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Structural basis of initial RNA polymerase II transcription

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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 29 August 2011

Thank you for submitting your manuscript for consideration by The EMBO Journal. I have now received the report from the final referee and I enclose their reports below. As you will see from their comments the referees provide mixed recommendations, referee #1 clearly does not support publication and has concerns if the structures are true representations of the OC and ITC as well as their resolution. However, referee #2 and #3 who both strongly support publication in The EMBO Journal do not support these concerns. Referee #2 would like to see the presentation and discussion of the structures to be significantly extended to emphasize the significance and originality of the current work. From the reports it is important to incorporate the suggestions made by referee #2 and also comment on the issues raised by referee #1, including a more detailed comparison with the Liu et al paper in Science. Given the strong support form two referees I would like to invite you to submit a revised version of the manuscript. Given that no additional experimental work is required I would like to work with a deadline of resubmission of one month, please let me know if you feel that this is possible.

I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. When you submit a revised version to the EMBO Journal, please make sure you upload a letter of response to the referees' comments. Please note that when preparing your letter of response to the referees' comments that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website: http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

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Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS:

Referee #1 :

In this paper, Cramer's group crystallized and solved structures of yeast pol2 with DNA with or without short RNAs to obtain structural insights into the open complex (OC) and initially transcribing complexes (ITC) that has been less well studied in any multi-subunit cellular RNA polymerases. Although Cramer's group is a leader in this field and reported many important structures of polymerases, this reviewer was not impressed by this work thereby suggests sending this work to more specialized journals such as Protein science or Acta crystallographica.

Here are this reviewer's problems:

1) Authors claimed their structures are OC and ITC mimics, this may not be true. There is no data supporting this assumption. For me, their structures are just polymerase transcribing short RNAs from non-promoter DNA. As authors described in this paper and also previous Nature paper (reporting the Pol2-TFIIB complex), pol2 requires general transcription factors for making open complex and initiating transcription. Especially, TFIIB N-terminal domain plays multiple roles in these processes. Lacking TFIIB in their structures makes this reviewer wonders what they observed are also true in the biologically relevant transcription processes.

2) Based on structure comparisons between OC and ITCs from this work and transcription elongation complexes (TECs) from their previous works, they proposed that the pol2 positions DNA/RNA hybrid of ITCs slightly different (~1.5 Angstroms) from one observed in TEC. However, all structures were determined ~3.5 Angstroms resolutions, thereby discussing ~1.5 Angstroms difference is not appropriate using non-high resolution structures. Authors claimed that their structures have very good R-factors and stereochemistry, but it doesn't mean good structures of DNA and RNA. Their contributions of R-factors and stereochemistry can be easily diluted within ~500 kDa molecular weight pol2 structure. If authors want to tell good structures of DNA and RNA, they should show B-factor plot of them that can convince crystallographers to support the structures, if the B-factor is not over 100.

3) Nearly the same structures and conclusion reported in this work was already reported from Kornberg's group in Science last week that substantially decreased novelty of this work. At the end of this manuscript, authors stated some differences between their and Kornberg's works but their discussion are not meaningful. Below are their clams and my responses.

First, we used the complete 12-subunit Pol II and the downstream duplex was visible in all our structures.
--Using 12-subunit pol2 makes their resolution worse and having downstream DNA in the structure doesn't tell any new insight.

Second, TFIIB was not required in our experiments to generate ITCs.
--This means their structures are not ITCs as I explained in 1).

Third, in contrast to Liu et al., we could resolve structures with DNA alone and with 2nt RNA, both with and without NTP, providing evidence that Pol II is able to bind free DNA and a very short hybrid with some stability.
--This is obvious and we knew already otherwise pol2 cannot initiate de novo transcription.

Forth, although the ITC hybrids also appeared tilted, we did not position a +1 templating base in the active site and hence did not observe the unusual deviation from DNA-RNA base pairing described by Liu et al.

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--Cannot discuss such a small difference with lower resolution structures.

Finally, we did not observe a fraying of the RNA 3'-end in the ITCs.
-- Cannot discuss such a small difference with lower resolution structures.

Referee #2 :

This is a nice piece of work that I believe may provide a significant contribution to the field. However, in spite of a huge body of experimental structural data included in this MS (11 crystal structures) the MS is surprisingly concise (26K vs 54K characters allocated by the Journal). In my opinion, the compressed style of presentation did not allow the authors to emphasize significance and originality of their work on a full scale.

Specific comments

1. Title (and also in the abstract).

"Open promoter complexes" look somewhat confusing as they are likely not limited to only PolII and a short ssDNA template.

2. Abstract.

In my understanding, the authors used the non-hydrolyzable NTP analog that should be acknowledged here.

It is also strange that the Abstract does not mention the trigger-loop (TL) configuration in the NTP+ complexes that I feel is an interesting and essential part of the work.

3. Introduction, par. 1
"...a stable elongation complex (EC) that contains a 8-base pair (bp) DNA-RNA hybrid".

I would speak about the "~8-9bps" as the actual length of the hybrid seems to be not yet elucidated and, in fact, may possibly vary in a course of elongation.

4. Introduction, par. 2
"Initial RNA synthesis apparently does not require additional protein factors, since .."

Do the authors mean that nucleotide addition in the natural initiation complexes do not "require additional protein factors" (and upstream DNA?)? If so, more introductory materials/discussion should be provided here as a reference to a single (quite early, 1982) paper does not look very decisive.

5. Page 5, par. 1
"The structures were refined at resolutions between 3.4 and 3.8 ≈ and show very good R-factors and stereochemistry (Table 1)".

There is no stereochemistry in the Table 1.

6. Page 6, par. 1
"... we soaked ITCs with the non-reactive NTP analog, (AMPCPP, or GMPCPP in case of the 2nt RNA), and solved the structures at resolutions between 3.4 and 3.8 ≈ (Table 1)".

GMPCPP is not shown in the Table 1.

7. Section: "Conserved mechanism of NTP selection"
So far, the two experimental structural models (for bacterial and yeast RNAP) have been published showing the significant structural differences for the two key elements of the NTP+ closed, "insertion" ECs, the NTP binding configuration/position and conformation of the folded TL. In my understanding, the authors now report a third, "hybrid" model in which the NTP position and

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conformation is reminiscent of that in the bacterial EC, while the TL fold looks (at least in the Fig. 6b) more similar to that of the previously reported for the yeast enzyme. This is an essential section of the MS that provides an additional independent structural view on the NTP+ closed configuration in multi-subunit RNAPs. I, therefore, believe that these results should be presented and discussed in more details and suggest that the authors provide following additional information for this section (that may be presented in Supplement):

a) the local Ramachadran statistics (% residues in the 4 regions as defined by the program PROCHECK) and B-factors for the flexible portion of the folded TL;
b) the stereo views showing the folded TL (flexible part) with the side chains superimposed on the omit difference ED produced before and after refinement;
c) the stereo views showing superposition of the bacterial and previously published yeast (if different from that in the authors' structure) folded TL configurations on the authors' initial omit ED.
d) for additional proofreading, it would be worth showing the omit maps after refinement of the authors' complex structure using the alternative TL configurations.

7.1 Page 6, last par.

Here (and throughout the MS where the NTP loading is discussed), citations of Vassylyev et al. included in the reference list is not appropriate in the context; the NTP binding to the bacterial EC was reported in the other paper.

7.2 Page 7, par. 2
"The NTP position and interactions are essentially identical in the previous structure of a bacterial RNA polymerase EC with AMPCPP (Vassylyev et al, 2007) (Fig. 6c), indicating a universally conserved mechanism of nucleotide selection and incorporation fidelity in all cellular RNA polymerases".

To strengthen this authors' conclusion, it is essential to schematically show NTP interactions in both (yeast and bacterial) complexes along with the local sequence alignment of the respective protein structures.

7.3 Page 7, par. 2
"In addition to N479, NTP/dNTP discrimination may involve the trigger loop residue Q1078, which may couple the presence of an NTP with a 2'-OH group to closing of the trigger loop, thereby restricting catalysis to NTPs".

This is an interesting and important prediction that I feel would benefit from illustration of the supportive experimental data, i.e. the ED in vicinity to the interacting groups.

8. Discussion.
The first half of this section is largely redundant to the previous text and, frankly, I did not see much discussion in this section. A small piece of new information provided by the authors here might be well compressed to a single paragraph or, may be even better, appended to the respective sections of the Results. In a second half of Discussion, the authors briefly list up the differences between their work and the similar published results (Liu et al, 2011) providing almost no comments and/or comparison. It is a bit disappointing provided, in particular, that the overall size of the MS is ~2 times smaller than that allocated for the Research Articles in the Journal. In addition, some statements in discussion appear to be quite vague (at least in my reading) and sometimes detract from (rather than emphasize) the originality of the work. I suggest that this section is thoroughly re-written to provide more mechanistic implications and better focus on the comparative analysis with the published competing results (Liu et al, 2011).

8.1 Page 10, par. 1
"Comparisons with previous structures of ECs with bound NTP (Kettenberger et al, 2004; Vassylyev et al, 2007; Wang et al, 2006) indicate that the exact positioning of the NTP depends on the experimental design".

In my reading, with no subsequent discussion, this statement hints that both experimental designs are not physiologically relevant.

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8.2 Page 10, par. 2
"Transcription initiation in vivo requires ..."

This paragraph seems to be loosely related to the discussion content and looks more as an introductory one.

8.3 Page 10, par.3
"Liu et al. revealed densities of DNA-RNA hybrids in complex with PolII that are overall similar to the ones revealed here and describe a similar transition in hybrid conformation between the ITC and EC, ...".

and Page 11, par. 1
"...although the ITC hybrids also appeared tilted, we did not position a +1 templating base in the active site and hence did not observe the unusual deviation from DNA-RNA base pairing described by Liu et al.".

Did the authors look at the ED of Liu et al.? Does this ED accommodate the RNA/DNA hybrid model (in particular, at the 4/5 bps level) presented by the authors?

In addition, Liu et al. also report the NTP+ complex. Is the TL conformation (and ED) is similar to that observed by the authors?

9. Table 1.

This Table should be re-worked to include all standard crystallographic information describing each of the complexes under study (i.e. data collection and refinement statistics). It is critical that, in the revised Table, the authors include the Ramachadran statistics and B-factors for the overall structure (see Comment #7).

Referee #3 :

This manuscript reports a set of Pol II - nucleic acid structures that reveal the transition of Pol II from the open complex state through initial transcribing complexes of 2-7 nt RNA. The open complex mimic showed that Pol II contains a binding sites for downstream double stranded DNA and the template strand opposite the active site - previously modeled based on the stable elongation complex structure. The 2 nt - Pol II structure reveals key contacts with Rpb2 that likely stabilize short RNAs in initial transcribing complexes. The 4-5 nt complexes reveal a tilted hybrid with a shifted position of the DNA template strand that can be translocated by addition of the next NTP. Finally, the results add more detail to the mechanism of how NTPs are selected by Pol II and comparison of these results to the literature show that this mechanism is conserved. The manuscript is very well written and the results are an important advance.

A recent paper from the Kornberg group overlaps with some of these results. However, the methods and structures in the Cramer group paper are sufficiently different and the results are of high importance, so that this paper should be published in EMBOJ. As outlined in the discussion, there are at lest 5 differences in this work with the Kornberg paper. Together, these two papers reveal key mechanisms in the transition from initiation to elongation and nicely explain many previous biochemical results.

1st Revision - Authors' Response 04 October 2011

Detailed list of responses to referee concerns

Referee #1 :

In this paper, Cramer's group crystallized and solved structures of yeast pol2 with DNA with or without short RNAs to obtain structural insights into the open complex (OC) and initially transcribing complexes (ITC) that has been less well studied in any multi-subunit cellular RNA polymerases. Although Cramer's group is a leader in this field and reported many important structures of polymerases, this reviewer was not impressed by this work thereby suggests sending this work to more specialized journals such as Protein science or Acta crystallographica.

We understand some of this reviewers' concerns and have addressed them in the revised version as described below. We however disagree with the conclusion that the work should be published in a technical journal since it does contain many biological implications of high significance and broad interest.

Here are this reviewer's problems:

1) Authors claimed their structures are OC and ITC mimics, this may not be true. There is no data supporting this assumption. For me, their structures are just polymerase transcribing short RNAs from non-promoter DNA. As authors described in this paper and also previous Nature paper (reporting the Pol2-TFIIB complex), pol2 requires general transcription factors for making open complex and initiating transcription. Especially, TFIIB N-terminal domain plays multiple roles in these processes. Lacking TFIIB in their structures makes this reviewer wonders what they observed are also true in the biologically relevant transcription processes.

We agree that TFIIB is required for open complex formation and transcription initiation in vivo. However, it is not required for transcription initiation in vitro when a tailed template is used. We additionally argue our crystallised complexes have bypassed the stages of initiation that require TFIIB and thus represent biologically relevant states post recruitment. Since this reviewer might have been disturbed that we did not describe the differences between factor-dependent in vivo initiation and factor-independent in vitro initiation we have carefully edited the title, abstract and text to better reflect that complexes without general factors are structurally investigated.

2) Based on structure comparisons between OC and ITCs from this work and transcription elongation complexes (TECs) from their previous works, they proposed that the pol2 positions DNA/RNA hybrid of ITCs slightly different (~15 Angstroms) from one observed in TEC. However, all structures were determined ~3.5 Angstroms resolutions, thereby discussing ~1.5 Angstroms difference is not appropriate using non-high resolution structures. Authors claimed that their structures have very good R-factors and stereochemistry, but it doesn't mean good structures of DNA and RNA. Their contributions of R-factors and stereochemistry can be easily diluted within ~500 kDa molecular weight pol2 structure. If authors want to tell good structures of DNA and RNA, they should show B-factor plot of them that can convince crystallographers to support the structures, if the B-factor is not over 100.

We disagree. The diffraction limit of a protein crystal is never equal to the optical resolution of the features observed in the electron density map or the coordinate error. The optical resolution is generally much better (smaller) than the diffraction limit (for details see Stout and Jensen (1989) X-Ray Structure Determination: A Practical Guide) and is also dependent on data quality. As a general rule, the coordinate error in well-defined parts of a crystal structure such as the active centre region of Pol II is 1/7 of the nominal resolution. Thus, diffraction data extending to 3.5 Å resolution result in a model with a coordinate error in the range of 0.5 Å. We therefore can without doubt discuss structural differences in the range of 1-2 Å and be confident about the conclusions we draw.

In regard to the B-factors plot, we know that crystallographic programs like the early XPLOR assumed B-factors cannot be higher that 100 Å^2. However, especially with 3rd generation sources and highly sensitive detectors B-factors can be much higher than 100 Å^2 and if so this does not mean the location of nucleic acid atoms would be ill-defined. Pol II-nucleic acid structures generally have nucleic acid B-factors well above this value, and remain highly informative with respect to nucleic acid conformation.

3) Nearly the same structures and conclusion reported in this work was already reported from Kornberg's group in Science last week that substantially decreased novelty of this work. At the end of this manuscript, authors stated some differences between their and Kornberg's works but their discussion are not meaningful. Below are their clams and my responses.

First, we used the complete 12-subunit Pol II and the downstream duplex was visible in all our structures.

--Using 12-subunit pol2 makes their resolution worse and having downstream DNA in the structure doesn't tell any new insight.

In accordance with the consensus in the field, we used a stricter I/SigI cut-off in the outer resolution shell to define our resolution limits, which explains some of the resolution differences when compared to the recent Science paper, which uses cut-offs as low as 1.2. If the same cut-offs are used, our structures compare favourably. In addition, 12-subunit Pol II crystallizes in a different space group and has a far larger unit cell and higher solvent content (80% instead of 50%). This dramatically increases the number of reflections observed and improves the observations to parameter ratio, which is crucial in refinement, generally leading to very good stereochemistry. Thus although our structures have a more conservative lower nominal resolution, we report considerably lower R_{free} factors (~3%) and a considerably smaller R_{free}-R_{factor} gap than any of the structures from the recent Science publication, rendering our structures at least as reliable as the ones published in Science. In addition, the 12-subunit pol II is the biologically relevant initiation-competent form of Pol II in vitro (Edwards et al., JBC 1991), and is associated with the genome in vivo (Jasiak et al., JBC 2008). Thus, our structures contain the biologically relevant form of Pol II.

Second, TFIIB was not required in our experiments to generate ITCs.

--This means their structures are not ITCs as I explained in 1).

As explained above, we do not claim our structures are ITCs. We rather claim the structures mimic ITCs and represent minimal ITCs that occur during initial transcription in vitro. We made sure the text reflects this properly. Also, we have crystallized complexes that have apparently bypassed the need for TFIIB by emulating a melted promoter, and by inclusion of short transcripts. We argue that whilst our structures lack TFIIB, they are also highly likely to represent features of ITCs in vivo. In addition, Liu et al. explained in their Science paper that they required TFIIB to generate the complexes, but TFIIB was not resolved in any of their reported structures and thus may have had an indirect non-biologically relevant effect during crystallisation rather than supporting any protein-nucleic acid contacts.

Third, in contrast to Liu et al., we could resolve structures with DNA alone and with 2nt RNA, both with and without NTP, providing evidence that Pol II is able to bind free DNA and a very short hybrid with some stability.

--This is obvious and we knew already otherwise pol2 cannot initiate de novo transcription.

Obvious it may be, but this was not observed directly before and only these structures provide direct evidence of the location of DNA and an RNA dinucleotide. We believe these are important contributions to the understanding of different states accessed by Pol II during initiation. The template DNA in the binary complex is strikingly similar to the elongation complex and allows us to speculate that the general transcription factors may not actually position the template strand for initiation, since Pol II is able to stably position it without additional factors. It should be noted that the recent Science paper also mentions a dinucleotide and trinucleotide ITC was crystallised yet there is no structure described or deposited. In contrast, difficulties to trap these early intermediates crystallographically were taken as evidence for an inherently greater instability.

--Cannot discuss such a small difference with lower resolution structures.

The lower resolution of our structures is amply compensated by the fact that we used a brominated uracil marker in the template strand in every complex we crystallised. By calculating anomalous difference Fourier maps with data collected at the bromine absorption edge, we could accurately and unambiguously assign the sequence of all the nucleic acid strands in every complex and showed that Watson-Crick base pairing is maintained within the hybrid. This was not done in the deposited structures by Liu et al. Although the ITC hybrids are tilted in our work and theirs, we did not observe the unusual deviation from DNA-RNA base pairing described by Liu et al. Indeed, we fear that in some of the structures by Liu et al. there are out-of-register errors in the DNA template strand that led to incorrect conclusions. Please refer to the new paragraph at the end of our discussion for details.

Finally, we did not observe a fraying of the RNA 3'-end in the ITCs.

-- Cannot discuss such a small difference with lower resolution structures.

Our RNA densities are unambiguous, with individual bases and phosphates resolved. The short RNAs are generally observed in their entirety, and thus base fraying is excluded. In addition, as

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discussed above, the bromine peak allowed us to accurately define the nucleic acid sequence. We have carefully rewritten the discussion to include a more detailed comparison between our work and the work published by Liu et al. and hope that this clarifies the similarities and differences.

Referee #2 :

This is a nice piece of work that I believe may provide a significant contribution to the field. However, in spite of a huge body of experimental structural data included in this MS (11 crystal structures) the MS is surprisingly concise (26K vs 54K characters allocated by the Journal). In my opinion, the compressed style of presentation did not allow the authors to emphasize significance and originality of their work on a full scale.

We wish to thank the referee for the kind words and support. We have extended the manuscript as suggested by the referee. In particular, we have included a more detailed comparison with the published work in the discussion. Although there is still some space left, we refrain from further extending the paper, as we believe all important points have been addressed now.

Specific comments

1. Title (and also in the abstract).

"Open promoter complexes" look somewhat confusing as they are likely not limited to only PolII and a short ssDNA template.

We agree and have changed the title and abstract to make this point clear. We make clear now that the DNA complex mimics part of the open complex.

2. Abstract.

In my understanding, the authors used the non-hydrolysable NTP analog that should be acknowledged here.

done

It is also strange that the Abstract does not mention the trigger-loop (TL) configuration in the NTP+ complexes that I feel is an interesting and essential part of the work.

We agree with the referee that this is a major point. We included this point in the abstract in the context of a new contact that may explain NTP/dNTP discrimination.

3. Introduction, par. 1

"...a stable elongation complex (EC) that contains a 8-base pair (bp) DNA-RNA hybrid".

I would speak about the "~8-9bps" as the actual length of the hybrid seems to be not yet elucidated and, in fact, may possibly vary in a course of elongation.

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4. Introduction, par. 2

"Initial RNA synthesis apparently does not require additional protein factors, since .."

Do the authors mean that nucleotide addition in the natural initiation complexes do not "require additional protein factors" (and upstream DNA?)? If so, more introductory materials/discussion should be provided here as a reference to a single (quite early, 1982) paper does not look very decisive.

We extensively edited the text accordingly to clarify this.

5. Page 5, par. 1

"The structures were refined at resolutions between 3.4 and 3.8 Å and show very good R-factors and stereochemistry (Table 1)".

There is no stereochemistry in the Table 1.

We added a second table that contains all the statistics.

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"... we soaked ITCs with the non-reactive NTP analog, (AMPCPP, or GMPCPP in case of the 2nt RNA), and solved the structures at resolutions between 3.4 and 3.8 Å (Table 1)".

GMPCPP is not shown in the Table 1.

We edited the table to include GMPCPP

7. Section: "Conserved mechanism of NTP selection"

So far, the two experimental structural models (for bacterial and yeast RNAP) have been published showing the significant structural differences for the two key elements of the NTP+ closed, "insertion" ECs, the NTP binding configuration/position and conformation of the folded TL. In my understanding, the authors now report a third, "hybrid" model in which the NTP position and conformation is reminiscent of that in the bacterial EC, while the TL fold looks (at least in the Fig. 6b) more similar to that of the previously reported for the yeast enzyme. This is an essential section of the MS that provides an additional independent structural view on the NTP+ closed configuration in multi-subunit RNAPs. I, therefore, believe that these results should be presented and discussed in more details and suggest that the authors provide following additional information for this section (that may be presented in Supplement):

a) the local Ramachadran statistics (% residues in the 4 regions as defined by the program PROCHECK) and B-factors for the flexible portion of the folded TL;

b) the stereo views showing the folded TL (flexible part) with the side chains superimposed on the omit difference ED produced before and after refinement;

c) the stereo views showing superposition of the bacterial and previously published yeast (if different from that in the authors' structure) folded TL configurations on the authors' initial omit ED.

d) for additional proofreading, it would be worth showing the omit maps after refinement of the authors' complex structure using the alternative TL configurations.

We have addressed these issues and attach 7 figures that we refer to as supplemental figures (although we do not think they should be published because they do not provide new insights). In particular we have added a figure that shows that the part of the TL that binds the NTP is well ordered, whereas the other part remains mobile, and that there is a minor fraction of complexes with open TL. Note this is the same for the published structures by Liu et al. We have also added a figure that compares the three different closed TL structures. Note we have been very conservative in what we include in our final model. We have not included the H1085 residue as it is ill-defined and also took out the TL residues that face away from the NTP site. The residues that face the NTP, including the Q1078 residue that is discussed as recognizing the 2'-OH group, have been included and show very well-defined electron density. We have also downloaded electron density maps (from the Uppsala Electron Density Server) for the recent NTP-containing structures in the competing study and see that they also have very weak trigger loop density in the same region and also clear difference density that would fit an open TL, similar to that shown in figure S2.

7.1 Page 6, last par.

Here (and throughout the MS where the NTP loading is discussed), citations of Vassylyev et al. included in the reference list is not appropriate in the context; the NTP binding to the bacterial EC was reported in the other paper.

We are sorry for the mistake. We have changed the reference accordingly.

7.2 Page 7, par. 2

"The NTP position and interactions are essentially identical in the previous structure of a bacterial RNA polymerase EC with AMPCPP (Vassylyev et al, 2007) (Fig. 6c), indicating a universally conserved mechanism of nucleotide selection and incorporation fidelity in all cellular RNA polymerases".

To strengthen this authors' conclusion, it is essential to schematically show NTP interactions in both (yeast and bacterial) complexes along with the local sequence alignment of the respective protein structures.

We have added a corresponding figure to the manuscript.

7.3 Page 7, par. 2

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"In addition to N479, NTP/dNTP discrimination may involve the trigger loop residue Q1078, which may couple the presence of an NTP with a 2'-OH group to closing of the trigger loop, thereby restricting catalysis to NTPs".

This is an interesting and important prediction that I feel would benefit from illustration of the supportive experimental data, i.e. the ED in vicinity to the interacting groups.

We have added such a figure panel. See also above.

8. Discussion.

The first half of this section is largely redundant to the previous text and, frankly, I did not see much discussion in this section. A small piece of new information provided by the authors here might be well compressed to a single paragraph or, may be even better, appended to the respective sections of the Results. In a second half of Discussion, the authors briefly list up the differences between their work and the similar published results (Liu et al, 2011) providing almost no comments and/or comparison. It is a bit disappointing provided, in particular, that the overall size of the MS is ~2 times smaller than that allocated for the Research Articles in the Journal. In addition, some statements in discussion appear to be quite vague (at least in my reading) and sometimes detract from (rather than emphasize) the originality of the work. I suggest that this section is thoroughly re-written to provide more mechanistic implications and better focus on the comparative analysis with the published competing results (Liu et al, 2011).

We agree and wish to thank the referee for pointing this out. We have carefully rewritten the discussion to account for the referee's concerns. We have also very carefully compared our structures to the ones published by Liu et al and discuss the details of this comparison at the end of the discussion. We have also added discussion of abortive transcription to the manuscript.

8.1 Page 10, par. 1

"Comparisons with previous structures of ECs with bound NTP (Kettenberger et al, 2004; Vassylyev et al, 2007; Wang et al, 2006) indicate that the exact positioning of the NTP depends on the experimental design".

In my reading, with no subsequent discussion, this statement hints that both experimental designs are not physiologically relevant.

We have deleted this statement.

8.2 Page 10, par. 2

"Transcription initiation in vivo requirs ..."

This paragraph seems to be loosely related to the discussion content and looks more as an introductory one.

We have shortened and moved this paragraph to the introduction.

8.3 Page 10, par.3

"Liu et al. revealed densities of DNA-RNA hybrids in complex with PolII that are overall similar to the ones revealed here and describe a similar transition in hybrid conformation between the ITC and EC, ...".

and Page 11, par. 1

"...although the ITC hybrids also appeared tilted, we did not position a +1 templating base in the active site and hence did not observe the unusual deviation from DNA-RNA base pairing described by Liu et al.".

Did the authors look at the ED of Liu et al.? Does this ED accommodate the RNA/DNA hybrid model (in particular, at the 4/5 bps level) presented by the authors?

We have carefully examined these structure. Most importantly the conformation of the hybrid is very similar, being tilted as we observe it, but we feel the Liu structures contain an out of register error in the DNA template strand, since all base pairs are mismatched. We added this observation to the discussion and suggest that essentially identical results are obtained if the DNA register is adopted in the Liu structures.

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In addition, Liu et al. also report the NTP+ complex. Is the TL conformation (and ED) is similar to that observed by the authors?

Please add text

9. Table 1.

This Table should be re-worked to include all standard crystallographic information describing each of the complexes under study (i.e. data collection and refinement statistics). It is critical that, in the revised Table, the authors include the Ramachadran statistics and B-factors for the overall structure (see Comment #7).

We have included a second table with these statistics.

Referee #3 :

This manuscript reports a set of Pol II - nucleic acid structures that reveal the transition of Pol II from the open complex state through initial transcribing complexes of 2-7 nt RNA. The open complex mimic showed that Pol II contains a binding sites for downstream double stranded DNA and the template strand opposite the active site - previously modelled based on the stable elongation complex structure. The 2 nt - Pol II structure reveals key contacts with Rpb2 that likely stabilize short RNAs in initial transcribing complexes. The 4-5 nt complexes reveal a tilted hybrid with a shifted position of the DNA template strand that can be translocated by addition of the next NTP. Finally, the results add more detail to the mechanism of how NTPs are selected by Pol II and comparison of these results to the literature show that this mechanism is conserved. The manuscript is very well written and the results are an important advance.

A recent paper from the Kornberg group overlaps with some of these results. However, the methods and structures in the Cramer group paper are sufficiently different and the results are of high importance, so that this paper should be published in EMBOJ. As outlined in the discussion, there are at lest 5 differences in this work with the Kornberg paper. Together, these two papers reveal key mechanisms in the transition from initiation to elongation and nicely explain many previous biochemical results.

We wish to thank the referee for his/her kind words. We have carefully revised the manuscript and agree it will provide an important reference for the future.

2nd Editorial Decision 10 October 2011

Thank you for submitting your revised manuscript for consideration by the EMBO Journal. It has now been seen by one for the original referees who finds that you have satisfactorily addressed the initial technical concerns of all the reviewers. I am therefore happy to accept the manuscript for publication in The EMBO Journal pending the changes to the Supp Material suggested by the referee. These changes should be incorporated as soon as possible.

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

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS:

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Referee #2 :

In my opinion, the manuscript was substantially improved providing now more critical experimental details and better discussion. Though the resolution and data quality are modest, and crystallographic statistics hints a certain overfitting during refinement, the major conclusions of the authors are well supported by experimental data (electron density, ED) and do not go beyond the actual (effective) resolution. Judging by quality of the ED shown in the revised manuscript I believe that the authors may distinguish the reported alterations in the overall shape of the nucleic acid duplex, base pairing, flipping of the DNA/RNA bases, etc.. As I noted on the first round of review the conclusions of this manuscript are of general interest and I agree now with the reviewer #3 that together with the recently published paper of Liu et al. this manuscript provides important insights in the mechanisms of transcription initiation.

Comment

In the Supl. Figs. S4 and S5, it seems some side/main chain atoms from the flexible portion of the TL are not well defined by the ED. I, therefore, suggest that in the final model, the authors remove the side chains from the respective residues. I also feel that it is worth adding to the model a C-alpha trace for the alternate "open" configuration of the TL that the authors observe in the ED.

Author Correspondence 10 October 2011

Many thanks indeed. I am very happy that the concerns could be addressed.

Note we did not plan to deposit any supplemental materials. Rather, the few density figures submitted were only meant for the referee, as said in our responses (I did not see how to upload the files and therefore chose supplemental). We have described in detail in the main text what can be seen in the density and what can not. Also, everyone can download electron densities for all PDB entries via the density server.

Please advise whether you agree we do not need to deposit the density figures as supplemental materials.

Editorial Correspondence 10 October 2011

I have looked through the relevant figures and aggree that thex do not need to be included in the manuscript.

Yours sincerely,

Editor
The EMBO Journal

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