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Sister chromatid interactions in bacteria revealed by a site-specific recombination assay

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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   09 February 2012

Thank you for submitting your study on the new E. coli sister chromosome cohesion assay to The EMBO Journal. I apologize for the delay in its evaluation, related to the fact that your submission coincided with the holiday office break and a peak submission period. We have in the meantime received the reports from three referees, which I am copying below, as well as informal feedback from our Editorial Advisory Board, so that I am now in a position to provide you with a decision.

As you will see, the referee reports are somewhat equivocal: on one hand, the reviewers all appreciate the elegant assay you developed to show that sister chromosomes remain in close proximity following replication. On the other hand, there are concerns regarding (a) the limited amount of new biological insight and unclear significance of the observed bacterial sister chromosome cohesion or proximity, and (b) the technical/experimental set-up and conclusiveness of the assay, especially regarding faithful read-out of cohesion time.

Upon careful considerations of these points, we conclude that given the importance of the topic and the potential value of your system for the field, we would not hold concern (a) as an overriding criterium against publication, as long as you should be able to decisively rule out the technical concerns related to point (b), including convincing demonstration that your assay may indeed faithfully report on cohesion times. Since this may only be judged based on data and explanations after substantial revisions, I am at this point not able to make any strong commitments regarding the eventual acceptance of the study. Nevertheless, should you be confident you may be able to address those specific concerns as requested by all three referees, then I would encourage you to conduct these experiments and submit a revised version for our further consideration. When revising the

manuscript, it will be important to also pay close attention to aspects of interpretation, presentation, writing and reference formatting. In this respect, I wonder if an (admittedly) less catchy yet more explicit title could be chosen, to reflect the point made especially clear by referee 2: that the observed bacterial cohesion is different from eukaryotic bona fide 'sister chromatid cohesion' that keeps sisters close for extended periods and establishes/counteracts tension forces. On the other hand, I don't agree with referee 2 that 'cohesion' necessarily requires specific cohesin proteins, since I feel that topological DNA contacts would in principle also well suited to generate 'cohesion'. I'd be happy to discuss these points or title suggestions informally prior to resubmission.

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your 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):

The manuscript by Lesterlin and co-authors describes a novel tool for studies of intracellular alignment of newly replicated fragments of bacterial chromosomes. This is an important question that still lacks adequate high resolution methods and the new approach offers a significant advance to the field. The approach is based on monitoring the frequency of Cre-loxP recombination within a cassette that is placed in various sites along the chromosome. The cassette consists of a lacZ gene with two loxP sites that disrupt its promoter. Recombination within the locus restores the promoter and can be monitored using the blue/white colony test. Recombination is initiated by short pulses of Cre induction at various times after initiation of DNA replication. The arrangement of the two loxP sites is such (they are too close to each other) that only intermolecular recombination is possible, when the chromosomal locus has just replicated and the two sister copies of the cassette remain in close proximity. The authors refer to the existence of such proximity as sister chromosome cohesion and explore the duration and extent of such cohesion at various times after initiation of DNA replication. This is an elegant insightful system and some of their findings are quite exciting (e.g. that colocalization as observed by microscopy does not always correlate cohesion as defined in their assay). I enthusiastically recommend publication of the manuscript once the authors address the concerns below.

1. The manuscript is very difficult to read. The authors must use separate paragraphs to describe distinct experiments and ideas. This applies to the entire manuscript from Introduction to Discussion. The author must also avoid introducing a new method or experiment and its results in one sentence. I noted a few such places below but the problem is more widespread than that. In few cases, controls that validate an experiment are described way after the results, which is highly confusing. Finally, experiments do not always include sufficient detail needed to reproduce them or, sometimes, to judge them. In particular, it is not always clear which strains were used in a given experiment.
2. A key premise in many experiments is that Cre induction results in recombination only during the time of induction. This is an unexpected result and the authors show reasonable but not exhaustive

evidence in its support. The authors must also exclude the possibility that the previously produced Cre can induce recombination in subsequent generations of cells (see also below).

3. Given the novelty of this approach, the authors need to make a more concerted effort to validate their system. For example, why frequency of recombination exceeds at times 50% (see below)? The authors also need to be precise when describing and discussing their results: greater recombination does not necessarily mean greater cohesion.

4. The authors must move essential data from the Supplement into the main text. For example, all plots on the frequency of intermolecular recombination in Figs 2-4 must be accompanied with the plots for the intramolecular frequencies and both sets of data must be discussed in parallel.

Other comments:

1. Intro, paragraph 1, sentence "Some SMC complexes have a condensing activity". Unclear- change to "Some SMC complexes have a DNA condensing activity" "

2. Intro, paragraph 1: "Cohesion is mediated by cohesin, a specific multi-subunit complex that interacts with structural maintenance of chromosomes (SMC) proteins" Please rephrase: Cohesin consists of two SMCs and two other proteins.

3. Fig.1A. Unclear what the photographs show- please enlarge them a bit.

4. Fig. 1B: What is NI?

5. P.6: "The rate of Laclox recombination was also measured by scoring white (Lac-) and blue (Lac+) clones on X-Gal containing plates (Figure 1A)". No data or experimental details are shown here. Please rephrase or remove the sentence.

6. Insufficient details of the experiment in Fig. 1D: When cephalexin was added and why was it needed at all? DNAC2 mutants are supposed to stop replication regardless of cephalexin and thus should yield no Cre-lox recombination even in its absence. The authors need to show the results without cephalecin and explain the difference if there is any. Please also include details on the 30C data in Fig.1D. Were these points obtained after temperature shift from 40C?

7. pp.6-7, paragraph beginning with "We first ensured that Laclox recombination..." describes several ideas and is way too long. Please split it in parts to help follow the logic.

8. Experiment in Fig. 1E needs more discussion and perhaps control experiments. Why is the observed frequency of recombination more than 50%? One might expect repeated rounds of recombination in this system (e.g. Fig 1A). If so, the even number of interchromosomal recombination events would resolve the concatameric chromosome back into dimers. In this case, the highest frequency of recombination should not exceed 50%. The authors need to explain why this does not happen in their system. Moreover, the control experiment in Fig. 2A must be shown before the data in Fig. 1E.

9. p8, "The cells were synchronized under slow growth conditions..." please say how (e.g. by temperature shift to 40C).

10. p.8. Please indicate which panel of the figures is under discussion (e.g. Fig S1A, not simply FigS1). Second, Fig. S1B-E is not described anywhere in the paper.

11. Fig. 1F. Please indicate the time of each pulse either on the figure or in the legend (or both).

12. "This shows that cohesion occurred immediately after replication and only for a limited time, 30 minutes for Ori-1 and 20 minutes for Ori-3, Ter-1 and Ter-6." The authors show in Fig S1A that the entire chromosome is replicated in 40 to 50 min and cells divide by 70 min. Thus, the "limited time" is about the time needed to replicate half of the chromosome. Perhaps a better estimate can be achieved by quantifying extent of recombination.

13. Experiment in Fig. 2A is an important control. It must be shown earlier. I would be convinced by it if the authors provided more experimental details. What was the doubling time at these conditions? How many cell divisions occurred during the time course? This is important because the previously produced Cre might be able to capture loxP sites during the next round of DNA replication, when DNA is stripped of other proteins.

14. typo in Fig 2B."rcombination"

15. The authors must provide all sequences of the generated constructs, especially for the chromosomal mutants. Where exactly were loxlac and rifampicin (Fig. 2B) cassettes inserted? Please also show the sequence or organization of the rifampicin cassette.

16. p. 10. "We performed kinetic experiments of Cre induction for the four loci coupled with the intermolecular or intramolecular assay". Please define the intermolecular and intramolecular assays and describe how they were done before describing their results.

17. Fig. 2C. The color of dashed lines is difficult to tell. Please increase their thickness.

18. Fig. 2C, 2D. Quantitative analysis of the shown kinetics is a bit clumsy. The authors should try fitting to more realistic equations. All shown data sets could be fit to either single-exponential

(intramolecular events) or double-exponential (intermolecular events) decays, which would be traceable to the Michaelis-Menten mechanism. This would also simplify discussion of the result.

19. The experiment in Fig. 3 is inadequately described. Legend to Fig. 3A: "Representative pictures of the parS/ParBP1-GFP tag were inserted at the Ori-1, Ori-3 and Ter-1 locus". There is a problem with this sentence; please rephrase.

20. ParS/ParBP1-GFP system is not described anywhere in the paper. Please include it in the Methods and introduce in the main body of the paper as well.

21. Fig. 3B: No details are given for measurements of colocalization events, or how the C and D periods were determined, or how the colocalization period was deduced. These issues must be explicitly addressed in the body of the paper. The text on p.10 is too generic.

22. Fig. 3C. MatP mutants do display a reduction in Cre-lox recombination although to a lesser extent than the colocalization of the ter regions. Thus, correlation between colocalization and cohesion does exist. Please acknowledge it in the text.

23. p13 "...were measured in wt and parC1215ts (Topo-IVTS) strains (Figure 4A)." Confusing- Delete "(Figure 4A)" and put a reference to Figure S4A instead in the next sentence.

24. Please provide more details in the legends to Figs. S3 and S4. Are the left bars in Fig S4A the no-arabinose controls?

25. p13. "Interestingly, Topo-IV alteration only moderately affected recombination frequency at the Ori-1 locus." Please explain why this is interesting.

26. p13, bottom. "...we observed a reduction of SCI for the Ori-3 and Ter-1 respectively, 0.72 +/- 0.05 and 0.76 +/- 0.04". The numbers are within the experimental error and, therefore, are the same.

27. p14, second line. Should not it be "(Figure 4B)"?

28. Fig. S4B. Please show quantification of the micrographs and describe them in greater detail in the main text of the paper.

29. p14, top half. Please summarize the main result of the experiment and remove needless details: Why do we need to know the average number of foci per cell in the entire population?

30. p14 "The strong influence of Topo-IV transient inactivation..." Given the long rationale for the next experiment, this might be a good place to start a new section.

31. p14, bottom "This result also suggested that the alteration of the decatenating activity of Topo-IV, rather than the positive supercoil removal, was involved in the modulation of recombination between sister chromatids." This is consistent with, not supportive of the model. Given major chromosome packing defects in mukB mutants, many other mechanisms can be envisioned.

32. p15. After the sentence "Therefore, we tested if alteration of the gyrase activity in the gyrB203ts allele led to an increase in the intermolecular recombination rate....", please describe the phenotype of this mutant at permissive and non-permissive conditions.

33. p15; "After a 20 minutes shift to a non-permissive temperature (42{degree sign}C), a strong decrease in the intermolecular rate was observed (Figure 4D)". As shown in Fig S5, the intermolecular recombination rate did not change upon the temperature shift, the normalized rate did. However, I was not convinced that the used normalization is appropriate in this case. Indeed, both the wt and the mutant show almost 100% intramolecular recombination rate (and why is this different from the wt in Fig. S4?). It is difficult to compare, therefore, the overall level of Cre activity in the wt and the mutant at 42C. The authors should show both the normalized intra- and intermolecular recombination rates in figures 2, 3 and 4 to avoid such confusion. In addition, the authors need to repeat the experiment in Fig. S5B using shorter Cre induction times, so that their recombination rates are well below 100%.

Referee #2 (Remarks to the Author):

Unlike in eukaryotes, where sister chromatid cohesion (SCC) is a must for proper chromosome segregation during mitosis, the occurrence or the importance of SCC has remained controversial in the bacterial chromosome segregation field. The occurrence of SCC so far has been claimed from low-resolution optical microscopy, since sister loci could not be resolved for a while after they were formed by replication. Here, the authors have devised a recombination assay to demonstrate "cohesion" at the molecular level based on the premise that proximity of loci brought about by cohesion would promote recombination. The assay has been elegantly designed and rigorously evaluated (Fig. 1). The results do support the view that the loci stay in close proximity a significant fraction of the cell generation following their duplication. In the following I have a few comments that should be addressed to clarify the presentation.

1. How real time is the assay- meaning how long did it take for the appearance of recombination products after the presumed cohesion? From Fig. 1C, it appears that the lag time to record cohesion could be 20 min. In Fig. 1F, it appears more like 10 min.
2. The authors should mention what was the basis of choosing the four loci that they have analyzed. In particular, only at the very end of the paper it is mentioned that the Ori-3 tag is within the SNAP2 region. In that case, I would have expected more recombination at Ori-3 than at Ori1. But the results obtained were opposite.
3. The authors have ignored migS from the discussion. Please note that migS effect was found in fast growing cells. Later studies that discounted the importance of migS were done in very slow growing cells. This locus should be tested in the Lac/lox assay.
4. The authors should discuss their finding that "cohesion" was seen only between newly replicated regions and NOT when replication is completed (Fig 1D). This is unlike the situation in eukaryotes, where dedicated proteins (cohesins) keep the sisters together until mitosis. These features of cohesion thus do not apply to E. coli. SCC in E. coli could be a trivial outcome of the proximity of newly replicated DNA. Unfortunately, the biological relevance of the so-called "SCC" in bacteria remains questionable even after this novel approach.

Minor comments

5. P.6, l.11: Change event 4 to event 3.
6. P.7: Change Ter-1 to Ter-6 in l.2 and Figure 1F to 1E in l.16.
7. P.8: Change "less than 50" to {less than or equal to} 50 in l.10, and mention that recombination at Ori-1 could be seen till T40 in the last line.
8. P.9, l.18: Delete from "In order to quantitatively---Ter-1 locus (Fig 2 and S2)." In other words, describe the experiment first and then talk about the results. Add the recombination values of Fig. 2 (44%, 80% etc.) before "This observation indicates---" in the next page.
9. P.11, top para: I could not follow how the rates 33% etc (p.11, l.3) and 26% etc. on (p.11, l.8) were deduced. Explain the deduction in the Figure legends. The numbers in Fig. 2D convey the basic take home messages of this paper and should be presented more clearly. An alternative could be to show the normalized plots so that the final values can be read out directly.
10. P.16: Change "not cohere at all" to 'not cohere extensively' in l.20 (See Fig. 5 of Nielsen 2007), and in the 2nd line from the bottom, mention generation time while discussing cohesion periods. It should be pointed out that, to the extent analyzed, there may not be any special loci for cohesion as was found by Nielsen and not by Bates.

In summary, unless the molecular mechanism of cohesion is demonstrated (by discovery of "cohesin", for example) we might all be chasing a wild goose.

Referee #3 (Remarks to the Author):

In the manuscript under review, Lesterlin, Gigant and colleagues investigate sister chromatid cohesion in E. coli. In previous studies, the existence of cohesion between replicated chromosomes was inferred by the lag between replication of a locus and visual separation of the sister loci. Here, an elegant genetic assay that takes advantage of site-specific recombination is used to assess a physical interaction between sister chromatids. In accordance with the co-localization study (Wang, Reyes, Sherratt 2008 Genes & Dev), the authors provide evidence for physical interaction (recombination) between sister loci for a limited time after their replication. Moreover, consistent with the earlier co-localization study, TopoIV was found to affect the frequency of these interactions, suggesting that DNA topology (pre-catenanes) is a key component of cohesion.

The recombination assay is quite creative and provides molecular evidence of sister chromatid interaction. I am not sure that the assay in its present form can be used to quantitatively assess cohesion time (see below). More importantly, I think the conclusions of the paper (taken at face value) do not significantly extend beyond the current thinking in the field. As such, I cannot recommend publication in EMBO Journal.

specific concerns and comments are below.

To quantitatively relate the level of inter-molecular recombination to cohesion lifespan, the authors

sought to show that the rate of recombination (really the total number of blue recombinants) strictly reflected the substrate availability within the induction window and was not due to recombination during AND after the induction pulse.

To test this, they induced Cre for 10 minutes and analyzed Lac+ colonies after dilution (of the inducer) and direct plating compared to dilution and waiting 10min or 30min. In all cases they saw the same number of Lac+ colonies. From this, they conclude that intermolecular-recombination (Lac+ colonies) reflected substrate availability during the induction window. I don't understand how they can conclude this unless Cre is not active in cells that have been plated and only in media. To my mind the number of recombinants reflects inter-molecular recombination during induction and after - until Cre is degraded or diluted to a significant degree. After a 10min induction pulse the authors report 44% recombination for their origin tag (Ori-1). If recombination only occurred during the induction window, this would indicate that 44% of the cells were cohesed at Ori-1 in this 10min. window. They are working with an asynchronous culture grown in minimal medium with an ~80 min doubling time. This doesn't sound right to me. It seems much more likely that the 44% reflects recombination during induction and after - whether it is on plates or in liquid.

With this in mind, it makes is hard to think about "rates of recombination" and thus extremely hard to use these experiments to infer cohesion times. I think we can say from Figure 2C that the amount of cohesion is likely to be longer (or replication slower) at Ori-1 compared to the other sites because there is more recombination after 10 minutes. But other than this qualitative conclusion I don't see how you can use asynchronous cultures and the terminal phenotype of blue colonies to establish cohesion time. Maybe I'm thinking about this incorrectly, if so, the authors should spend more time in the text explaining the logic of these experiments.

A western blot examining Cre stability after a 10min induction pulse would be informative. Maybe if they had a degradable allele of Cre these experiments would be more interpretable.

The explanation for what was normalized in the intra and inter molecular recombination experiments and why this normalization makes sense needs to be made more clearly. I found this section very confusing.

Section entitled "Distinction of SCI and colocalization of loci":
"In growing conditions used, we estimated that sister Oir-1, Ori-3 and Ter-1 loci were co-localized for 30, 20 and 35 minutes." Was this estimate based on Helmstetter? If yes, this should be mentioned here - not in following section.

In the MatP section:
"14 to 18% decrease, respectively, for the Ori-3 and Ter-1 loci" Should this be Ter-1 and Ter-6 loci?

Ori-3 tag is located in SNAP 2 a region reported to have extended co-localization (Joshi et al). However, this region does not appear to have increased recombination between sisters nor do the authors report prolonged co-localization. A discussion of these results in light of previous reports seems important.

Section entitled: "Sister chromatids were cohesive for a limited time window after their replication" Description of synchronization method is not mentioned until second to last sentence of section. The section would be easier to read if this was described earlier.

1st Revision - authors' response   08 June 2012

Thank you for your comments and those of the referees on our manuscript « Sister chromatid cohesion in E. coli».
We have now performed new experiments that reinforce the conclusiveness of the new recombination assay that we have developed to test molecular cohesion of the sister chromatids in E. coli. We have also added a number of results that clearly illustrate the important biological insights that can be obtained by our recombination assay. In the purpose to highlight the fact that bacterial cohesion differs from the cohesion mechanisms observed in eukaryotes we propose to change the

title of the manuscript: "Topological links between sister chromatids in bacteria revealed by a site-specific recombination assay".

Briefly, in the revised manuscript, we provide new experimental evidences strongly suggesting that Cre recombinase only catches the sister chromatids in contact during the time window of the induction pulse. We have performed kinetic analysis of the recombination reactions to refine our estimation of the cohesion. These experiments strongly suggest that recombination assay faithfully reports on cohesion times. We have also analyzed recombination in different regions of the chromosome that are suspected to present either extensive or short cohesion period, according to past cellular biology experiments. These experiments demonstrate that the colocalization of the sister chromatids corresponds to the sum of at least two events : i) topological links that maintain the sister chromatids in close contacts and allow Cre recombination; ii) colocalization of the sister chromatids in a subcellular territory, where sister chromatids are not competent for Cre recombination.

We believe that thanks to the comments of each referee, the revised manuscript has been strongly improved. The importance of the finding and the novelty of the assay should interest a large audience. Thank you for considering our work for publication in the EMBO journal.

Detailed description of the responses to the referees.

Referee 1:

1. The manuscript is very difficult to read. The authors must use separate paragraphs to describe distinct experiments and ideas. This applies to the entire manuscript from Introduction to Discussion. The author must also avoid introducing a new method or experiment and its results in one sentence.

We have performed an extensive rewriting of the manuscript. The manuscript has been reorganized as follow:

The first paragraph of the results "*The Laclox assay probes sister chromatid interactions*" is now split into two parts: i) *The Laclox assay probes sister chromatid interactions*"; ii) *Recombination events between sister chromatid are rapidly detected by a PCR assay*

To simplify the manuscript the demonstration that SC recombination provokes the formation of chromosome dimers and therefore requires XerCD or RecA to allow viability has been removed.

The paragraph "*Variability in the frequency of Laclox recombination revealed variability in the lifespan of sister chromatid cohesion in steady state cells*" is now split into two parts: i) *Lac + colonies formation is an accurate measure of SCI; ii) Variability in the frequency of laclox recombination revealing variability in the extent of sister chromatid cohesion.*

The paragraph "*Topo-IV activity modulated sister chromatid cohesion*" is now split into two parts :i) *Topo-IV activity modulated sister chromatid cohesion and colocalization; ii) Regulation of DNA topology around the replication fork controls SCI.*

In the discussion the paragraph "*Sister chromatid cohesion at the molecular level in E. coli*" is now split into two parts : i) *Sister chromatid cohesion at the molecular level in E. coli*; ii) *Roles for sister chromatid cohesion in bacteria.*

In few cases, controls that validate an experiment are described way after the results, which is highly confusing. Finally, experiments do not always include sufficient detail needed to reproduce them or, sometimes, to judge them. In particular, it is not always clear which strains were used in a given experiment.

We have taken into accounts these points in the revised version of the manuscript (see below for details)

2. A key premise in many experiments is that Cre induction results in recombination only during the time of induction. This is an unexpected result and the authors show reasonable but not exhaustive evidence in its support. The authors must also exclude the possibility that the previously produced Cre can induce recombination in subsequent generations of cells (see also below).

This is a key point for every referee; we have taken special attention to this aspect. I have summarized here the experiments that we have performed to control that most of the recombination events involve only SC that are close enough to recombine during the Cre induction pulse.

a) We have tried to monitor Cre production and turnover during and after the induction pulse by western blot analysis. Unfortunately, none of the commercially available Cre antibodies

that we tried, revealed any Cre protein on western blot experiments

b) We have extended the dilution period following the Cre induction pulse up to over night before plating. The results of this experiment are now presented on Figure 2C and S2. Even after overnight incubation the frequency of Lac+ colonies is similar to that observed immediately after a 10 min induction pulse. This observation confirms that newly formed SC in the cell cycles following the pulse are not captured by the Cre recombinase.

c) We analyzed the formation of recombination products by PCR and Southern blot, it shows that following induction recombination is not immediately observed at the frequency that was observed on plate. However 30 min after the 10 min induction pulse the recombination reaches a plateau similar to that observed on plate (Figure S2B). This experiment (presented on Figure 2D) shows that recombination products accumulate during the dilution delays but their amount does not exceed the amount of Lac+ colonies.

d) We have blocked the translation by addition of chloramphenicol immediately after the induction. The incubation with chloramphenicol was performed in the presence of arabinose to induce *cre* mRNA synthesis. As observed for the dilution, the addition of chloramphenicol blocks the accumulation of recombined SC. This experiment (presented on Figure 2E) strongly suggested that Cre synthesized during the induction pulse present a rapid turnover and therefore will not be efficient to produce recombination of the SC formed subsequently.

e) We have plotted the kinetics of recombination frequency as a function of the extent of induction according to an exponential decay. After the induction lag, the data are perfectly fitted a single exponential decay (Figure 3A). This allowed us to obtain catalytic constant for the inter molecular and the intramolecular recombination, and to directly estimate the cohesion time according to the ratio K_{inter} / K_{intra} (Figure 3B). We are particularly thankful to referee 1 to have noticed that the kinetics follows exponential decay rules.

3. Given the novelty of this approach, the authors need to make a more concerted effort to validate their system. For example, why frequency of recombination exceeds at times 50% (see below)?

The recombination frequency exceeds frequently 50% because intramolecular recombination between the external loxP sites of the *lac3loxP* configuration is not prohibited any more (event 3 on Figure 1A, observed on Figure 1C). We have added the following sentences to precise this aspect:" After 120 minutes, *lac3lox*, the reciprocal recombination product, was hardly detected, indicating that it was converted into the *lac1loxP* product. In *lac3lox,* the external *lox*P core sequences were separated by 105 bp and therefore supported intramolecular recombination *in vivo* (event 3, Figure 1A). Because of event 3 on the *lac3lox* substrate, the amount of SC in the *lac1lox* configuration can nearly reach 100% for long Cre induction."

4. The authors must move essential data from the Supplement into the main text. For example, all plots on the frequency of intermolecular recombination in Figs 2-4 must be accompanied with the plots for the intramolecular frequencies and both sets of data must be discussed in parallel.

This is a complex task to do taking into account the limited space available for publication. We have chosen not to add the raw data in the figures and kept them as supplementary material. However we have clearly specified in the text the experiments where significant changes of the intramolecular recombination frequency were observed.

5. All the typos were modified as suggested by referee 1

6. Insufficient details of the experiment in Fig. 1D: When cephalexin was added and why was it needed at all? DNAC2 mutants are supposed to stop replication regardless of cephalexin and thus should yield no Cre-lox recombination even in its absence. The authors need to show the results without cephalecin and explain the difference if there is any. Please also include details on the 30C data in Fig.1D. Were these points obtained after temperature shift from 40C?

As suggested by referee 1, we have reformulated the text corresponding to Figure 1D. Cephalexin was essential in this experiment to prevent cell division. Otherwise, as suggested by referee1 the DnaC blocks would be sufficient to block recombination (i.e this is actually observed on figure 1E pulse N°2 and N°3). The purpose of this experiment was to test if fully replicated and segregated chromosome maintained in the same cell because of filamentation could recombine. This experiment demonstrates that recombination is replication dependant even if multiple chromosomes are present in the same cell.

7. pp.6-7, paragraph beginning with "We first ensured that Laclox recombination..." describes several ideas and is way too long. Please split it in parts to help follow the logic.

Modified as suggested, see point n°1

8. Experiment in Fig. 1E needs more discussion and perhaps control experiments. Why is the observed frequency of recombination more than 50%? One might expect repeated rounds of recombination in this system (e.g. Fig 1A). If so, the even number of interchromosomal recombination events would resolve the concatameric chromosome back into dimers. In this case, the highest frequency of recombination should not exceed 50%. The authors need to explain why this does not happen in their system.

In the purpose to simplify the reading of the manuscript we have chosen to remove this experiment. The frequency of Lac+ colonies is not limited to 50% because of the event 3 (see point n°3). However, we agree with referee 1, if multiple round of intermolecular recombination are allowed then the frequency of dimers (lethality in the XerC-RecA-) should not exceed 50% of the recombined cells. The only explanation that we could provide to this observation is that two consecutive events of intermolecular recombination are rare enough to be missed by the lethality assay. Taking into account the short length of the cohesion period (20 min), the rapid inactivation of free Cre enzyme (may be few minutes) and the extent of time required to complete one reaction (>6 min) this is reasonable.

Moreover, the control experiment in Fig. 2A must be shown before the data in Fig. 1E.

For the clarity of the manuscript we preferred not to make the change suggested by referee1

9. p8, "The cells were synchronized under slow growth conditions..." please say how (e.g. by temperature shift to 40C).

The text has been modified as suggested.

10. p.8. Please indicate which panel of the figures is under discussion (e.g. Fig S1A, not simply FigS1). Second, Fig. S1B-E is not described anywhere in the paper.

The text has been modified as suggested.

12. "This shows that cohesion occurred immediately after replication and only for a limited time, 30 minutes for Ori-1 and 20 minutes for Ori-3, Ter-1 and Ter-6." The authors show in Fig S1A that the entire chromosome is replicated in 40 to 50 min and cells divide by 70 min. Thus, the "limited time" is about the time needed to replicate half of the chromosome. Perhaps a better estimate can be achieved by quantifying extent of recombination.

A quantification of the recombination observed for each pulse is now presented on figure 1E.

13. Experiment in Fig. 2A is an important control. It must be shown earlier. I would be convinced by it if the authors provided more experimental details. What was the doubling time at these conditions? How many cell divisions occurred during the time course? This is important because the previously produced Cre might be able to capture loxP sites during the next round of DNA replication, when DNA is stripped of other proteins.

This aspect is discussed in point n°2. The legend and the text corresponding to this figure have been modified as suggested.

14. typo in Fig 2B."rcombination"

The text has been modified as suggested.

15. The authors must provide all sequences of the generated constructs, especially for the chromosomal mutants. Where exactly were loxlac and rifampicin (Fig. 2B) cassettes inserted? Please also show the sequence or organization of the rifampicin cassette.

We have provided a list of the primers used for the construction on table S2. The insertion of the rifampicine resistance gene is now described in the material and methods section, the orientation of the *rif* gene is described on Figure 2A.

16. p. 10. "We performed kinetic experiments of Cre induction for the four loci coupled with the intermolecular or intramolecular assay". Please define the intermolecular and intramolecular assays and describe how they were done before describing their results.

The text has been modified as suggested.

17. Fig. 2C. The color of dashed lines is difficult to tell. Please increase their thickness.

The intramolecular recombination kinetics (dashed lines) are now also presented on the fitted curves on Figure 3A.

18. Fig. 2C, 2D. Quantitative analysis of the shown kinetics is a bit clumsy. The authors should try fitting to more realistic equations. All shown data sets could be fit to either single-exponential (intramolecular events) or double-exponential (intermolecular events) decays, which would be traceable to the Michaelis-Menten mechanism. This would also simplify discussion of the result.

Thanks to referee 1 we have fit the data according to an exponential decay (Figure 3). It gives robust results that allowed us to extract more precise estimation of the cohesion periods (Figure 3B).

19. The experiment in Fig. 3 is inadequately described. Legend to Fig. 3A: "Representative pictures of the parS/ParBP1-GFP tag were inserted at the Ori-1, Ori-3 and Ter-1 locus". There is a problem with this sentence; please rephrase.

The text has been modified.

20. ParS/ParBP1-GFP system is not described anywhere in the paper. Please include it in the Methods and introduce in the main body of the paper as well.
21. Fig. 3B: No details are given for measurements of colocalization events, or how the C and D periods were determined, or how the colocalization period was deduced. These issues must be explicitly addressed in the body of the paper. The text on p.10 is too generic.

We modified completely this section of the manuscript to take into account suggestion of each referee. The ParS-ParB system is described in the text, the determination of the C and D period using SSB-YPet fusion to monitor replication is presented on Figure 4A-C . The estimation of sister loci colocalization according to Helmstetter equations is described in the text. The results are summarized on Figure 4C on a cartoon describing E. coli cell cycle in the conditions of the experiments performed in this manuscript.

22. Fig. 3C. MatP mutants do display a reduction in Cre-lox recombination although to a lesser extent than the colocalization of the ter regions. Thus, correlation between colocalization and cohesion does exist. Please acknowledge it in the text.

The text has been modified as suggested.
23. p13 "...were measured in wt and parC1215ts (Topo-IVTS) strains (Figure 4A)." Confusing-Delete "(Figure 4A)" and put a reference to Figure S4A instead in the next sentence.

The text has been modified as suggested.
24. Please provide more details in the legends to Figs. S3 and S4. Are the left bars in Fig S4A the no-arabinose controls?

The legends to Fig S3 and S4 have been modified as suggested.

25. p13. "Interestingly, Topo-IV alteration only moderately affected recombination frequency at the Ori-1 locus." Please explain why this is interesting.

Our new measure of intramolecular recombination for Ori-1 (performed with shorter induction pulse) allows us to detect a small (1.45 x) increase in the recombination at the Ori-1 locus upon inactivation of Topo IV . Therefore we removed this paragraph.

26. p13, bottom. "...we observed a reduction of SCI for the Ori-3 and Ter-1 respectively, 0.72 ± 0.05 and 0.76 ± 0.04". The numbers are within the experimental error and, therefore, are the same.

The text has been modified to clarify our point.

27. p14, second line. Should not it be "(Figure 4B)"?

The text has been modified as suggested.

28. Fig. S4B. Please show quantification of the micrographs and describe them in greater detail in the main text of the paper.

The text and figure S4 have been modified as suggested.

29. p14, top half. Please summarize the main result of the experiment and remove needless details: Why do we need to know the average number of foci per cell in the entire population?

The average number of foci is the value taken into account in the earlier studies (Nielsen et al 2007; Wang et al, 2008; Espeli et al, 2008) to estimate the colocalization. We have modified the text to point out that this measure is really misleading when the cell cycle is modified.

30. p14 "The strong influence of Topo-IV transient inactivation..." Given the long rationale for the next experiment, this might be a good place to start a new section.

The paragraph "*Topo-IV activity modulated sister chromatid cohesion*" is now split into two parts :i) *Topo-IV activity modulated sister chromatid cohesion and colocalization; ii) Regulation of DNA topology around the replication fork controls SCI.*

31. p14, bottom "This result also suggested that the alteration of the decatenating activity of Topo-IV, rather than the positive supercoil removal, was involved in the modulation of recombination between sister chromatids." This is consistent with, not supportive of the model. Given major chromosome packing defects in mukB mutants, many other mechanisms can be envisioned.

The text has been modified as suggested.

32. p15. After the sentence "Therefore, we tested if alteration of the gyrase activity in the gyrB203ts allele led to an increase in the intermolecular recombination rate....", please describe the phenotype of this mutant at permissive and non-permissive conditions.

The text has been modified as suggested.

33. p15; "After a 20 minutes shift to a non-permissive temperature (42{degree sign}C), a strong decrease in the intermolecular rate was observed (Figure 4D)". As shown in Fig S5, the intermolecular recombination rate did not change upon the temperature shift, the normalized rate did. However, I was not convinced that the used normalization is appropriate in this case. Indeed, both the wt and the mutant show almost 100% intramolecular recombination rate (and why is this different from the wt in Fig. S4?). It is difficult to compare, therefore, the overall level of Cre activity in the wt and the mutant at 42C. The authors should show both the normalized intra- and intermolecular recombination rates in figures 2, 3 and 4 to avoid such confusion. In addition, the authors need to repeat the experiment in Fig. S5B using shorter Cre induction times, so that their recombination rates are well below 100%.

As the referee 1 pointed out it is crucial for the normalization that the Cre induction pulses were not producing saturating amount of Lac+ colonies. Therefore we performed intra and inter molecular recombination test with various induction time according to the locus, the mutant and the temperature considered. The normalization of the data is always based on one time pulse giving

non-saturating (<80%) frequency of recombination for the wt and the mutant strain for the intramolecular recombination and one time pulse for the intermolecular. We have performed new experiments to remove data that were above or close to 80%.

Referee 2:
1. How real time is the assay- meaning how long did it take for the appearance of recombination products after the presumed cohesion? From Fig. 1C, it appears that the lag time to record cohesion could be 20 min. In Fig. 1F, it appears more like 10 min.

To evaluate the real time potential of the recombination assay we performed several new tests that are described in response to referee 1 point 2.
The difference between Figure 1C and 1F comes from the synchronization (Figure 1F) that produce in a small time scale a bigger amount of SC able to recombine that in the non synchronized culture (Figure 1C). We performed PCR and Southern blot (Figure 2D and Figure S2B) experiments that show that recombination products accumulate after Cre induction pulse because complete recombination reaction is slow.

2. The authors should mention what was the basis of choosing the four loci that they have analyzed. In particular, only at the very end of the paper it is mentioned that the Ori-3 tag is within the SNAP2 region. In that case, I would have expected more recombination at Ori-3 than at Ori1. But the results obtained were opposite.

To reinforce the biological significance of our work we added the analysis of several new loci to the manuscript. First, since the definition of the SNAP region is not yet very precise we have chosen to introduce a *laclox* cassette in the same position as a locus from SNAP1 (*gln* in Joshi et al 2011), The Ori$_{SNAP}$ locus produce strong recombination frequency, and an the higher estimated cohesion period of the Ori region. Our new estimation of the cohesion period for the Ori-3 locus (see referee 1 point 2 and 18, Figure 3B) indicates that it is also cohesive for a longer period than Ori-1. We have also measured the cohesion for a locus near the dif site (Ter$_{dif}$) which presented an extended cohesion and colocalization. Interestingly for this locus cohesion extend after the end of the C period. Finally we have chosen 3 loci located in the middle of the replication arms that show various amount of SCI, confirming that cohesion might be spotty.

3. The authors have ignored migS from the discussion. Please note that migS effect was found in fast growing cells. Later studies that discounted the importance of migS were done in very slow growing cells. This locus should be tested in the Lac/lox assay.

We have now analyzed SCI for the ori-1 and ori-3 loci in the absence of migS, their frequency of SCI remains unchanged (data not shown)
The authors should discuss their finding that "cohesion" was seen only between newly replicated regions and NOT when replication is completed (Fig 1D). This is unlike the situation in eukaryotes, where dedicated proteins (cohesins) keep the sisters together until mitosis. These features of cohesion thus do not apply to E. coli. SCC in E. coli could be a trivial outcome of the proximity of newly replicated DNA. Unfortunately, the biological relevance of the so-called "SCC" in bacteria remains questionable even after this novel approach.

Clearly sister chromatids interactions that can be detected by the laclox assay is limited to the S phase (excepted for the Ter$_{dif}$ locus). This is not the case when we measure of cohesion with the standard cell biology approach (Figure 4C, Bates and kleckner 2005, Adachi et al 2008, Espeli et al 2008). These experiments demonstrate that the colocalization of the sister chromatids corresponds to the sum of at least two events : i) topological links that maintain the sister chromatids in close contacts and allow recombination; ii) colocalization of the sister chromatids in a subcellular territory, where sister chromatids are not competent for Cre recombination. Since cohesion, in eukaryotes, is also monitored thanks to FROS tags that are observed at a poor spatial resolution, it is possible that similar hierarchical organization exist during G2 phase (region of close contact between SC and regions where the SC are physically separated but maintained in the same diffraction limited focus). These aspects are now discussed in the manuscript.
5. P.6, l.11: Change event 4 to event 3.

6.   P.7: Change Ter-1 to Ter-6 in l.2 and Figure 1F to 1E in l.16.
7.   P.8: Change "less than 50" to {less than or equal to} 50 in l.10, and mention that recombination at Ori-1 could be seen till T40 in the last line.
8.   P.9, l.18: Delete from "In order to quantitatively---Ter-1 locus (Fig 2 and S2)." In other words, describe the experiment first and then talk about the results. Add the recombination values of Fig. 2 (44%, 80% etc.) before "This observation indicates---" in the next page.

The text has been modified as suggested.
9.   P.11, top para: I could not follow how the rates 33% etc (p.11, l.3) and 26% etc. on (p.11, l.8) were deduced. Explain the deduction in the Figure legends.

The frequency of recombination according to the induction time follows exponential decay fits. We have analyzed them on Figure 3A. The single exponential fit gives access to the catalytic constants of the inter and intra molecular reactions. Considering that the intermolecular reaction is only limited by the availability of SC, the K_{inter} / K_{intra} ratio allows the direct estimation of the cohesion period.
The numbers in Fig. 2D convey the basic take home messages of this paper and should be presented more clearly. An alternative could be to show the normalized plots so that the final values can be read out directly.

The figure 2D has been removed and the results (described above) presented on Figure 3B.

10.   P.16: Change "not cohere at all" to 'not cohere extensively' in l.20 (See Fig. 5 of Nielsen 2007), and in the 2nd line from the bottom, mention generation time while discussing cohesion periods. It should be pointed out that, to the extent analyzed, there may not be any special loci for cohesion as was found by Nielsen and not by Bates.

The analysis of several new loci suggests that cohesion is indeed spotty. The differences are not huge but we observed cohesion ranging from 10 to 32 minutes. This point is discussed in the manuscript.

Referee #3
1) To quantitatively relate the level of inter-molecular recombination to cohesion lifespan, the authors sought to show that the rate of recombination (really the total number of blue recombinants) strictly reflected the substrate availability within the induction window and was not due to recombination during AND after the induction pulse.

To test this, they induced Cre for 10 minutes and analyzed Lac+ colonies after dilution (of the inducer) and direct plating compared to dilution and waiting 10min or 30min. In all cases they saw the same number of Lac+ colonies. From this, they conclude that intermolecular-recombination (Lac+ colonies) reflected substrate availability during the induction window. I don't understand how they can conclude this unless Cre is not active in cells that have been plated and only in media. To my mind the number of recombinants reflects inter-molecular recombination during induction and after - until Cre is degraded or diluted to a significant degree.

        This is a key point for every referee; we have taken special attention to this aspect. I have summarized for the referee1 point 2 the experiments that we have performed to control that most of the recombination events involve only SC that are close enough to recombine during the Cre induction pulse.

2) After a 10min induction pulse the authors report 44% recombination for their origin tag (Ori-1). If recombination only occurred during the induction window, this would indicate that 44% of the cells were cohesed at Ori-1 in this 10min. window. They are working with an asynchronous culture grown in minimal medium with an ~80 min doubling
time. This doesn't sound right to me. It seems much more likely that the 44% reflects recombination during induction and after - whether it is on plates or in liquid.

The intermolecular recombination frequency cannot be used directly to extract the cohesion time of a define locus. Because each locus present a specific reactivity for the Cre recombinase the only way to estimate the period when the two SC are close enough to recombine is the ratio inter molecular /

intra molecular. We believe that the more accurate way to estimate the cohesion from the frequency of recombination is the exponential decay fit described on figure 3A, it allows the determination of the catalytic constant for each reaction. We proposed that intra molecular reaction is possible at any step of the cell cycle, while intermolecular is only limited by the availability of the SC, therefore the Kinter / Kintra ratio gives the proportion of the cell cycle when SC are close enough to recombine, i.e. 0.23 X 80 min = 18 min for the Ori-1 locus

3) With this in mind, it makes is hard to think about "rates of recombination" and thus extremely hard to use these experiments to infer cohesion times. I think we can say from Figure 2C that the amount of cohesion is likely to be longer (or replication slower) at Ori-1 compared to the other sites because there is more recombination after 10 minutes. But other than this qualitative conclusion I don't see how you can use asynchronous cultures and the terminal phenotype of blue colonies to establish cohesion time. Maybe I'm thinking about this incorrectly, if so, the authors should spend more time in the text explaining the logic of these experiments.

A western blot examining Cre stability after a 10min induction pulse would be informative. Maybe if they had a degradable allele of Cre these experiments would be more interpretable.

The referee 3 is right, a western blot analysis monitoring Cre turnover would be a great control for the point we'd like to make on the figure 2 and 3. Unfortunately, we could not find any commercial Cre antibody that reveals Cre protein on western blot from E. coli protein extract. Some of them did not even recognize the purified Cre protein. We provide on Figure 2 a number of experiments suggesting that only the Cre protein able to catch *loxP* sites during the induction pulse are able to recombine SC. These elements are described on the referee 1 point 2 paragraph

The explanation for what was normalized in the intra and inter molecular recombination experiments and why this normalization makes sense needs to be made more clearly. I found this section very confusing.

The description of the intramolecular assay has been modified to make it easier to read. This assay is critical to be able to compare the intermolecular frequency for different loci, genetic background or temperature. *"Differential abilities of chromosome regions to promote laclox recombination could reflect either differential abilities for sister chromatids to interact or the difficulty for Cre to form synapsis between loxP sites in some regions of the chromosome. To test these possibilities, we measured for the same loci the capacity of intermolecular and intramolecular loxP/Cre recombination (Figure 2A)."*

Section entitled "Distinction of SCI and colocalization of loci":
"In growing conditions used, we estimated that sister Oir-1, Ori-3 and Ter-1 loci were co-localized for 30, 20 and 35 minutes." Was this estimate based on Helmstetter? If yes, this should be mentioned here - not in following section.

We modified completely this section of the manuscript to take into account suggestions of each referee. The determination of the C and D period using SSB-YPet fusion to monitor replication is presented on Figure 4A-C . The estimation of sister loci colocalization according to Helmstetter equations is described in the text. The results are summarized on Figure 4C on a cartoon describing E. coli cell cycle in the conditions of the experiments performed in this manuscript.

In the MatP section:
"14 to 18% decrease, respectively, for the Ori-3 and Ter-1 loci" Should this be Ter-1 and Ter-6 loci?

The text has been modified as suggested.
Ori-3 tag is located in SNAP 2 a region reported to have extended co-localization (Joshi et al). However, this region does not appear to have increased recombination between sisters nor do the authors report prolonged co-localization. A discussion of these results in light of previous reports seems important.

To reinforce the biological significance of our work we added the analysis of two new loci to the manuscript. First, since the definition of the SNAP region is not yet very precise we have chosen to introduce a *laclox* cassette in the same position as a locus from SNAP1 (*gln* in Joshi et al 2011), the Ori$_{SNAP}$ locus produces strong recombination frequency, and the higher estimated cohesion period of the Ori region. Our new estimation of the cohesion period for the Ori-3 locus (see referee 1 point 2 and 18, Figure 3B) indicates that it is also cohesive for a longer period than Ori-1. Our work reveals the molecular cohesion in the SNAP is not dramatically longer than in the other Ori loci, this is different from what was observed by standard cellular biology approach. We made similar observations in the Ter region. These experiments demonstrate that the colocalization of the sister chromatids corresponds to the sum of at least two events : i) topological links that maintain the sister chromatids in close contacts and allow recombination; ii) colocalization of the sister chromatids in a subcellular territory, where sister chromatids are not competent for Cre recombination.
The role of the SNAP regions in the topological links between SC detected in this work is discussed in the manuscript.

Section entitled: "Sister chromatids were cohesive for a limited time window after their replication" Description of synchronization method is not mentioned until second to last sentence of section. The section would be easier to read if this was described earlier.

The text has been modified as suggested.

2nd Editorial Decision   25 June 2012

Thank you for submitting your revised manuscript for our consideration. Two of the original reviewers have now assessed it once more - see comments below - and I am pleased to inform you that they both consider the paper now in principle suited for publication in The EMBO Journal. Referee 2 lists a number of specific points such as text corrections or requests for further discussion/altered interpretation in some instances, which I would like to ask you to address in a final round of minor textual revision. When re-revising, you may also want to consider the title change as proposed by this referee.

Once we will have received the final version incorporating these additional changes, we should then be able to swiftly proceed with formal acceptance and production of the article!

Yours sincerely,
Editor
The EMBO Journal

_____

Referee #1 (Remarks to the Author):

The authors have done an excellent job addressing all concerns of the reviewers. The manuscript is ready for publication.

Referee #2 (Remarks to the Author):

1. Title: The topic of the paper is Sister Chromatid Interactions (SCI) - the title might as well say that directly. I would change "Topological links between sister chromatids" to SCI. Topological linking is only a part of the paper and the evidence for "linking" is at best indirect.
2. P.3, l.4: Change "chromatids" to chromatid.
3. P.4, l.17: This paper does a good job in demonstrating SCI but NOT SSC - no effect of MukB was seen. It would be best to stay away from using "cohesion" as far as possible.

4. P.7, l.16: Change "at 5" to five more.

5. P.8, 8-12: Delete. What you mean by low? What was the basis for expecting higher values?

6. P.9, l.4: Change "rifampicine" to rifampicin.

7. P.9, l.17: 135 or 140 min?

8. P.9, l.21: Change "S3A" to S2A and "suggested" to suggest- inferences are generally made in the present tense. Change also, showed to shows in p.13, l.20.

9. Fig. 2D: Define "A.I" in the ordinate. Shouldn't the last two points of the grey line be 35 and 50 min, not 40 and 50 min? I would also write the abscissa as Cre (min) or 10 min Cre + delay (min), otherwise +delay may refer to the both the Cres.

10. P.11, l.2: Change S-1 to sec-1. It would be clearer what you mean.

11. P.13, l.3: Add 'period of' after "estimated".

12. P.13, l.23: Either elaborate or delete the sentence starting with "Strikingly,--." It is confusing the (next) concluding sentence of the section on p.14 - which is an important message of this paper.

13. P.14, l.19: " Similar reduction" confused me. First say that upon overproduction of TopoIV there was a reduction of SCI and then say that the degree of reduction was similar for the two loci. This would clarify that the results at the nonpermissive temperature and under overproducing conditions are dissimilar.

14. P.16, top: I see a bit of contradiction here: If MukB affects removal of positive supercoils, it should also affect precatenane formation, which depends on positive supercoils. In mukB mutant, my expectation would be lower SCI values compared to the wt (Fig. 5C). It appears that the expectation from in vitro results is not borne out here. This may be discussed.

15. P.16, l.16: Mention 'at the permissive temperature' after "Compared with the wt strain.

16. P.16, last three lines: Fig. 5D 42{degree sign}C results are opposite of what I would predict. Line 5 of this page says that precatenanes help to increase SCI. In the absence of gyrase, precatenanes should increase. Why then SCI is less?

17. P.17, l.22: Why "slightly"? In Fig. 4C, the difference between red and green lines are about two-fold for some markers. Since this is the main topic of this paper, be more specific.

18. P.18, l.6: Change SSC to SCC?

2nd Revision - authors' response   28 June 2012

I would like to thank you and the referees for the processing of the manuscript. I believe that the manuscript has been greatly improved by your advices and the comments of the referees.
I have taken into account every aspect pointed out by referee 2. I have summarized below the important points that have been modified in the new version of the manuscript

1) I have modified the title of the manuscript: "**Sister chromatid interactions in bacteria revealed by a site-specific recombination assay.**"

2) As suggested by referee 2, I have replaced SCC (sister chromatid cohesion) by SCI (sister chromatid interaction) in the result section and limited the use of SCC to the discussion to describe to potential of topological links to promote SCC in bacteria.

3) I have chosen not to develop the fact that MukB deletion did not modify the level of SCI (referee 2 14th point). I believe that revealing the connection between MukB and Topo-IV in vivo requires an in depth analysis and that our assay was just brushing the topic.

4) We agree with referee 2 the results of the gyrase mutant experiment were opposite of what we predicted. We believe that we have a too simple view of the topological challenges encountered by the replication fork. We have modified the text (page 16) to give an interpretation of this apparent contradiction: " We propose that a slight decrease in the DNA gyrase activity at a permissive temperature was sufficient to increase precatenation without impeding replication (Grompone et al, 2003). At a non-permissive temperature, excess positive supercoils ahead of the fork trigger rapid replication fork blockage, and ongoing decatenation by Topo-IV reduces the sister chromatid interaction frequencies."