Structure of the SSB-DNA polymerase III interface and its role in DNA replication

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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 28 April 2011

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by three referees whose comments are enclosed. As you will see, all three referees express interest in your manuscript and are generally in favour of publication, pending satisfactory revision of a limited number of specific issues. Among these, the most significant one is the concern that different mutants were used in the in vitro and in vivo experiments (see referees 2 and 3), which will have to be addressed by comparing these mutants side-by-side in replication assays.

Should you be able to satisfactorily address this point, as well as the more specific technical and presentational issues, then we shall be happy to consider the study further for publication in light of the reviewers' positive recommendations. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version.

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

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.

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):

This paper was a delight to read. It is well written and presents some very interesting results concerning the role of the SSB-chi interaction in DNA replication. The identification of the SSB binding site on chi enabled the authors to mutate that site on chi and thus disrupt the interaction. Previously, that interaction was probed via changes to the SSB c-terminus, which not only disrupted chi binding, but also SSB binding to nearly 12 other proteins. Thus it was impossible to determine the effect of disruption of the SSB-chi interaction. The current ms. has overcome this problem and demonstrates that the SSB chi interaction is important for stabilizing the replisome and in coupling leading and lagging strand synthesis. Furthermore, the results reported here rule out a previous suggestion that the SSB-chi interaction is important for lagging strand primer handoff. The results reported here are very clear and represent a significant advance in our understanding of the important role of SSB in DNA replication.

I have only one minor concern about the ITC experiments. The stoichiometries are given in terms of SSB monomer-chi stoichiometries, yet the SSB is clearly tetrameric. Thus these should be reported as chi bound per tetramer. In this regard, the actual model used to analyze these data should be reported.

Referee #2 (Remarks to the Author):

The paper presented by Marceau A.H. et al addresses the role of Chi interaction with SSB in DNA replication and presents important for the field of DNA replication findings. High resolution structure of the Psi-Chi complex with the C-terminal tail of SSB is of a high significance in light of the growing support for previously underappreciated active role of SSB in DNA metabolism. While the existence of multiple SSB-binding proteins underscores the importance of SSB in DNA replication, the common binding motif of SSB makes it difficult to delineate the functional role of specific interactions. The presented structure provided opportunity to design specific mutants to exclusively target Chi-SSB binding without compromising other potential functions of Chi and SSB, particularly in vivo. In vitro and in vivo experiments with these mutants complement previously published results, where SSB mutant was employed to address similar questions. Therefore, the manuscript is certainly worth publishing.

At the same time, few points of uncertainties of either experimental design and interpretation or disagreement with previously published results are not sufficiently explained and discussed. The major drawback of the paper is utilization of different mutants for in vivo and in vitro experiments. This makes it difficult to directly connect results. Will double mutant support partial lagging strand synthesis as well?

Interpretation of in vitro replication assay is somewhat confusing and additional discussion would be very helpful. The lack of binding of R128A mutant was demonstrated by binding to free SSB. Within replisome, this interaction can be stabilized due to high local concentration or additional contacts of other DNA binding subunits. While authors made this argument to explain design of a more severe mutant for in vivo studies, this issue is not discussed in respect to in vitro data.

The connection between primase concentration and salt dependence is not clear. Can it be explained by inability to "recycle" primase?
In this respect, more specific explanation of differences with previously published results is essential to appreciate significance of presented studies. The authors simply stated that previously employed SSB113 mutant affect additional interactions beyond Chi, while omission of Chi could be disruptive for Psi function and stability. Yet, previous studies have addressed multiple interactions and reaction steps and those results have to be discussed. For example, the reaction without Chi resulted in apparently smaller or no effect on leading strand synthesis in rolling replication and no lagging strand synthesis, yet, obliteration of one specific Chi-SSB contact resulted in opposite effect on both leading and lagging strands. Is it a consequence of the potential residual interaction of R128A mutant within replisome? Does it mean that without Chi leading and lagging strand synthesis is simply uncoupled, while with Chi mutant both polymerases are destabilized? Can it be that the stabilization of leading strand polymerase is not essential at low salt?

In respect to in vivo experiments, why simple dilution of cells growing in log phase disrupts active replication (log to lag phase transition)?

Overall, the structural part is of a great significance for understanding the specific role of Chi-SSB interaction, and, even beyond structure-guided mutagenesis, for understanding general mechanism of SSB function in replication, recombination and repair. While overall conclusion about the importance of Chi-SSB contact for stability of the active replisome is sound, it would be helpful to discuss results and interpretation of biochemical experiments in connection to previously published studies of similar interactions.

Additional points:

Page 11 - 'slightly shorter Okazaki fragments' are almost twice shorter accordingly to Fig. 3E

Page 12 - 'variatiu'

Page 17 - 'the most impaired variant'. R128A mutant utilized in vitro is likely to be less impaired than KR/EE mutant.

Page 23 - PolIII* purification is in SSB section.

Fig. 3C and F - is titration by DnaG started at 0?

Referee #3 (Remarks to the Author):

The manuscript submitted by A. H. Marceau et al. investigates the interaction between an E. coli DNA polymerase III holoenzyme subunit, chi, and single-stranded DNA binding protein, SSB. The authors solve the structure for a co-crystal containing a C-terminal peptide from SSB consisting of 5 amino acids bound to the chi subunit of a chi-psi complex. Based on interactions observed in the co-crystal, the authors engineer site-directed mutations in the chi subunit and analyze the effects of these mutations on chi-SSB binding, DNA replication, cell growth, viability, and morphology, and SOS induction. The approach the authors used to determine the importance of chi-SSB interactions on DNA replication is elegant because the mutations made to chi are specific for this interaction and do not affect other interactions that chi or SSB make at the replication fork. Previous studies have used holC-null strains in which chi is completely absent or a ssb113 strain which contains a ts mutation in SSB that may have effects other than the interaction with chi. This work has the potential to be an outstanding publication if an additional experiment is done. The main criticism is that in vitro experiments including binding studies and replication assays are performed with the chi-R128A mutant, but this mutant did not differ from wild-type chi in vivo. A chi double mutant, chi-R128E/K132E, showed a marked difference in cell growth and replication, and induced SOS in comparison with wild-type chi, but this mutant was not characterized in biochemical assays. The authors assume that the double mutant will show the same biochemical deficits in vitro as the R128A single mutant, but this may not be the case given the difference in phenotypes in vivo. The authors should repeat replication assays with the chi-D128E/K132E mutant to determine whether this mutant behaves the same as chi-R128A.
Specific comments:
1) In general, the Figure legends do not contain enough information to easily understand the experiment and data being presented. Readers will have to work to determine what is being shown and how experiments have been performed. Figure 3 would benefit from a schematic diagram illustrating the rolling circle replication assay.

2) Is the interaction between the four C-terminal residues of SSB and chi relevant to the interactions between intact proteins? The N-terminal Trp residue of the SSB peptide is not shown in Fig. 1. Was there density present for this residue?

3) Why does the sequence of the pentapeptide (Trp-Asp-Ile-Pro-Phe) given in the Methods differ from the sequence of the C-terminal tail of SSB shown in Fig. 1 (Asp-Asp-Asp-Ile-Pro-Phe)?

4) Fig. 2 - What model was used to fit the binding isotherms? Are the errors reported errors in the fit or standard errors in individual experiments? How many replicates were done?

5) Fig. 3 - How many replicates were done? Error bars should be added to panels B, C, E and F.

6) It would be nice if the authors could comment on how the in vivo activity of the chi-D128E/K132E mutant compares to a holC-null strain based on data in the literature. Are they similar? Or, do the mutations in chi have some effect that is not observed in the absence of chi?

We are pleased to submit our revised manuscript, "Structure of the SSB-DNA Polymerase III interface and its role in DNA replication" (EMBOJ-2011-77778) for publication in EMBO Journal. We appreciate the reviews of our original manuscript and have addressed the issues raised by the reviewers as detailed below.

Please don’t hesitate to contact me with any questions regarding our revised submission. I look forward to hearing from you.

Summary of changes:

Reviewer 1

(1) The stoichiometries are given in terms of SSB monomer-chi stoichiometries, yet the SSB is clearly tetrameric. Thus these should be reported as chi bound per tetramer. In this regard, the actual model used to analyze these data should be reported.

We have now included information in the Experimental Procedures and in the legend for Figure 2 that more clearly described the overall arrangement of the SSB// complex and the model used to fit the calorimetry data. The simplest possible model, independent binding of a single // complex to each SSB-Ct element, was used in this analysis.

Reviewer 2

(1) The major drawback of the paper is utilization of different mutants for in vivo and in vitro experiments. This makes it difficult to directly connect results. Will double mutant support partial lagging strand synthesis as well?

We have now constructed, purified, and analyzed the biochemical activity of the R128E/K132E-containing Pol III* complex. This complex has activities that parallel that of the R128A-containing Pol III* complex: lagging-strand DNA replication is supported whereas leading-strand
DNA replication is even more inhibited by the addition of 70 mM KCl than was observed with the R128A-containing Pol III* complex. Moreover, this analysis bridges our in vitro observations with our in vivo data on the R128E/K132E-encoding holC mutant. The in vitro R128E/K132E Pol III* data are included in the revised Figure 3 and revised Supplementary Figures 2 and 3.

(2) Interpretation of in vitro replication assay is somewhat confusing and additional discussion would be very helpful. The lack of binding of R128A mutant was demonstrated by binding to free SSB. Within replisome, this interaction can be stabilized due to high local concentration or additional contacts of other DNA binding subunits. While authors made this argument to explain design of a more severe mutant for in vivo studies, this issue is not discussed in respect to in vitro data.

We have now included additional discussion of this point in the Results section.

(3) The connection between primase concentration and salt dependence is not clear. Can it be explained by inability to “recycle” primase?

The primase concentration dependence of Okazaki fragment lengths is consistent with previous observations cited in our manuscript. Namely, the average length of Okazaki fragments decreases as primase concentration is increased. The earlier work on this subject showed that primase dissociates and reassociates with the DNA replication machinery. As is discussed in the manuscript, the salt effects observed with Pol III* variants instead appear to affect the overall stability of the replisome. There is a fractional offset of the Okazaki fragment size, but this is true across all salt concentrations tested (Figure 3F).

(4) In this respect, more specific explanation of differences with previously published results is essential to appreciate significance of presented studies. The authors simply stated that previously employed SSB113 mutant affect additional interactions beyond Chi, while omission of Chi could be disruptive for Psi function and stability. Yet, previous studies have addressed multiple interactions and reaction steps and those results have to be discussed. For example, the reaction without Chi resulted in apparently smaller or no effect on leading strand synthesis in rolling replication and no lagging strand synthesis, yet, obliteration of one specific Chi-SSB contact resulted in opposite effect on both leading and lagging strands. Is it a consequence of the potential residual interaction of R128A mutant within replisome? Does it mean that without Chi leading and lagging strand synthesis is simply uncoupled, while with Chi mutant both polymerases are destabilized? Can it be that the stabilization of leading strand polymerase is not essential at low salt?

[related point made later in the review] Overall, the structural part is of a great significance for understanding the specific role of Chi-SSB interaction, and, even beyond structure-guided mutagenesis, for understanding general mechanism of SSB function in replication, recombination and repair. While overall conclusion about the importance of Chi-SSB contact for stability of the active replisome is sound, it would be helpful to discuss results and interpretation of biochemical experiments in connection to previously published studies of similar interactions.

We have now added additional discussion of our results with respect to earlier results to address these questions.

(5) In respect to in vivo experiments, why simple dilution of cells growing in log phase disrupts active replication (log to lag phase transition)?

We have addressed this question to the best of our ability in the manuscript. We agree that this observation is puzzling and will require additional experimentation that is beyond the scope of this manuscript to answer. An important observation that is included in our manuscript is that inactivation of the recA suppresses some of the phenotypic problems, indicating that recombinational repair of aberrant replication and/or accumulated DNA damage is important for establishing these phenotypes.

(7) Minor points: (a) Page 11 - 'slightly shorter Okazaki fragments' are almost twice shorter accordingly to Fig. 3E, (b) Page 12 - 'variant' (c) Page 17 - ‘the most impaired &fx03C7; variant’. R128A mutant utilized in vitro is likely to be less impaired than KR/EE mutant. (d) Page 23 -PolIII*
purification is in SSB section. (e) Fig. 3C and F - is titration by DnaG started at 0?

These are all addressed or repaired in the revised manuscript. (a) The word "slightly" was struck in the revised manuscript. (b) Misspelling is corrected (c) The new experiments with the double-variant chi protein help address this point. (d) The Pol III* purification is actually in the "Protein Purification" section of Experimental Procedures and simply follows the subsection on SSB. (e) The first [DnaG] is 0, as is indicated in the original figure and in the Experimental Procedures section of the revised manuscript.

Reviewer 3

(1) This work has the potential to be an outstanding publication if an additional experiment is done. The main criticism is that in vitro experiments including binding studies and replication assays are performed with the chi-R128A mutant, but this mutant did not differ from wild-type chi in vivo. A chi double mutant, chi-R128E/K132E, showed a marked difference in cell growth and replication, and induced SOS in comparison with wild-type chi, but this mutant was not characterized in biochemical assays. The authors assume that the double mutant will show the same biochemical deficits in vitro as the R128A single mutant, but this is may not be the case given the difference in phenotypes in vivo. The authors should repeat replication assays with the chi-D128E/K132E mutant to determine whether this mutant behaves the same as chi-R128A.

See response (1) to Review 2.

(2) In general, the Figure legends do not contain enough information to easily understand the experiment and data being presented. Readers will have to work to determine what is being shown and how experiments have been performed. Figure 3 would benefit from a schematic diagram illustrating the rolling circle replication assay.

We have added additional information to the figure legends and a schematic of the rolling circle replication assay to Figure 3 in the revised manuscript.

(3) Is the interaction between the four C-terminal residues of SSB and chi relevant to the interactions between intact proteins? The N-terminal Trp residue of the SSB peptide is not shown in Fig. 1. Was there density present for this residue?

[related point made later in the review] Why does the sequence of the pentapeptide (Trp-Asp-Ile-Pro-Phe) given in the Methods differ from the sequence of the C-terminal tail of SSB shown in Fig. 1 (Asp-Asp-Asp-Ile-Pro-Phe)?

We have now discussed this more thoroughly in the revised manuscript. Longer SSB-Ct peptides did not work in crystallization experiments but the more limited peptide reported in our manuscript did. The Trp residue is not part of the natural SSB-Ct sequence but was added for quantitation purposes, as is described in Experimental Procedures. Electron density was not observed for this residue.

(5) Fig. 2 - What model was used to fit the binding isotherms? Are the errors reported errors in the fit or standard errors in individual experiments? How many replicates were done?

See response (1) to Review 1. The reported errors are errors in the fit. Each titration was done several times using different concentrations of SSB and / variants and the final data are consistent with each of these binding experiments. Single experiments are reported in the revised manuscript as is typical for ITC.

(6) It would be nice if the authors could comment on how the in vivo activity of the chi-D128E/K132E mutant compares to a holC-null strain based on data in the literature. Are they similar? Or, do the mutations in chi have some effect that is not observed in the absence of chi?

We wish that this was possible, but the descriptions of holC deletion strains have been very limited. One report is simply unpublished data that are described in a review (Kelman and O’Donnell, 1995) whereas another reports temperature-sensitive viability of the holC-deletion strain but not additional
detail on strain phenotypes (Saveson and Lovett, 1997). This latter study focused on a screen examining the effects of many DNA replication mutants on DNA deletion formation; holC was not the major focus of the work. We have described the relationship of the point mutations in our study to the prior limited description of holC mutants in the revised manuscript.

Acceptance letter
29 July 2011

Thank you for submitting your revised manuscript for our consideration. It has now been seen once more by two 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

(Remarks to the Author)
This is an excellent manuscript ready for publication.

note: the sentence "Pol III* complexes were purified .... as described in Supplemental Information" in SSB purification sub-section should be removed.

Referee #3

(Remarks to the Author)
The authors adequately addressed the questions that I posed in their revised manuscript and now I believe the manuscript is suitable for publication in the EMBO Journal.