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The fission yeast MRN complex tethers dysfunctional telomeres for NHEJ repair

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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 25 October 2011

Thank you for submitting your manuscript for consideration by The EMBO Journal. We have now received the reports from three expert reviewers, which are copied below. As you will see, the referees generally acknowledge your findings of MRN-mediated telomere and plasmid end tethering for NHEJ in fission yeast as interesting and potentially important, and thus potentially suitable for publication in our journal. There are nevertheless a number of issues requiring clarification; among these, the most substantial concern is referee 1's criticism about the somewhat indirect evidence for the key role of Mre11-dependent tethering. I realize that this may not be a simple question to tackle, but any further data you may be able to gather to more directly support this model would clearly be helpful; minimally the extended mutant analysis suggested by referee 1 should be attempted to obtain further insight into this aspect.

In light of these opinions and recommendations, I would like to give you the opportunity to address the reviewers' comments by way of a revised manuscript. In this respect, please be reminded that it is our policy to allow a single round of major revision only, and that it will therefore be important to diligently and comprehensively answer to all the specific points raised at this stage in the process. Should you have any questions or concerns with regard to the referee requests (especially the main concern of referee 1), please do not hesitate to get back to me for further consultations.

We generally allow three months as standard revision time, and it is our policy that competing

manuscripts published during this period will have no negative impact on our final assessment of your revised study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

When preparing your letter of response to the referees' comments, please bear in mind 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, 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

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REFEREE REPORTS:

Referee #1 (Remarks to the Author):

Reis et al. show that Mre11 is required for NHEJ of unprotected telomeres in fission yeast. Interestingly, they find that the Mre11 nuclease activity is not required and, as shown previously, that Mre11 is not required for NHEJ of linear plasmids. Using a cleaver bi-molecular assay, they show that Mre11 is required for NHEJ between ends on different molecules. In addition, they show that mutations that affect the Mre11 dimerization domain disrupt the Mre11 NHEJ activity. From these results, they conclude that Mre11's major role in telomere fusion is to tether unprotected ends for NHEJ. The experiments are well designed and well executed. However, the conclusions are indirect and rely heavily on the inferred effect of the Mre11 dimerization-interface mutations. These mutations affect the dimerization of Mre11 monomers, but their effect on the dimerization within intact MRN complex is less clear (Williams, 2008). Moreover, they may well have other effects on MRN function. Although the authors conclusions are plausible and interesting, I think that to warrant publication in EMBO J, a more direct demonstration of telomere tethering is required.

At the very least, the authors should include a more complete panel of mutants in their bi-molecular ligation assay in Figure 5. One or both of the Ku mutations would be useful to test if the difference between the MRN mutants and the lig4 mutant is due to MRN-independent tethering or tethering-independent ligation. The rad32 nuclease mutations are critical controls to confirm that the Rad32 nuclease activity is not required for NHEJ, as predicted from the telomere experiments.

Minor points:

It would be useful to know how the rad3 tel1 strains were made and at approximately how many cell cycles post meiosis they were analyzed.

The PCR data cited on page 11 confirming NHEJ between distinct transformed molecules should be included.

The DNA damage assays described in the Methods do not appear to be used in any of the figures.

Referee #2 (Remarks to the Author):

The roles of the MRN complex and ATM/Tel1 in fusion of dysfunctional telomeres, and in NHEJ repair of DSBs in general, are matters of significant interest and uncertainty. Studies in budding yeast suggest that the MRX complex but not Tel1 are required for general NHEJ and for fusion of

dysfunctional telomeres. Mammals require both MRX and ATM for fusion of dysfunctional telomeres, but their roles in general NHEJ are less clear. For reasons unknown, Mre11 nuclease activity is also required for fusion of dysfunctional telomeres in mammals.
In this paper, the authors continue their work unraveling what happens to telomeres in the absence of the telomere-capping protein Taz1 in fission yeast. Their previous studies showed that telomeres are recognized as DSBs and trigger a DNA repair response in the absence of Taz1. During prolonged G1 periods, taz1 results in lethality generated by telomere fusion, via the NHEJ pathway. Based on findings in other organisms showing that the MRN/MRX complex is required for NHEJ-dependent telomere fusion in the absence of telomere protecting proteins, this paper investigates the role of MRN in the repair of taz1 dysfunctional telomeres. The main result described in their manuscript is that MRN (but not its nuclease activity) is indeed required for NHEJ repair. This result is surprising, as plasmid re-ligation assays have shown that MRN is dispensable for NHEJ in fission yeast. In order to explain the difference in the requirement for MRN during NHEJ at telomeres versus plasmid re-ligation, the authors redesigned the plasmid-based NHEJ assay. The new assay relies on the joining of DNA ends that are not closely linked together, and shows that MRN is indeed required for the ligation reaction. To further investigate how MRN contributes to NHEJ repair, the authors test whether the DNA damage signal transduction from MRN to Rad3 or Tel1 is required. Rad3, Tel1 or Crb2 appear not to be required for NHEJ repair in taz1 cells. Finally, the authors used different Mre11 mutants to try and dissect the function of MRN in NHEJ repair and find that dimerization mutants of Mre11 show reduced NHEJ at telomeres, as well as reduced NHEJ in their revised plasmid-assay. They therefore suggest that Mre11 is required to tether dysfunctional telomeres for NHEJ repair. This is the most novel and impressive result in the paper.
By establishing that the Mre11 dimerization is crucial for fusion of dysfunctional telomeres and efficient NHEJ repair of untethered DNA ends, this paper makes a significant contribution to the fields of telomere dynamics and DSB repair. The findings in this manuscript should be of general interest to the readership of EMBO Journal.
The quality and thoroughness of the experiments is excellent. With one possible exception the interpretation of the data seems to be quite fair. The exception involves the relative roles of classical NHEJ such as lig4 of Ku in comparison to MRN subunits. In the two-fragment ligation assay the MRN subunits are clearly important, but not as important as lig4. It is at least worth mentioning that some NHEJ is happening in the absence of MRN.
Only minor issues need to be addressed:
Page 4/6: Langerak et al., 2011 should be cited when mentioning that Ctp1 initiates 5'-end resection in fission yeast.
Page 6/7: Ctp1 is not expressed in G1-arrested cells (Limbo et al., 2007) and therefore would not be expected to be involved in NHEJ-dependent telomere fusion in G1-arrested cells.
Page 7: second to last line: "we observed a drastic reduction in rap1 chromosome-end fusions" should be: "we observed a drastic reduction in rap1 nbs1 chromosome-end fusions"/
Page 9: Crb2 and 53BP1 share some conserved domains but it may be a bit misleading to describe them as homologs. Maybe describe them as "structurally related".
Page 10: There is some uncertainty about the role of Mre11 nuclease activity in HR repair in fission yeast. Hartsuiker et al 2009 (Molecular Cell) reported that rad32 D65N (not D56N as typo'd in line 2, page 10) is "slightly" sensitive to IR, although their spot assays indicate that survival is decreased about 5-10 fold in the mutant. Porter-Goff and Rhind (2009) reported about the D65N and H125N mutants are about 10-fold more sensitive to IR in comparison to wild type. Williams et al (2008) reported that another nuclease deficient allele (H134S) is about 10-100-fold more sensitive to IR. From these data it appears that Mre11 nuclease activity is important for HR repair in fission yeast, as it is in mammalian cells.
Page 10: Has anyone shown that fission yeast Rad32-D65N lacks both endo- and exonuclease activities? Maybe this has been extrapolated from other studies.
Page 11: Limbo et al (2007) reported that Ctp1 is not required for plasmid NHEJ repair.
Page 11: Contrary to the statement in the text, the ctp1 mutant does seem to be significantly impaired in the two-fragment NHEJ assay (Figure 5B). Some comment on whether the difference are statistically significant should be added, along with a possible explanation.
Page 13: "Thus, we hypothesize that this function is critical for NHEJ repair in fission yeast." This is only true when the DNA ends are not in close proximity, as conventional plasmid re-ligation assays do not show a requirement for MRN.
Page 13: "MRN may unclench several events...". I don't know what this means.
Discussion: The recent evidence that MRN and Ctp1 remove Ku from DSBs is particularly interesting in light of these data (Langerak et al., 2011).

Referee #3 (Remarks to the Author):

In this manuscript, Reis and colleagues report a series of experiments starting with the observation that MRN is required for NHEJ at dysfunctional telomeres in fission yeast. The authors show that Ctp1, Dna2, Tel1, and, Rad3 are not required for NHEJ at dysfunctional telomeres. Interestingly, further analysis shows that the dimerization ability, but not nuclease ability of the Rad32, is required for NHEJ at dysfunctional telomeres. The authors also used a novel plasmid repair assay to show that MRN and dimerization ability of Rad32 are required for joining two DNA fragments in vivo by NHEJ. The authors conclude that MRN acts to tether unlinked DNA ends to support efficient NHEJ.

The experiments are straightforward and well described. The manuscript describes a logical series of experiments and will be appreciated by readers interested in telomeres and DNA repair.

Minor comments:

Page 7, line 9-10
The paper showing the role of Exo1 at DSB and telomere should be sited.

Page 9, line 10-13
If the lower intensity of the telomere signal in the fusion bands is due to shorter telomeres, the intensity of the original LMIC bands should be also low, because the telomere length of the chromosome ends should be short. It could be possible that Rad3 and Tel1 may be required for efficient NHEJ. The authors could state this possibility. Alternatively, a novel plasmid repair assay could be used to test if Rad3 and Tel1 are required for efficient NHEJ.

Page 21, line 9-11
Page and volume number are not shown
Mol Cell. 2001 Jul;8(1):137-47.

Page 21, line 16
Pages are wrong and volume number is not shown
Nature. 2009 Aug 13;460(7257):914-8

There are several similar mistakes in page 21 and 22.

1st Revision - authors' response 23 January 2012

Reviewer #1:
We thank Reviewer #1 for remarking on the importance of our findings, especially, highlighting the use of our bi-molecule assay that measures end joining of independent DNA molecules.
In an attempt to gather direct evidence for telomere tethering in our assays, as requested by Reviewer #1, we investigated microscopically whether telomere clustering (as proxy for telomere tethering) was disrupted in taz1 cells harboring mrn mutations. Unfortunately, the results were generally negative. Number of telomere foci per cell (marked using Pot1-mRFP) was similar despite cells were proficient or deficient in MRN function. Telomere clustering is a complex and still poorly understood phenomenon that relies on separate mechanisms (see S. Gasserís work in budding yeast as reference). Unlike newly generated DSBs, telomere tethering may encompass different mechanisms, thus making it difficult to study as other DNA ends.

We acknowledge Reviewer #1’s concern that our evidence for MRN role in tethering DNA free ends relied heavily on the "inferred effect of the Mre11 dimerization-interface mutations". Reviewer #1 rightly points out that these studies were performed on Rad32 monomers (Williams et al., 2008), thus the "effect on the dimerization within intact MRN complex is less clear".

As requested, to corroborate our findings, we have included a more complete panel of mutants in our bi-molecule ligation assay. Apart from lig4 , we have now included pku70 and pku80 in both single and bi-molecule NHEJ assays. Ku mutants re-enforced our conclusion that both assays rely on all NHEJ repair pathway. Specifically, the differences between lig4 and mrn mutants on the bi-molecule assay were similarly observed in pku70 and pku80 .

Reviewer #1's request for an extended analysis of Rad32MRE11 nuclease mutants revealed critical to our revised manuscript. Apart from the D65N allele, we now present data for motif II and motif III histidine mutants characterized in Williams et al., 2008. We further studied motif I rad32-D25A allele (budding yeast MRE11-D16A) and decided not to include it in our current study since this mutant was shown to behave as a loss-of-function mutant (Porter-Goff and Rhind, 2009). Analysis of H68S and H134S phosphoesterase mutants was at odds with D65N allele we had previously presented. Upon strain confirmation and repeating the results for reproducibility, we had a clear message - the nuclease domain was required for NHEJ both at telomeres and on our bi-molecule assay. This result is consistent with work on mouse telomeres showing that MRE11-H129N (homologous to H134) was defective in NHEJ at telomeres (Deng et al., 2009).
Our data showed we had a clear difference between two putative nuclease dead mutants (D65N and H134S) thought to be similar in studies of their homologues in S. cerevisiae (D56N and H125S respectively). Because in vitro studies of the nucleolytic activity of these mutations are lacking in fission yeast, we cannot corroborate a role for these sites in Rad32. Additionally, whereas the D65N allele is a clean gene replacement (Hartsuiker et al. 2009), both H68S and H134S are 13myc-tagged versions (Williams et al., 2008) that may interfere with functions not yet assessed in previous studies. Nevertheless, our simplest hypothesis implies that the nuclease activity is dispensable for NHEJ (inferred by the D65N disruption of the Mn2+ ion) even though nuclease domain integrity is required (data from motif II and III mutants). Given the uncovered interactions of Mre11 complex and DNA, we proposed in our manuscript that, rather than nuclease activity per se, DNA end coordination performed by the nuclease domain of MRN, is the important feature for the ensuing NHEJ repair reaction. This idea is strongly supported by our bi-molecule results showing that H68S and H134S fail to promote end-joining of independent DNA ends. In agreement, the dimerization-interface mutations were similarly required for NHEJ of free DNA ends. Advancing this hypothesis will lead to further directed studies and instigate a better knowledge of this multifaceted complex.

Minor points addressed:
- We have detailed how we have generated the rad3 tel1 strains in the results section.
- We have now included the PCR data, as requested, showing that we have NHEJ between different DNA molecules in our bi-molecule assay in Supplementary Fig. 3.
- We have now made clear in the manuscript that DNA sensitivity assays in Supplementary Fig. 4 served to show that none of the Rad32Mre11 point mutants used in our study behave as the null mutant.

Reviewer #2:

We were encouraged by Reviewer #2's comments and would like to thank the exciting remarks to our work. Indeed, we agree that the "most novel and impressive result" is our finding that MRN main function in NHEJ relates to its function in bringing together independent DNA ends. We have now extended this idea to include the function of the nuclease domain in NHEJ repair. In light of our new results covering three independent alleles in the nuclease domain, a new picture has emerged. In contrast to our previous finding showing that nuclease activity of Rad32Mre11 was dispensable (using the active site Mn2+ disruption allele D65N), both phosphoesterase motif II and motif III histide mutants (H68S and H134S) are partially required for NHEJ of dysfunctional telomeres and DNA free ends. Although this result opened a new line of research and further studies are needed, we propose that the nuclease domain per se, rather than its nuclease activity, is the required feature of Mre11 for NHEJ. This is strongly supported by data on our revised split molecule plasmid assay, showing that similar to NHEJ at telomeres, the phosphoesterase mutants are defective in joining free DNA ends and not for single molecule repair.

We believe this idea may help to explain the disparate results between mammalian cells and budding yeast. Mre11 role in cleaning DNA ends via its nuclease activity may be intimately related with its ability to tether and coordinate DNA ends for subsequent repair reactions such as NHEJ and SSA (as suggested by the work of Paull and Gellert, 1998). Our observation that the D65N allele serves as separation of function mutant between Mre11 role nucleolytic processing and tethering of free DNA ends may help to clarify this issue.

Minor points addressed:
Page 6/7: We have now mentioned the cell cycle regulation of Ctp1 via protein expression levels in Limbo et al., 2007 and its absence in G1 to be consistent with not being involved in telomere NHEJ.
Page 7: We have correct according to suggestion.
Page 9: Weíve substituted it by "structurally related" as suggested.
Page 10: Apart from D65N, we have now included H68S and H134S alleles. We have compared DNA sensitivities directly in Supplementary Figure 4 and we discuss nuclease mutants as an important feature of our manuscript. (We have also corrected D56N to D65N, thanks for spotting it.)
Page 10: Indeed, exonuclease activity of D65N and others have been primarily extrapolated from studies in S. cerevisiae and P. furiosus. As stated before, in vitro studies in fission yeast are vital to ascertain the nucleolytic function of Rad32, as differences may exist.
Page 11: We have now acknowledged Limbo et al., 2007 for Ctp1 being dispensable for NHEJ repair.
Page 11: In light of Reviewer 2ís remark, we re-made the ctp1 KO strain and repeated the plasmid repair experiments. For several reasons we noticed that, over time, this strain could accumulate suppressors.
Page 13: We have changed from "MRN may unclench several events..." to "MRN initiates several events… "
We have now incorporated Langerak et al. 2011 in our current version. This important paper was not published at the time we submitted our first manuscript.

Reviewer #3:

We are grateful to Reviewer #3 encouraging remarks. As requested, we have now cited Tomita et al. 2003 and modified our references according to EMBO Journal standards. We thank the Reviewerís corrections.
In regards to Reviewer #3's comment related to LMIC probing: "If the lower intensity of the telomere signal in the fusion bands is due to shorter telomeres, the intensity of the original LMIC bands should be also low, because the telomere length of the chromosome ends should be short." We would like to clarify that the sequences used for probing the L, M, I and C terminal restriction fragments of chromosomes I and II of fission yeast are several Kbps internal (see Baumann & Cech, 2000 as example). Thus, unlike telomeres in cells deficient for telomerase activity, L, M, I and C signal do not become less visible unless the whole chromosome fragment is lost.

2nd Editorial Decision 10 February 2012

Thank you for submitting your revised manuscript for our consideration. Two of the original referees have now assessed it once more (see comments below), and in addition, I have also carefully looked at your response as well as your new data and their interpretations. Unfortunately, our conclusion from these considerations is that our final decision cannot be a positive one in this case.

We appreciate all the efforts that have gone into this revision, and in agreement with the referees continue to find the presented model a potentially very attractive and interesting one. However, the overriding main concern is that the necessary extension, especially of the mutant analysis, has not lead to the required substantiation of the model, but rather provided findings that at face value confound the main conclusions on Mre11/Rad32 roles as tethering factor in NHEJ. While I realize

that your tentative explanations for the new observations may well be plausible, I feel that the absence of rigorous biochemical characterization of the various employed Rad32 mutants now becomes really problematic - to the point where I have to agree with referee 1 that it is not clear how much dimerization defects and/or nuclease defects contribute to the clearly documented phenotypes in telomere fusion and bi-molecular plasmid joining assays. Admittedly, definitively clarifying these issues will require substantial further efforts and complementary analyses, but I am afraid that for publication in The EMBO Journal, as opposed to a more genetics-oriented journal, we have to insist on decisive reconciliation of these uncertainties and discrepant observations at this stage. In light of our editorial policy to allow only a single round of major revision, I therefore see little choice but to return the manuscript to you at this point with the decision that we cannot offer publication in the journal in this case. I nevertheless hope that you will at least find our referees' comments helpful, and that this negative decision does not prevent you from considering The EMBO Journal for publication of other studies in the future.

Yours sincerely,

Editor
The EMBO Journal

Referee reports:

Referee #1 (Remarks to the Author):

My concern about the indirect nature of the support for the authors conclusions remains. The fact that the nuclease domain is required for a nuclease-independent function leads credence to the concern that the dimerization-domain mutations may have effects independent of dimerization. Furthermore, the fact that telomere clustering is not affected demonstrates, as the authors concede, that telomere tethering is likely to be a complicated affair. So, I stand by my earlier analysis that, while the model presented is a compelling one, the data supporting it is not sufficiently strong.

Referee #2 (Remarks to the Author):

The revised manuscript has addressed my concerns. The new data on the additional Rad32 nuclease alleles complicates matters, but overall I remain enthusiastic about the significance and general interest of the work.

There are typos on page 12, lines 5 and 22.

Table I needs to be updated to include the new nuclease mutants.

Additional correspondence (author) 08 October 2012

It was very nice to talk with you in the meeting and I really appreciated you having the time to reconsider our manuscript on MRN.

I've looked into the question you raised on whether the dimerization domain mutants of Mre11/Rad32 affected just the dimerization or whether other functions were also affected.

The Tainer paper, where they describe the mutants (Williams Cell 2008), says it quite explicitly in the title of Figure 3: "Dimerization Is Key to Stable DNA Binding but Not Endonuclease Activity". In the text they say: "Mre11-L61K and Mre11-L97D retain high levels of ssDNA endonuclease activity (Figure 3G) and 30-50 dsDNA exonuclease activity (data not shown), suggesting that a high-affinity DNA interaction across the dimeric interface is not essential for efficient exo- and endo- nucleolytic catalysis."

I hope this helps.

Additional correspondence (author) 29 October 2012

I am trying to follow up on our manuscript.
I was very encouraged by our conversation on the activity of the dimerisation mutants of MRN. As I had the chance to write on my last email, it turns out that the mutants that affect the dimerization of MRE11 and NHEJ do not appear to have defects in DNA binding or nuclease activity, thus asserting that a separation of function between NHEJ and HR.

I really think this makes a strong case to our current model.

Additional correspondence (editor) 30 October 2012

Thank you for your patience while we have been consulting with an additional expert advisor regarding the conclusions on Mre11 mutations and MRN complex biochemical activities in your earlier submission, EMBOJ-2011-79668. I am pleased to inform you that this advisor not only considered the study in principle of high interest and suitable for a broad general journal such as ours, but that s/he importantly also found the conclusions on a nuclease-independent tethering role of the MRN complex sufficiently supported by your data with the original D65N mutant of Mre11 - which given its manganese-coordination role is considered by the advisor the cleanest possible nuclease-specific mutation. The advisor further agreed that the two other supposed nuclease-deficient mutations (which surprisingly did affect tethering and telomere fusion), analyzed in the revised manuscript, are much less clear based on structural considerations, and likely to additionally also affect DNA binding and/or positioning.

Regarding the supposed dimerization mutants of Mre11, our advisor agreed with referee 1 that it is unlikely that they are straightforward separation-of-function mutations as originally proposed based on structural work; instead they may more generally affect the architecture of the complex and consequently the end tethering function. The expert's advice was therefore that prominently referring to a role of dimerization in the abstract would be overinterpretation; while this could certainly be treated in the discussion section, the safer (and somewhat more conservative) interpretation would be that mutations that affect the general architecture of the complex lead to the defects in end tethering and telomere fusion; whereas a 'pure' nuclease mutation (D65N) does not.

In this light, we shall be happy to reconsider a resubmission of the paper, incorporating these points, for ultimate publication in The EMBO Journal. Before resubmission, please carefully rewrite the paper in the spirit of the advisor's input:
by removing the prominent claims about Mre11 dimerization roles and replacing them with more cautious conclusions on general architectural problems; and by discussing the differential effects of the distinct 'nuclease' mutants, emphasizing the significance of the dispensability of D65 for tethering and fusion.

To avoid further delays in the publication of this work, I would appreciate if you would manage to resubmit before the end of this week, making sure to upload all files as well as the relevant licenses and authorization forms already in production- ready format. This means a text file, individual production-quality figure files, a single supplementary information PDF, as well as a brief cover letter referring to our communications and detailing the changes made in comparison to the previous version. Should you need any further information, please don't hesitate to get back to me.

Yours sincerely,

Editor
The EMBO Journal

Re--submission 02 November 2012

Please find enclosed our revised version of the manuscript entitled "MRN tethers dysfunctional telomeres for NHEJ repair" by Clara Correia Reis, Sílvia Batista and myself.

We were pleased to hear of your latest consultation with an additional advisor concerning our manuscript. We agree with your assessment that our major point became diluted in the extensive mutant analysis of MRN. We also concur that more work is needed to make structural claims over the individual functions of the MRN complex. These mutations have not yet been studied fission yeast (as we refer in the discussion), neither have they been analysed in structural detail so that we may be certain of the mechanism behind their phenotype.

Overall, we have developed two independent assays to measure NHEJ repair: one using endogenous dysfunctional telomeres and a novel assay using exogenous plasmid DNA ends. Both assays are in agreement with the general conclusion. MRN has a major role in tethering unbound DNA ends for NHEJ repair.

In our current version, we have modified the text to the consensus of our reviewers and the final advisor:

- We removed the strong claim in the abstract concerning the so-called dimerization mutants and substituted by: "Rad32MRE11 mutations that affect binding and/or positioning of DNA ends also impaired NHEJ at telomeres and restored the viability of taz1 in G1."
- Where we previously attributed the phenotypes to defects in dimerization, we now refer to defects in complex architecture (e.g. "Rad32MRE11 complex architecture is required for efficient NHEJ").
- We highlighted the fact that even though they were required for NHEJ repair, these mutations "disrupted homodimerization and stable DNA interaction leaving both exo and endonuclease function largely unaffected."
- In the discussion, we highlighted the role of the D65N mutant in our conclusion: "A strong nuclease dead mutant rad32-D65N (responsible for Mn2+ coordination) was clearly proficient in NHEJ repair at telomeres and in split-molecule plasmid assays, supportive of a nuclease-independent tethering role of the MRN complex for NHEJ." and referred to differences to other nuclease mutants as possible general architectural defects

Acceptance letter 05 November 2012

Thank you for submitting a new and re-revised final version of your manuscript on telomere tethering by the fission yeast MRN complex for our consideration. As discussed in our previous correspondence, we are in light of the positive comments of an expert editorial advisor happy to now accept the manuscript for publication in The EMBO Journal. Thank you very much for this contribution to our journal, and congratulations to a successful publication - I hope you will consider us again in the future for submission of your most exciting work.

With best regards,
Editor
The EMBO Journal

