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Recognition of the amber UAG stop codon by release factor RF1

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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 25 March 2010

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has now been seen by three referees whose comments to the authors are shown below. You will see that the referees are generally positive about your work and that they would support its ultimate publication in The EMBO Journal after appropriate revision. I would thus like to invite you to prepare a revised manuscript in which you need to address the referees' points in an adequate manner. I should also add that - as pointed out by referee 2 - the PDB accession details for your novel structural data need to be included at revision stage. I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Peer 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.

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1 (Remarks to the Author):

In this work by Korostelev et al., Noller and collaborators present a crystal structure of the class-1 release factor 1 (RF1) in complex with a UAG-programmed ribosome with a deacylated tRNA in P site and an empty E site. This structure complements previous structural reports from Noller's group on RF1 (Laurberg et al., 2008) and RF2 (Korostelev et al., 2008) bound to UAA-programmed ribosomes and from Ramakrishnan's group on RF2 bound to a UGA-programmed ribosome (Weixlbaumer et al., 2008). These four structures have now provided a basis for quantitative interpretation of the speed and accuracy of codon reading by class-1 release factors in bacteria and therefore fill a long existing gap in our knowledge of how codon reading can be done by proteins in a ribosomal context.

The present structure appears to be of somewhat lower quality than the previous ribosomal structures of class-1 RFs. The highest resolution shell has a signal to noise ratio of 1.1 (Table 1), so that the resolution should preferentially be given as 3.7 Å (signal to noise 2), rather than 3.5 Å. For this very reason, the placement of 1600 ions in the structure may, in addition, be a bit uncertain.

The present structure lacks E-site tRNA, but this seems to have little influence on the overall 70S ribosome structure. The authors also conclude that all termination complexes have slightly different structures than the 70S elongation complex, in that there is a small relative rotation of the subunits, a small inward twisting of the head of the 30S subunit and other minor differences, but the functional meaning of these differences is unclear.

The main contribution of the paper relates to the recognition of both UAG and UAA codons by RF1, which is of considerable interest.

The paper also provides confirmatory information about the role of Gln 230 in the GGQ motif for how catalysis of the ester bond in peptidyl-tRNA is induced by the presence of the release factor.

The authors have also made functional experiments, probing the effects of mutations in the so called switch loop between domains 3 and 4 of the RF and its putative role to position domain 3 in the peptidyl transfer center (PTC) in a stop codon dependent way. They find that deletion of helix 69 (h69) of 23S ribosomal RNA in conjunction with a deletion of the switch loop in RF1 gives a synergistic reduction in the rate of termination (1000-fold), while the h69 deletion by itself gives a 10-fold and the RF1 deletion by itself gives a 3-fold reduction. This is in line with their proposal that RFs enter the ribosome in closed conformation, as in the crystal structure of the free factors, and then unfolds to the extended conformation seen on the ribosome (U. Rawat et al., 2003; 2006) as first suggested by Rawat et al. (2003). Here, I think that the discussion would benefit from mentioning the small angle xray scattering (SAXS) data (B. Vestergaard et al., 2005), suggesting that the conformation of RFs in solution is open, rather than closed, and that the closed form may have functional relevance only during methylation of the class-1 release factors (M. Graille et al., 2005). It has, in addition, been argued that there is a sterical hindrance to the entry of the closed RF form to the A site (Weixlbaumer et al., 2008). Whether or not the RFs experience a major global change as they enter the ribosome and the putative relation between this conformational change and the high accuracy of codon recognition by RFs (D. Freistroffer et al., 2000) is so important that a fuller discussion would, to my opinion, be motivated.

Referee #2 (Remarks to the Author):

The paper by Korostelev et al. entitled "Recognition of the amber stop codon by release factor RF1"

addresses the specific recognition of the UAG codon within the bacterial ribosomal termination complex containing release factor RF1. The work is essentially based on crystallography and is complemented by some functional tests with mutants of the release factor and of the large subunit helix h69. Several structural studies have been published over the past years: cryo-EM studies allowed localising the class-I release factors RF1 and RF2 on the ribosome, and identified the position of domains 2/4 and 3 involved in stop codon recognition and tRNA-peptidyl bond hydrolysis respectively, and also revealed a structural difference between the isolated RF2 and the ribosome-bound state. More recently, several crystal structures extended the understanding of the protein synthesis termination process by providing details into the recognition of the three stop codon bases, the role of key residues in helix 44 of the small subunit and the possible mechanism of hydrolysis with the help of the conserved GGQ motif. From the three standard termination codons UAG, UGA and UAA, structures with either RF1 or RF2 have been determined with the RF2-specific UGA or the non-specific UAA, but the RF1-specific UAG recognition had not been addressed yet. The present work thus completes the investigation of termination complexes with standard stop codons. While the study is of potential interest, especially with regards to the specific molecular recognition of the third base, there are several points that should be addressed prior to publication. The deposition of structure factors and coordinates within the appropriate data bases should be mandatory for publication.

Comments in more details:
- the paper contains only a short discussion combined with the results, but it would benefit from a more extensive discussion and in particular from a clear comparison of all four complexes with respect to the recognition specificity (including additional, possibly supplementary, figures)
- the abstract is very succinct; it should at least contain a short description of the mechanism of UAG-specific RF1 recognition.
- the figures should be rendered in a clearer way (figures 1, 2, 3), with a clearer coloring (pink/grey, fade blue etc. is hardly visible), full labels in figure 2 and 3, a comparison with RF2 (Val / Gln in figure 2D), a comparison of the 4 available structures with RF's.
- in the introduction, the cryo-EM and early crystal structures should at least be mentioned.
- the relevance of the E-site tRNA is not mentioned, are the peptide-release rates the same in presence and absence of this tRNA? The reconstituted 70S complex might be an opportunity to test this as most crystal structures until now contain a co-purified E-site tRNA.
- Thr194: from the figure it is not clear what the H-bond geometries are, are the angles and distances (should be indicated as labels) suggesting an equivalent importance of the bonds to N6 and N7 of the purine?
- in the discussion on the role of the Gln residue, the possible modulation of the side-chain activity through methylation should be mentioned.
- the structural reorganisation of the switch loop could be illustrated with a comparison with the crystal structure of the isolated factor.
- peptide release assays: were the experiments repeated at least three times, what are the error bars for these experiments?
- table 1: the high redundancy of the data is clearly beneficial for the reliability of the data, however an I/sigma of 1.1 in the last shell seems rather optimistic. This is also supported by the fact that at 3.7Å resolution there is still 2 I/sigma, indicating a fast drop beyond 3.7 Å resolution, the resolution seems therefore overestimated. A simple test would be to calculate a map at different resolutions between 3.5 and 3.7 Å and validate at which cutoff features significantly appear/disappear.
For the reader, it might be better to put the R pim in the table (total and in the last shell) and indicate the Rmerge in the legend. Also, the definition of all R factors should be indicated.
- structure factors and coordinates should be deposited within the PDB data base.

Referee #3 (Remarks to the Author):

In this paper Korostelev and colleagues present the X-ray structure of RF1 bound to a 70S ribosome in order to analyze how RF1 recognizes an UAG termination codon. This, this paper complements previous work on similar complexes with UAA and UGA termination codons. The X-ray structure is complemented by some functional tests. The results are interesting and the paper is well written. It is suggested to take the following points into account for a revised version of the paper.

Specific points:

1. The crystallographic table shows an unusually low value for I / Sigma I in the highest resolution shell (3.65-3.52A) and indicates that data with a very poor signal to noise ration have been included. The authors should either carefully reprocess their data to reduce the resolution to a realistic and accurate value of I / Sigma I = 2.0 (3.7-3.85A). Or they should discuss this point in the paper and justify the inclusion of data with a value of I / Sigma I = 1.0.
2. A larger part of the electron density should be shown in a supplemental figure in order to demonstrate the overall quality of the electron density map.
3. The conformational changes described at the end of the first section of the Results ("Conformation of the L1 stalk ...), e.g. the rearrangement of bridge B2a, are interesting. It would be useful to have an additional figure for this, maybe in the supplement.
4. Figure two should contain also a part of the RF2 structure in order to have a comprehensive comparison of termination codon recognition in bacteria.
5. In the Material and Methods section the author refer to their previous work (Laurberg et al., 2008) for initial crystallization conditions. As these conditions are essentially the ones worked out by the Ramakrishan lab it would be appropriate to cite here also the Selmer et al., 2006 paper.

Minor points:

6. The Laurberg et al., 2008 paper in the reference list appears to be duplicated.
7. In the legend to figure 2 or in M&M the abbreviation "NCS-averaged" should be explained for the general reader.

1st Revision - authors' response 29 April 2010

Reviewer #1:

1. The highest resolution shell has a signal to noise ratio of 1.1 (Table 1), so that the resolution should preferentially be given as 3.7 Å (signal to noise 2), rather than 3.5 Å.

Response: Due to availability of good molecular replacement models for the structure of the *T. thermophilus* 70S ribosome, it is now common practice to include data up to I/sigma of 1.0 - 1.5 for the structures of 70S ribosome complexes (see recent structures by the Steitz and Ramakrishnan labs (Gao et al., 2009; Stanley et al., 2010). However, we have now reprocessed our data to a resolution of 3.6 Å, at which I/sigma has a value of 1.5, and have re-refined the structure. Accordingly, all statistics are now reported at 3.6 Å, which we feel is a conservative description of the resolution (Table S1).

2. The placement of 1600 ions in the structure may, in addition, be a bit uncertain.

Response: We have removed the majority of them and left only 410 ions that were placed at positions observed in higher-resolution structures of the termination complexes (Korostelev et al., 2008; Laurberg et al., 2008). Table S1 has been updated accordingly.

3. I think that the discussion would benefit from mentioning the small angle xray scattering (SAXS) data (B. Vestergaard et al., 2005), suggesting that the conformation of RFs in solution is open, rather than closed, and that the closed form may have functional relevance only during methylation of the class-1 release factors (M. Graille et al., 2005). It has, in addition, been argued that there is a sterical hindrance to the entry of the closed RF form to the A site (Weixlbaumer et al., 2008).

In the revised manuscript, we show that there is a potential hydrophobic binding pocket for the methyl group in the ribosome (Figure S5), in keeping with the observed slightly higher binding affinity of the methylated factor. We have also incorporated the references mentioned by the reviewer into our discussion.

4. Whether or not the RFs experience a major global change as they enter the ribosome the putative

relation between this conformational change and the high accuracy of codon recognition by RFs (D. Freistroffer et al., 2000) is so important that a fuller discussion would, to my opinion, be motivated.

Following the reviewer's recommendation, we have expanded discussion of the relation between the conformational change of the release factor and fidelity of stop codon recognition on page 7.

Reviewer #2:

1. The paper contains only a short discussion combined with the results, but it would benefit from a more extensive discussion and in particular from a clear comparison of all four complexes with respect to the recognition specificity (including additional, possibly supplementary, figures)

Response: Following the reviewer's suggestion, we have incorporated a discussion of how the two factors recognize the three stop codons. Figures 2 and 3 now show how RF1 is able to recognize both UAG and UAA and RF2 UAA and UGA. Figure 4 also shows a superimposition of all four structures in the catalytic site. Further comparative details are also presented in Figures S3, S5 and S6.

2. The abstract is very succinct; it should at least contain a short description of the mechanism of UAG-specific RF1 recognition.

Response: We have revised abstract to include a description of the mechanism of recognition of the UAG codon.

3. The figures should be rendered in a clearer way (figures 1, 2, 3), with a clearer coloring (pink/grey, fade blue etc. is hardly visible), full labels in figure 2 and 3, a comparison with RF2 (Val / Gln in figure 2D), a comparison of the 4 available structures with RF's.

Response: The figures have been re-rendered and (as mentioned above) additional panels have been added to Figures 2 and 3 that now include mechanisms of stop codon recognition in all four structures.

4. In the introduction, the cryo-EM and early crystal structures should at least be mentioned.

Response: The cryo-EM and early crystal structures are now discussed on page 2, in the introduction.

5. The relevance of the E-site tRNA is not mentioned, are the peptide-release rates the same in presence and absence of this tRNA? The reconstituted 70S complex might be an opportunity to test this as most crystal structures until now contain a co-purified E-site tRNA.

Response: We have addressed the relevance of the E-site tRNA in termination on page 4.

6. Thr194: from the figure it is not clear what the H-bond geometries are, are the angles and distances (should be indicated as labels) suggesting an equivalent importance of the bonds to N6 and N7 of the purine?

Response: We have included a figure with appropriate labels (Fig. S3) and a discussion concerning the equivalence of the hydrogen bonds in Supplementary Information.

7. In the discussion on the role of the Gln residue, the possible modulation of the side-chain activity through methylation should be mentioned.

Response: A discussion of the role of methylation of the Gln side-chain (page 6) as well as a figure showing a potential methyl binding pocket (Fig. S5) have been added.

8. The structural reorganisation of the switch loop could be illustrated with a comparison with the crystal structure of the isolated factor.

Response: A figure depicting the structural reorganization of the factor has been added to

Supplementary information (Fig. S7).

9. Peptide release assays: were the experiments repeated at least three times, what are the error bars for these experiments?

Response: We have added a note to the caption for Figure 7. The updated figure includes error bars.

10. Table 1: the high redundancy of the data is clearly beneficial for the reliability of the data, however an I/sigma of 1.1 in the last shell seems rather optimistic. This is also supported by the fact that at 3.7 Å resolution there is still 2 I/sigma, indicating a fast drop beyond 3.7 Å resolution, the resolution seems therefore overestimated. A simple test would be to calculate a map at different resolutions between 3.5 and 3.7 Å and validate at which cutoff features significantly appear/disappear.

Response: See response to point #1 of reviewer #1.

11. For the reader, it might be better to put the R pim in the table (total and in the last shell) and indicate the Rmerge in the legend. Also, the definition of all R factors should be indicated.

Response: We have reported Rpim in the table (Table S1) and have defined all R factors in the caption.

12. Structure factors and coordinates should be deposited within the PDB data base.

Response: This has been done.

Gao, Y.G., Selmer, M., Dunham, C.M., Weixlbaumer, A., Kelley, A.C. and Ramakrishnan, V. (2009) The structure of the ribosome with elongation factor G trapped in the posttranslocational state. *Science*, **326**, 694-699.

Stanley, R.E., Blaha, G., Grodzicki, R.L., Strickler, M.D. and Steitz, T.A. (2010) The structures of the anti-tuberculosis antibiotics viomycin and capreomycin bound to the 70S ribosome. *Nat Struct Mol Biol*, **17**, 289-293.

Response to Reviewer #3:

1. The crystallographic table shows an unusually low value for I / Sigma I in the highest resolution shell (3.65-3.52A)...etc.

Response: See response to point 1 of reviewer #1.

2. A larger part of the electron density should be shown in a supplemental figure in order to demonstrate the overall quality of the electron density map.

Response: We have added a supplemental figure to show this (Fig. S4).

3. The conformational changes described at the end of the first section of the Results ("Conformation of the L1 stalk ...), e.g. the rearrangement of bridge B2a, are interesting. It would be useful to have an additional figure for this, maybe in the supplement.

Response: We have added additional figures to show these changes (supplemental Figures S1 and S2).

4. Figure two should contain also a part of the RF2 structure in order to have a comprehensive comparison of termination codon recognition in bacteria.

Response: Figures 2 and 3 have been redone to provide a comprehensive view of recognition of all

three stop codons by RF1 and RF2.

5. In the Material and Methods section the author refer to their previous work (Laurberg et al., 2008) for initial crystallization conditions. As these conditions are essentially the ones worked out by the Ramakrishan lab it would be appropriate to cite here also the Selmer et al., 2006 paper.

Response: We have incorporated the reference to Selmer et al, 2006 in this context.

Minor points:

6. The Laurberg et al., 2008 paper in the reference list appears to be duplicated.

Response: The second copy has been removed from the reference list.

7. In the legend to figure 2 or in M&M the abbreviation "NCS-averaged" should be explained for the general reader.

An explanation of the abbreviation NCS has been added to Materials and Methods.

2nd Editorial Decision 25 May 2010

Thank you for sending us your revised manuscript. Our original referees 1 and 2 have now seen it again, and you will be pleased to learn that in their view you have addressed the criticisms in a satisfactory manner, and that the paper will therefore be publishable in The EMBO Journal.

Before this will happen, however, I was wondering whether you would like to consider addressing the minor issues suggested by referee 2 (see below). Please let us have a suitably amended manuscript text via e-mail as soon as possible. We will upload it for you, and I will then formally accept the manuscript.

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1 (Remarks to the Author):

The manuscript has been significantly improved in response to the comments of the reviewers.

Referee #2 (Remarks to the Author):

The paper by Korostelev et al. entitled "Recognition of the amber stop codon by release factor RF1" in its revised version addresses all of the points discussed in the initial feedback and thus appears suitable for publication. As mentioned initially, the work on 70S ribosome termination complexes nicely completes the investigation of the molecular basis of recognition of all three stop codons by the two bacterial release factors RF1 and RF2.

The deposition of structure factors and coordinates within the appropriate data bases has been started and should be regarded as mandatory for publication.

Suggestion for the title: maybe include "UAG" as "amber" alone may not be known to the general readership, for example: Recognition of the amber UAG stop codon by release factor RF1.

Minor point: on the bottom of page 7 there is a revision discussion left over.

2nd Revision - authors' response 26 May 2010

We are sending you the finalized manuscript with the following changes:
1. Modified title, as suggested by referee 2
2. In acknowledgments, we now specify all 4 PDB ID codes for the structure uploaded at RCSB.

As for the minor point raised by referee 2, we could not identify any revision discussion left over on page 7; thus we did not modify the main text of the article.

Please let us know if you have any questions.

