THE EMBO JOURNAL

Manuscript EMBO-2009-72794

Positional dependence of transcriptional inhibition by DNA torsional stress in yeast chromosomes

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Review timeline:

Submission date:	06 October 2009
Editorial Decision:	28 October 2009
Revision received:	23 November 2009
Accepted:	04 December 2009

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 28 October 2009

Thank you for submitting your manuscript for consideration by The EMBO Journal. We have now received the comments of three reviewers, and I am pleased to inform you that all of them find your study interesting and in principle suitable for publication in The EMBO Journal, pending appropriate revision of a certain number of specific issues, including aspects of presentation and interpretation. Should you be able to adequately address these various points, we should be happy to consider a manuscript revised along the lines suggested by the reviewers ' comments for publication. In this respect, you may want to also consider a somewhat more cautious title as indicated by some of the referees, and please also carefully edit and revise the manuscript text with regard to language issues as well as format and correctness of the reference list.

Please be reminded that it is EMBO Journal policy to allow a single round of revision only, and that it is therefore essential that you diligently answer all the points raised at this stage. When preparing your letter of response to the referees' comments, please also 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 initiative, please visit our website: http://www.nature.com/emboj/about/process.html. In any case, please do not hesitate to get back to us should you need feedback on any issue regarding your revision.

Thank you for the opportunity to consider your work for publication. I look forward to reading the revised manuscript.

Yours sincerely,

Editor
The EMBO Journal

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REFEREE REPORTS:

Referee #1 (Remarks to the Author):

DNA topology is mostly studied in closed topological domains such as circular molecules. The novelty of this paper by Joshi and co-workers rests in the fact that it is an attempt to analyze the effects changes in DNA topology cause on linear chromosomes. It is likely that this by itself will attract a wide spectrum of readers.

The manuscript is clearly written and the results are neat. The title: "Evidence that DNA helical stress dissipates at the telomeres", however, appears an overstatement. Here and elsewhere (Salceda, 2006) the authors have demonstrated that in yeast top1 top2ts double mutants with the ectopic expression of the TopA gene from E. coli, circular minichromosomes accumulate (+) helical tension. In addition, here they showed a distinct transcriptional response at the telomeres under the same conditions. Despite their efforts to prove this functional response is neither related to a reduced transcriptional activity at subtelomeric regions nor to the telomeric silencing caused by the SIR3 gene, they showed no direct evidence that helical tension actually dissipates at the telomeres. They infer this might be the cause for genes situated at less than 100 kb from the chromosomal ends to gradually escape the overall down-regulation of transcription. This effect, however, may or may not be a direct one. In other words, this observation by itself does not prove telomeres are topologically open. In this respect, it would be nice to see whether or not the introduction of nicks far apart and near the telomeres affects transcription in the same way. It would also be nice to see whether or not all YACs (Yeast Artificial Chromosomes) of less than 100-200 kb behaves indeed as topologically open structures and whether or not any specific gene responds in a different way depending on its location in the chromosome.

In my opinion this is a very interesting paper that deserves to be published. Notwithstanding, I strongly suggest the authors to revise the manuscript in order to make conclusions directly based on their experimental observations and speculate about their possible significance in the discussion separately.

Other minor mistakes:

- page 15, second line of the paragraph corresponding to Yeast Strains and Growth Conditions: references are not numbered and should be quoted alike throughout the text.
- page 17 and 18, the publication years are not correctly spelled for Eissenberg et al., Freeman et al. and Huang et al.
-page 18, the reference Liu, LF, Wang JC (1987) appears twice.

Referee #2 (Remarks to the Author):

In this manuscript, the authors investigated changes the local abundance of transcript levels across the genome of the yeast S. cerevisiae in response to the generation of excess positive DNA helical stress (or positive changes in local DNA linking number). They observed that transcripts from genes located at the ends of all yeast chromosomes were significantly overrepresented in the presence of (+) topological stress. This effect is independent of transcript orientation or length, independent of the transcriptional activity of the affected sub-telomeric genes, and independent of the sub-telomeric chromatin structure. The authors thus draw the conclusion, largely by an argument of exclusion, that the dissipation of helical stress at chromosome ends is responsible for these general chromosome-end-specific effects. The wider implications of these data for chromosome and nuclear structure and function are critically discussed.
This is a highly original and interesting manuscript! In my opinion, this is a nice piece of work, which is relevant for a general readership. The data presented are sound and support the authors' conclusions. However, in an ideal world, I would have also expected a demonstration of local DNA helical tension across the genome independently of the indirect measurement of transcript levels, but

I am not aware of a currently available technique that would allow this direct measurement. Therefore, I have only a few relatively minor points with this manuscript as detailed below that should be addressed.

1) In Figure 1A, only about 60% of all 2µ plasmids become highly positively supercoiled in the top2-ts, delta-top1, TopA strain; the remainder, however, are unaffected. In contrast, in the delta-sir3 background of Fig. 5, all plasmids are affected. How can this be explained? Do only 60% of plasmids serve as templates for transcription to generate the positive supercoiling in the presence of Sir3p? Does heterochromatin influence template efficiency, or is this just experimental variation (albeit significant)? This issue should be discussed, at least briefly.

2) It is surprising to see that only modest changes of transcript levels are observed in the top2-ts, delta-top1, TopA strain in this work, whereas Gartenberg and Wang observed 'greatly diminished' ones in 1992. This discrepancy should be discussed.

3) In the discussion section on page12, first lane, literature references need to be given to support the authors' statement that changes in sub-telomeric traits affect expression of genes only within 10-20kb from chromosome ends.

4) In the legend to Fig. 1A, the 'linking number distribution' is indicated as 'T'. This is inaccurate. These topoisomers on the left arm of the 2D gel belong to the distribution of (-) supercoiled plasmids, the ones on the right arm to (+) supercoiled ones. This should be corrected/clarified.

5) The legend to Fig. 2B is unclear/incomplete. What do the authors mean by qRT-PCR adjustment? The grey zone in the plot also requires a definition.

6) The labelling of Fig. 4A is inconsistent between main text and figure. In the text, the authors refer to 'decile classes' of transcript abundance, whereas the figure displays expression class as 'percentile'. The authors should resolve this confusing situation.

Referee #3 (Remarks to the Author):

The manuscript by Joshi, Pina & Roca is very interesting. The authors apply an ingenious combination of experiments to conclude that chromosomes in the investigated mutant cells of S. cerevisiae are not organized into topological domains that can restrict axial rotation of the DNA. The authors propose that yeast chromosomes devoid of active yeast topo I and topo II release their positive torsional stress arising due to transcription (and also DNA replication as 120 min after topo II inactivation all cells should at last attempt to replicate their DNA, since yeast generation time is ca 80 min) by rotations that are transmitted along the entire length of chromosomes.
The authors have some problems with explaining how the telomeres, that are known to be attached to the nuclear envelope, can undergo rotation. They therefore propose that periodic detachments of telomeres from the envelope may be required for permitting the rotation. It seems to me that such detachments are not needed since telomeres' attachment is apparently very "fluid". Several studies from S. Gasser's lab have shown that yeast telomeres maintain freedom of motion while remaining attached to nuclear envelope. See for example Rosa et al., Measuring limits of telomere movements on nuclear envelope. Biophysical Journal, 90, L24-L26, 2006. It would be good to mention in the text the fluidity of telomeres' attachment and the relevant references.
I would also like to see at least a short discussion that the arising positive torsional stress is likely to be caused by DNA replication. DNA replication in the absence of DNA topoisomerases capable of relieving the positive torsional stress will generate very high positive torsional stress and it dissipation will require one rotation of chromosome ends for every 10 bp of replicated DNA (A topological view of the replicon. EMBO Reports, 5, 256-261, 2004).

The text would profit from corrections by an English speaker. Not being one, I can only suggest some changes.
1. In the title I would replace "helical stress" by " torsional stress"
2. Page 12. 4th line from bottom. I would replace "pivot" by "rotate".
1. Page 14, 5th line from bottom. I would replace "topologically" by "torsionally".

1st Revision - authors' response 23 November 2009

Referee #1

We acknowledge the referee's positive evaluation of the work by its novelty, general interest and neat results.

We agree with the referee and the editor in that the former ms title was an overstatement. Certainly, we cannot provide direct evidence of the dissipation of DNA helical tension at telomeres. Experiments proposed by referee 1 are clever and could further support our conclusions, but still provide indirect evidence. As denoted by referee 2, currently available techniques might not be able to carry out this direct measurement.

As requested, we changed the title to 'Positional dependence of transcriptional inhibition by DNA torsional stress in yeast chromosomes'; and the running title to 'DNA torsional stress in yeast chromosomes'. We also removed the term 'evidence' along the abstract, introduction and discussion sections, to replace it by terms such as 'indication', 'suggestion' or 'proposition'.

Mistakes found in the format of references are fixed.

Referee #2

We acknowledge the referee's opinion of our work as highly original, interesting, and relevant for a general readership. The referee raised points that could certainly lead to confusion. We addressed them as follows:

1-- We often observe some experimental variation on the fraction of plasmid that becomes positively supercoiled. Not always all molecules change conformation. But as long a significant amount does (i.e. >50%), we validate that cells are accumulating DNA helical stress.

After examining the 3 replicates of each set of experiments, we can state that both in SIR3 and sir3 strains the amount of plasmids becoming highly supercoiled were comparable. Experimental variation among replicates might be related to the fact that the amplification and activities of yeast multi-copy plasmids can easily fluctuate, especially in topoisomerase mutants.

To avoid confusion on this issue, we retyped in the first paragraph of the results: 'most minichromosome molecules appeared highly positively supercoiled'; and then explained in the last paragraph of the results: 'In the different replicates of the experiment, the amount of plasmid becoming highly supercoiled in the sir3 cells at the 120 min time point was comparable to that of SIR3 cells'.

2 - Certainly, Gartenberg and Wang observed 'greatly diminished' transcript levels in their study published in 1992 (though they did not report quantifying data). We attribute this apparent discrepancy to the fact that they mostly analyzed plasmid-borne transcripts and just only one chromosomal transcript. We believe that the accumulation and effects of DNA helical stress might be more pronounced in small circular domains such as yeast minichromosomes. In addition, there are other differences between their experimental setting and ours: the yeast strains, a longer inactivation of topoisomerase II (up to 4 hours), and the use of primer-extension and run-on transcription to detect transcripts (in contrast to DNA arrays and qRT-PCR used in our study).

As requested, we better explain this issue by typing in the first paragraph of the discussion: 'The overall reduction of transcript levels essentially corroborates the observations of Gartenberg and Wang (1992), who reported that transcription is greatly diminished in highly positively supercoiled yeast circular minichromosomes'.

3 - As requested, we mention references when we say, 'These traits vary substantially from telomere to telomere and are responsible for transcriptional silencing of genes located at less than 10-20 kb from chromosomal ends'.

4 - As requested, we modified figures 1A and 5A (and their corresponding legends) to replace the misleading 'T' by '(-)' to indicate the gel position of negatively supercoiled DNA.

5 - As requested, we explained better the qRT-PCR adjustment in the legend to figure 2B, and defined the grey zone depicted in the plot.

6 - As requested, we fixed the inconsistency of terms between main text and figure 4A by typing 'decile' at both places.

Referee #3

We acknowledge the referee's evaluation of our study as being ingenious and very interesting.

As the referee proposed, we added in the discussion (third paragraph): 'These movements could reflect a fluid attachment (Rosa et al, 2006), which could provide the telomere freedom to rotate while remaining anchored to the nuclear envelope.' This is an interesting proposition.

As the referee proposed, we comment more about DNA replication. We added in the introduction (second paragraph): 'Analogously, (+) DNA helical tension also builds up in front of DNA replication forks (Schvartzman and Stasiak, 2004)'. We also added in the discussion (before last paragraph): 'we expect that the positional dependence associated to DNA torsional stress reported here might apply also to other DNA transactions. Future studies can examine, for instance, how DNA replication is altered in the proximity of yeast chromosomal ends'.

We do not discuss further on DNA replication because the accumulation of (+) torsional stress in our system relies on the activity of bacterial topo I on the unwound DNA domain produced during gene transcription. Nonetheless, examining chromosome replication under DNA helical stress could be an interesting issue for future projects.

All refereeí suggestions regarding the use of some terms were adequate and have been satisfied.

As requested, the text has been corrected by native English speakers.

Acceptance letter 03 December 2009

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

You shall receive a formal letter of acceptance shortly.

Yours sincerely,

Editor
The EMBO Journal

Referee 2 (comments to authors):

The authors have addressed all of my original points and revised the manuscript accordingly. In my opinion, this revised manuscript reports highly original data and will be of interest for a general readership.

