Chromatin regulates DNA torsional energy via topoisomerase II-mediated relaxation of positive supercoils

Xavier Fernández, Ofelia Díaz-Ingelmo, Belén Martínez-García and Joaquim Roca

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Review timeline:

Submission date: 31 January 2014
Editorial Decision: 05 March 2014
Revision received: 11 April 2014
Accepted: 29 April 2014

Editor: Hartmut Vodermaier

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 05 March 2014

Thank you again for submitting your manuscript on topoisomerase roles on transcriptionally active yeast minichromosomes for our consideration. It has now been assessed by three expert referees, in light of whose comments we concluded that we would in principle be interested in considering this work further for publication. However, acceptance would be dependent on adequately clarifying several experimental concerns (raised mostly by referee 2), as well as addressing a number of important issues with the presentation of the results and conclusions, as indicated by all three reviewers. As you will see, especially referee 3 criticizes that many interpretations and conclusions (including in the title and abstract) overreach the presented data, and having read the manuscript myself, I agree that the real merits and advances conveyed by the presented work and data (as nicely summarized by referee 1) should receive stronger emphasis and better explanation, while the likely (but not demonstrated) functional implications should be more clearly presented as speculative (at least in the absence of more direct additional evidence) and covered mainly in the discussion section. In addition to revising the title (cf. referee 3’s comments), I therefore also encourage you to re-work especially the paper’s abstract, to make it less technical and to make the general significance of this work more easily accessible.

I am therefore inviting you to prepare a revised version of this manuscript, keeping in mind that our policy to allow only a single round of major revision makes it important to carefully answer to all points at this stage. We generally allow three months as standard revision time, and it is our policy that competing manuscripts published here or elsewhere 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 have any additional question regarding this decision or the referee reports, please do not hesitate to contact me. I would like to thank you once more for the opportunity to consider this work, and look forward to your revision.
REFEREE REPORTS:

Referee #1:

In eukaryotic cells transcription is complicated by the DNA being packed in nucleosomes and by the torsional stress generated in the DNA during the elongation by RNA polymerase. According to the classic view, "the twin domain model", positive and negative supercoils are generated ahead and behind the transcription machinery, and are relaxed by Topoisomerases (Topos), which favor processive elongation.

It has been suggested for long time that torsional stress is not just a by-product of transcription but an active contributor to many processes including nucleosome remodeling and promoter melting. Consequently, rather than acting as mere relaxases of torsionally stressed DNA, Topos may tune the rate of DNA supercoiling and indirectly modulate genetic transactions. Indeed the activity of Topos is not always interchangeable revealing different peculiarities in regulating DNA topological state. To test this idea in native chromatin, the authors use circular minichromosomes to compare Topo I and Topo II activities in relaxing positive and negative torsional stress. This is an understudied, but fundamental topic to understand the coupling between DNA topology and transcription of DNA in chromatin.

The observation that Topo I relaxes positive and negative supercoils with similar efficiency whereas Topo II relaxed positive more quickly than negative supercoiling allows the authors to conclude that the relaxation rate of positive torsional stress by Topos largely surpassed that of negative torsional stress leaving a net underwound state. Overall this suggests that the rapid relaxation of positive supercoils may facilitate the progression of DNA motors along the double helix while delays in relaxation of negative torsional stress may favor DNA structural transitions at specific regions often required for genomic transactions.

I really enjoyed reading this paper. It is well and clearly written. The data presented by the authors constitute an important contribution to the understanding of Topos biology in relationship to transcription. Moreover the findings will help to expose the concept that torsional stress might actively regulate transcription of chromatin fibers in vivo.

Therefore I believe the paper is suitable for publication in the EMBO Journal. The few comments listed below will improve the presentation of the data.

Minor comments:
1. Page 3. "Only in yeast top1 top2-ts double mutants is global RNA synthesis decreased". The author may want to change with "Only in yeast top1 top2-ts double mutants global RNA synthesis is decreased".
2. Page 5. "In the case of single-copy plasmids YCpTA1 and YCp50, the decrease in the Lk was incipient at 28°C and affected nearly all the plasmid molecules when the cells were shifted to 37°C" Figure 1D should be indicated at the end of this sentence.
3. Page 6. "was roughly -0.5 (-22/428)." There is a mistake in the calculation. The correct number 1 - 0.05.
4. Page 6. "YCpTRP1 from dtop1 top2-4 cells presented" should be changed in "YCpTRP1 from top1 top2-4 cells presented".
5. Page 8. "Therefore, the global relaxation rate of (+)TS effected by the endogenous topoisomerases largely surpassed that of (-)TS." Please correct with "...affected..."
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7. Figure 4 and Figure 5. The authors should clarify how quantification of populations under (-)TS and under (+)TS are performed.

Referee #2:

In this manuscript Fernandez and Roca investigate the ability of budding yeast DNA topoisomerase
I (topo I) and DNA topoisomerase II (topo II) to relax chromatinized yeast minichromosomes subject to (+) and (-) torsional stress (TS). The authors observe that minichromosomes accumulate (-)TS stress in topo I defective mutants upon topo II inactivation. This (-)TS accumulation is somewhat dependent on DNA transcription. By performing in vitro relaxation assays carried out with minichromosomes subject to either (+) or (-) TS the authors show that topo II is comparatively more efficient in relaxing chromatin under (+)TS. From these findings the authors conclude that this topo II bias towards relaxing (+)TS might contribute to the in vivo persistence of chromatin under (-)TS, which could in turn modulate certain DNA transactions (e.g. DNA bending, unwinding or secondary structures formation). This work also provides evidence hinting that chromatin architecture is likely to modulate DNA topoisomerase function in vivo.

This is an elegant study with conclusions that are, in general, well sustained by the experimental findings and are appropriately discussed in the context of previous literature. This work provides novel insight on DNA topoisomerase function on chromatin DNA and an appealing model is proposed for the resolution of torsional stress during transcription, which might have implications for several biological processes such as gene expression, DNA replication and chromosome organization. While most of the work is convincing, the experiments linking (-)TS accumulation following topo II inactivation to ongoing transcription should be explained in more detail and additional experimental evidence would be required to support the proposed model for (-)TS is a direct outcome of gene transcription.

Major concerns:

1.- Perhaps the least clear evidence in the manuscript regards the effect of transcription on (-)TS accumulation upon topo II inactivation. Authors should explain why in the experiment shown in figure 2B (-)TS accumulates upon topo II inactivation in cells grown in glucose in which the expression of the GAL1-LacZ gene is repressed. This observation is in apparent contradiction with the conclusion that (-)TS accumulation is dependent on transcription. An explanation should also be provided on why the minichromosome accumulates (-)TS in Dtop1TOP2 cells upon the temperature shift.

2.- The authors should provide more detailed evidence showing that transcriptional activity correlates with (-)TS accumulation in top1top2 mutants

3.- The logic of the experiment presented on figure 3C is not easy to follow for a lay reader. The authors should explain in which way could (-)TS be constrained within chromatin and how relaxation by viral topo I would be affected is such constraint existed in the minichromosome.

Minor concerns:

1.- In the introduction literature is sometimes inaccurately cited and should be reviewed carefully. For instance, fork stalling upon inactivation of both topo I and topo II was not shown by Baxter and Diffley in 2008, but was instead reported by Bermejo et al. in 2007. Also, the Bermejo et al. paper from 2009 cited does not report gene expression in top1D mutants.

2.- The way Lk distributions are analyzed in figure 3 should be explained in more detail in the methods section to guide a lay reader into how Lk is actually calculated from the relative position of the different topoisomers.

3.- On figure 3C a 2D gel showing the state of the control plasmid prior to incubation should be included to provide a reference of the relaxation by endogenous topo I and topo II activities suggested in the text.

Non-essential suggestions:

1.- The header of the third paragraph of the results section might read better as "Lk reduction in top1top2 cells doubles..."

2.- D in Dtop1 top2-4 is misspelled in the penultimate paragraph on page 6.
Referee #3:

Fernandez and Roca

This is a clearly written manuscript building on a large number of previous studies from both the Roca lab and others. Much of the data is confirmatory however all the results are well presented. A possible criticism is that the scope is limited to describing torsional stress in yeast mini-chromosomes and is almost entirely dependent on chloroquine gels to investigate supercoiling isomers.

Title:
I don't understand the title. At best it could be reworded as "Chromatin regulates DNA torsional energy via relaxation of positive supercoils by topoisomerase II" although this manuscript is not showing how "chromatin" might be doing this. A more accurate title would be "Topoisomerase II preferentially relaxes positive supercoils in chromatin"

Abstract:
The authors elude to how changes in supercoiling might influence RNA and DNA polymerases progression however no new data is presented in this manuscript to address this

Introduction:
Page 4. End of paragraph 2. The authors write "this comparison is important to address a fundamental issue, namely whether cellular topoisomerases remove with similar efficiency the twin domains of (+) and (-) TS generated during in vivo transcription." I agree with this statement but the authors are only addressing one specific aspect of the question - namely activity of topoisomerases on (-) and (+)TS templates. Surely a further critical factor is where topoisomerases bind with respect to the (-) and (+)TS domains and how the activities of TopoI and TopoII are regulated.

Page 5. The authors write "We postulate that chromatin plasticity promotes the rapid relaxation of (+)TS ...". What is chromatin plasticity? Furthermore the authors have not undertaken any experiments to investigate this in their manuscript. I am therefore concerned the story presented is becoming too speculative.

Figure 1-2
This data is largely confirmatory.

Figure 3
Panel A is unnecessary as is possibly confusing particularly as the -22 isomer from WT cells appears to align with the 0 isomer of relaxed plasmid.
Panel B is nice as it enables the linking number to be determined but the fundamental result is confirmatory. Likewise panel C data is nicely presented but from my understanding there was no real suggestion that the supercoiling in a plasmid was anything other than constrained within the plasmid.

Figure 4 and 5
How is the quantification done? Why is the graph in Figure 5 log % relaxed and why does it show a decrease? Relaxation should increase over time.

Figure 6
The model here is very general and does not relate to the data presented in the paper particularly as the authors have analysed torsional stress within the entire plasmid and not within specific supercoiled domains.

Minor comment
Page 6. Linking number of YCpTRP1 in Top1Top2 cells is given as 0.5. I think it should be 0.05
We are very pleased with the comments of the three referees and the editor, which allowed us to correct ambiguities and improve the overall quality of the manuscript. As detailed below (typed in blue), all the points raised by the referees have been addressed in the revised version (typed in red). We have revised also the title and the abstract to make the general significance of this work more easily accessible.

Referee #1:

In eukaryotic cells transcription is complicated by the DNA being packed in nucleosomes and by the torsional stress generated in the DNA during the elongation by RNA polymerase. According to the classic view, "the twin domain model", positive and negative supercoils are generated ahead and behind the transcription machinery, and are relaxed by Topoisomerases (Topos), which favor processive elongation.

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incipient at 28°C and affected nearly all the plasmid molecules when the cells were shifted to 37°C.”

Figure 1D should be indicated at the end of this sentence.

Done. We also indicated Figure 1A.

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We thank the referee for catching this mistake. We have corrected the text accordingly.

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The reason why (-)TS accumulates in YCp50pGAL1:LacZ even when the expression of the LacZ gene is repressed is because the plasmid has the URA3 gene. We thank the referee for raising this issue because it could appear also contradictory to other readers. As requested, we have clarified this matter in the revised text and have included a scheme of Yc50pGAL1:LacZ in figure 2B to recall the presence of URA3. Regarding why the minichromosome accumulates (-)TS in Δtop1TOP2 cells (as seen in Figure 2C), we developed the explanation in the discussion section. Namely, we reason that when the generation rate of (-) and (+)TS increases in vivo by high transcription activity, Lk reduction occurs in Δtop1 TOP2 mutants because even normal topo II activity is limiting to remove high levels of (-)TS.

2. The authors should provide more detailed evidence showing that transcriptional activity correlates with (-)TS accumulation in top1top2 mutants.

The correlation of (-)TS accumulation with transcriptional activity in top1top2 mutants was extensively demonstrated in the former study of Brill and Sternglanz (1988). Thus, our experiments just corroborated and complemented these previous observations. In figure 2A we show that (-)TS accumulation occurs exclusively in yeast minichromosomes that contain transcription units. In figure 2B we show that under high transcriptional activity (-)TS accumulation can occur even before topo II inactivation. Yet, as requested by the referee, we have included in the revision a control experiment to further substantiate that (-)TS accumulation associates to transcription. In the new panel C of figure 2, we show that the Lk reduction induced at permissive temperature in Yc50pGAL1:LacZ reverts when cells are shifted back from galactose- to glucose-containing media.

3. The logic of the experiment presented on figure 3C is not easy to follow for a lay reader. The authors should explain in which way could (-)TS be constrained within chromatin and how relaxation by viral topo I would be affected if such constraint existed in the minichromosome.

We agree with the referee in that the experiments in figure 3C could benefit from a more clear presentation and explanation. Thus, we have repeated these experiments and the 2D gels to provide a figure that is easier to follow. We used the same chloroquine concentration to compare the topology of minichromosomes and control plasmids, we show the naked minichromosome and control plasmid individually relaxed by topo I, and we included schemes of the samples examined in each gel lane. In the revised text, we mentioned how a gain of negative DNA supercoiling density could be constrained (i.e. by DNA unwinding proteins, DNA-RNA interactions, or non-B-DNA configurations). We indicated also that, after topo I incubation, the Lk distribution produced in Δtop1 top2-4 mutants would have remained unaltered in presence of such constraints.

Minor concerns:

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Diffley in 2008, but was instead reported by Bermejo et al. in 2007. Also, the Bermejo et al. paper from 2009 cited does not report gene expression in top1D mutants. We very much thank the referee for catching these inaccuracies. We have amended these citations accordingly.

2. The way Lk distributions are analyzed in figure 3 should be explained in more detail in the methods section to guide a lay reader into how Lk is actually calculated from the relative position of the different topoisomers.

As requested, we detailed in the methods section how DNA supercoiling density values are calculated from the results shown in figure 3.

3. On figure 3C a 2D gel showing the state of the control plasmid prior to incubation should be included to provide a reference of the relaxation by endogenous topo I and topo II activities suggested in the text.

As requested, we show in the new figure 3C the state of the control plasmid prior to incubation.

Non-essential suggestions:

1. The header of the third paragraph of the results section might read better as "Lk reduction in top1top2 cells doubles..."

We agree and have changed the wording accordingly.

2. D in Dtop1 top2-4 is misspelled in the penultimate paragraph on page 6.

We have amended this misspelling.

Referee #3:

This is a clearly written manuscript building on a large number of previous studies from both the Roca lab and others. Much of the data is confirmatory however all the results are well presented. A possible criticism is that the scope is limited to describing torsional stress in yeast mini-chromosomes and is almost entirely dependent on chloroquine gels to investigate supercoiling isomers.

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We agree in that the title was not easy, mainly due to the limitation of 100 characters. We pursued a title that transmits both the experimental findings and their significance. Thus, we prefer the first proposed by the referee "Chromatin regulates DNA torsional energy via relaxation of positive supercoils by topoisomerase II". The revised abstract states that our study does not show, only suggest, how chromatin might be doing this. Another title that we find suitable is: "Topoisomerase II quick relaxation of positive supercoils favors DNA unwinding in chromatin"
Abstract: The authors elude to how changes in supercoiling might influence RNA and DNA polymerases progression however no new data is presented in this manuscript to address this.

Our work does not present data on the stall of RNA and DNA polymerases produced by (+)TS since these effects have been already reported elsewhere by several labs (including ours). As requested, we have revised abstract to distinguish more clearly the new experimental findings from their likely functional relevance based on previous knowledge.

Introduction: Page 4. End of paragraph 2. The authors write "this comparison is important to address a fundamental issue, namely whether cellular topoisomerases remove with similar efficiency the twin domains of (+) and (-) TS generated during in vivo transcription." I agree with this statement but the authors are only addressing one specific aspect of the question - namely activity of topoisomerases on (-) and (+)TS templates. Surely a further critical factor is where topoisomerases bind with respect to the (-) and (+)TS domains and how the activities of TopoI and TopoII are regulated.

We agree with the referee in that, in addition to chromatin conformation, other critical (yet largely unknown) factors may regulate the localization and intrinsic activity of cellular topoisomerases. We have included this remark in the revised text.

Page 5. The authors write "We postulate that chromatin plasticity promotes the rapid relaxation of (+)TS ...". What is chromatin plasticity? Furthermore the authors have not undertaken any experiments to investigate this in their manuscript. I am therefore concerned the story presented is becoming too speculative.

We use the term "plasticity" to indicate the structural response of chromatin to physical forces acting on DNA, such as stretching and torsion. As explained in the discussion section, this plasticity has been inferred mostly by single-molecule studies and is consistent with our experimental observations with native minichromosomes. Nevertheless, to avoid confusion, we have removed this word from the introduction section.

Figure 1-2. This data is largely confirmatory.

Figure 3. Panel A is unnecessary as is possibly confusing particularly as the -22 isomer from WT cells appears to align with the 0 isomer of relaxed plasmid. Panel B is nice as it enables the linking number to be determined but the fundamental result is confirmatory. Likewise panel C data is nicely presented but from my understanding there was no real suggestion that the supercoiling in a plasmid was anything other than constrained within the plasmid.

We showed gels of panels A and B because it is not possible to resolve in a single 2D gel with specific chloroquine concentrations all the individual Lk topoisomers of relaxed plasmid (ΔLk about 0), of the normal minichromosome (ΔLk about -22), and of the minichromosome under (-)TS (ΔLk about -52). Therefore, the gel in panel A allows to calculate ΔLk of the normal minichromosome relative to relaxed DNA. After knowing this value, the gel in panel B allows to calculate ΔLk of the minichromosome under (-)TS relative to normal minichromosome. Regarding the data presented in figure 3C, we have provided a better description and new 2D gels such that the experiment is more easy to follow (referee 2 also requested this).

Figure 4 and 5. How is the quantification done? Why is the graph in Figure 5 log % relaxed and why does it show a decrease? Relaxation should increase over time.

As requested, we have detailed in the legends of Figure 4 and 5 how the populations under (-)TS and
under (+)TS were quantified. In Figure 5, we have changed "% relaxed" by "% chromosomes under TS". We thank the referee for catching this inconsistency.

**Figure 6. The model here is very general and does not relate to the data presented in the paper particularly as the authors have analysed torsional stress within the entire plasmid and not within specific supercoiled domains.**

The model in figure 6 relates to the data presented in the paper because the (-) and (+)TS that we accumulate in the yeast minichromosomes are by-products of in vivo DNA transcription. Therefore, these minichromosomes are likely to have conformations comparable to those of chromatin upstream and downstream the transcribing complexes. Accordingly, the model in figure 6 rationalizes all the in vitro and the in vivo observations of this study.

With our current technology it is hard to assess whether DNA torsional stress is equally distributed across the entire minichromosome or confined within sub-domains. Although this is an interesting issue for future research, we believe that it does not change the interpretation of the results and present conclusions.

**Minor comment:**

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We thank the referee for catching this mistake. We have corrected the text accordingly.

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**Acceptance letter**  
29 April 2014

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.

Referee #2

The authors have satisfactorily addressed my suggestions and criticisms. The manuscript presents novel findings that are adequately discussed to provide a reasonable model for the function of eukaryotic DNA topoisomerases in resolving torsional stress in the context of chromatin. I think it would be an important contribution to the EMBO Journal.