: We agree and have removed these data.

4) the western showing Rec8 levels is not all that convincing, especially the quantification

Response: We addressed this by repeating the western blots on new time courses, replacing Figure 4. Our results and new analysis reinforce our original finding of reduced phosphorylation in the quadΔ. Although there was not a statistically significant reduction in Rec8-GFP levels in our new analysis, there did appear to be a trend of the quadΔ having reduced Rec8-GFP 2 hours prior to MI. Please see amended presentation of the new results in lines 237-246.

5) Minor comments
Response: All the cited typos have been corrected as requested.

Fig1 Explain why rad3Δ is shown.
Response: Added this to figure legend (positive control for the drug plates).

Fig 2 why "DAPI focus" in the title? Number of nuclei would be more accurate, would it not?
Response: We have updated this to read "Number of Nuclei (DAPI Focus)" so that all information is present and more clear.

P6 line 131 the statement that "the quadΔ shows a less severe phenotype when challenged with all these drugs with the exception of MMS. It appears that in some situations the lack of all four of the polymerases is less deleterious than the absence of one." should be rephrased. This is only obvious for rev3Δ versus quadΔ in CPT and maybe rev1Δ and quadΔ in TBZ.
Response: We have reworded this to be specific regarding CPT and TBZ in comparison to some of the single mutants (lines 127-130).

P11 line 283 I don' t think it is correct to say that "in mitosis, the same trend is not observed…", when one compares sensitivity to various treatments. There is no obvious difference on the control plate, which is the only one providing information about phenotypes in the mitotic cycle - which is not even the point here.
Response: We have changed this to refer specifically to the mitotic viability shown in Fig 1B. It is simply to say that any loss in mitotic viability seen in single mutants is not additive in the quadΔ.

Second decision letter

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AUTHORS: Tara L. Mastro, Vishnu Tripathi, and Susan Forsburg
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

Comments for the author

The authors have satisfactorily addressed my concerns.

Reviewer 2

Advance summary and potential significance to field

This paper by Mastro and Forsburg describes intriguing phenotypes in meiosis associated with deletion of all four translesion polymerases in fission yeast. Deletion of all four TLSPs results in poor spore viability. The authors methodically examine each stage of meiosis and show defects in chromosome segregation in meiosis II. This is an exciting finding, not a phenotype one would expect to see in translesion polymerase mutants and thus an important contribution to the field. In an attempt to identify the mechanism responsible they show that chromatin association of Rec8 is not normal; first there is a delay in the removal of Rec8 from chromosome arms during MI, then it is removed prematurely from the centromeric region in meiosis II. They suggest that this is associated with reduced phosphorylation and higher turnover.

Comments for the author

The authors have addressed all my concerns and comments.