20th May 2013

Your manuscript entitled "Structure of the Ribosome with Elongation Factor G Trapped in the Pre-Translocation State" has now been seen by three referees, whose comments are attached below. In light of their advice we have decided that we cannot offer to publish your manuscript in Nature.

You will see that, while they find your work of some potential interest, our referees raise concerns about the advance your findings represent over earlier work, about the level of new insight regarding mechanism that is provided, and about the strength of the novel conclusions that can be drawn at this stage. We feel that these criticisms are sufficiently important so as to preclude publication of your work in Nature.

Although we regret that we cannot offer to publish your paper in Nature for editorial reasons, it might be appropriate for another journal in the Nature Publishing Group family such as Nature Structural and Molecular Biology. More information about this and other NPG journals can be found at www.nature.com/nature/about/family/index.html. Should you wish to have your paper considered by the editors of another NPG journal, please click on this link:

Using this link you will be able to transfer your manuscript, including the referees' comments and identities, to the editors of the journal you have chosen. As I know you are under time pressure, this may be an attractive option to you. Should you wish to have your manuscript considered at another journal without the referees' reports, you will need to submit the paper as a new submission directly to the journal.

All of our journals are editorially independent, and the editors of the chosen journal will make their own decision whether to send your manuscript for review.

I am sorry that we cannot be more positive on this occasion but hope that you will find our referees' comments helpful when preparing your paper for submission elsewhere.

With kind regards

Senior Editor
Nature

Referees' comments:

Referee #1:

In the manuscript entitled "Structure of the ribosome with elongation factor G trapped in the pre-translocation state," the authors determined the structure of the pre-translocation conformation of ribosome with EF-G, a state that evaded previous visualization due to its transient nature. This structure provides the key to understand EF-G catalyzed translocation. Through the clever use of antibiotics, the authors were able to for the first time visualize this transient state. Even though this paper presents an important structure of elongation, the analysis and conclusions presented are lacking in depth. The authors also failed to acknowledge and compare their results with many previous published data. Comments are outlined below:

1. The use of viomycin to trap the pre-translocation state is risky, because it is possible

that the antibiotic locks the ribosome in an out-of-pathway intermediate state. How does this structure compare with the ribosome with viomycin structure by Steitz, NSMB, 2010 17:289-93. doi: 10.1038??

2. Even though the ribosome is in the "pre-translocation" state, the EF-G has already hydrolyzed GTP and is in the EF-G-GDP form. Thus, the structure is not a true pre-translocation state. The comparison presented of EF-G conformation between this state and the post-translocation complex is not as significant as claimed in the paper, since the real important conformational change of EF-G on the ribosome is prior GTP hydrolysis and after GTP hydrolysis.

3. The authors state that the conserved loop at the tip of domain IV of EF-G maintains its interaction with the tRNA as it translocation from A to P site. Are there any specific conclusions on the mechanism of translocation that can be drawn from this observation? This problem of not going in depth in analysis exists throughout the paper.

4. The authors state that the EF-G adopts an intermediate conformation between free EF-G and EF-G bound to the post-translocation ribosome. What does this tell us? What is the significance of the 30A movement of domain IV?

5. The authors failed to cite some dynamic experiments on translocation. Chen et al. (Mol. Cell 2011, 42:367-77. doi: 10.1016) showed with single-moecule that translocation can occur when tRNAs are in the classical state or in the hybrid state. Chen et al. (NSMB 2013, doi:10.1038/nsmb.2567) also showed EF-G dynamics, as well as Munro et al. (NSMB 2010, 17:1470-7. doi: 10.1038/nsmb.1925). How does the structure presented here reconcile with these results?

6. The authors mentioned movements of L1 and L11 stalks. What role do the L1 and L11 stalks play in translocation?

7. The authors failed to cite an extremely relevant paper: Zhang et al., Science, 2009 325:1014-7. doi: 10.1126. How does the structure solved here compare with the ratcheting trajectory determined by Zhang? The structure of the ribosome here should be described in more detail, with respect to movements of different parts of the ribosome, since Zhang showed that multiple motions are responsible for translocation. Just simply saying "rotated, hybrid-state" is not sufficient for a structure paper.

8. The A/P* state is not previously unobserved. Though not stated as A/P*, Blanchard et al. (PNAS, 2004, 101 no. 35) observed a transient 500 ms state of intermediate FRET that likely corresponds to what the author here claims as A/P* state. How is this A/P* state significant to translocation?

9. What about other models of translocation? How does the presented model compare with those? The author never mentioned nor discussed any of the other models. Specifically, what is the role of GTP hydrolysis for translocation in the model presented?

10. The last concluding paragraph was poorly written. Instead of reiterating how the structure solved here is relevant to translocation, the authors simply repeated what is already known on the Brownian ratchet model. The Brownian ratchet mechanism is not novel; it has been proposed a decade ago. Currently, the authors only broadly stated the mechanism of translocation is a Brownian ratchet, which adds nothing to our current understanding of translocation. The authors should put into context of their new structure to show how translocation works mechanistically, not just broadly state a model that had been already proposed.

11. A more serious problem is that the authors seemed to conclude from their structure that it

supports the Brownian ratchet mechanism of translocation. However, none of their data and structure is in direct support of the model. In the concluding paragraph, please specifically relate what was new in this novel structure and how it provides new insights to the Brownian ratchet model.

12. A very interesting, and extremely important, structure to solve is the structure of ribosome and EF-G with viomycin and a non-hydrolyzable analog of GTP. Is there a specific reason why the authors did not solve that particular structure? If the authors are able to solve this structure and compare with what they currently have, this will definitely be a much stronger manuscript.

Overall, this is a worthy paper, but not really novel or deep enough to merit publication in Nature. The readers want a more detailed mechanism of translocation drawn from this novel structure. Instead, the authors only glanced over the results, without providing real insights and novel interpretations of the mechanism. The concluding paragraph, instead of stating novel insights, provided only a model that had been proposed a decade ago and generally accepted already.

Referee #2:

Grigorieff and colleagues present a reconstruction of the bacterial ribosome in complex with EF-G, tRNAs, mRNA and antibiotics viomycin and fusidic acid, in the pre-translocation state. There have been ribosome - EF-G complexes determined before by both X-ray crystallography and EM, but as the authors point out, there are no reports of a good quality, bona fide pre-translocation complex.

The manuscript is well written, the study is of high technical quality (without pushing the limits for resolution for ribosomal complexes), and the structure adds an interesting snapshot in the increasingly detailed movie of protein synthesis.

The authors show that there is a 20{degree sign} shift of EF-G between pre- and post-translocation conformations, and that there is a novel A/P* hybrid state of tRNA in their isolated, both interesting observations. It was anticipated that the pre-translocation complex would feature EF-G's domain IV next to, but not clashing with, the tRNA in the 30S A site; they observe such a conformation.

The authors conclude by supporting the previously proposed Brownian pawl role for EF-G, but don't actually explain how their current work makes the Brownian pawl more likely.

Also missing is discussion about two important points with respect to EF-G-catalyzed translocation:

1. If the role of EF-G as a Brownian pawl is to prevent back-translocation, what prevents back-translocation once EF-G has dissociated? The next step in translation is tRNA selection, which is the rate-limiting step in the elongation cycle and presumably there would be enough time for a competing back-translocation to occur at some rate. If the Brownian pawl is the correct model, one assumes something must prevent back-translocation at this stage as well or there would be no point of a having a Brownian pawl in the first place.

2. How does GTP hydrolysis fit into the cycle of conformational changes described for EF-G, tRNA and the ribosome, and what is the role of GTP hydrolysis role in translocation? At least some discussion seems appropriate.

Referee #3:

This paper describes the cryo-EM structure of a previously unobserved state in prokaryotic translation: the 70S ribosome with EF-G in the pre-translocation state. This state has been hypothesized, but had thus far eluded structure determination. Because this structure fills an important gap in our structural understanding of ribosome functioning, this paper is in principle worthy of publication in Nature. I do however have some reservations that should be addressed in a revised version of the manuscript. The paper seems to have been written in a great hurry, which has had a detrimental effect on its quality. In particular the figures are of poor quality and a lot of technical information appears to be missing.

The only figure from which the quality of the cryo-EM map of the new state can be assessed is Fig. 1. However, its most detailed panel c does not convey the quality one would expect for a 7.6 A map, in particular so in the most relevant region: the lower end of the tRNA and domain IV of EF-G. In particular for the latter, the density seems quite different from the atomic model fitted into it. The question is whether this is due to the poor figure, or due to remaining heterogeneity in the actual reconstruction. This question is even more important as the authors used an unpublished classification approach, and unresolved heterogeneity could potentially lead to incorrect fitting of the atomic models. A rotating movie of this area with the density and the fitted models could convey the quality of the fit much better. In addition, the authors only report a real-space correlation coefficient between the (entire) model and the map. Instead of this uninformative number, the authors
should provide a Fourier Shell Correlation (FSC) curve between the model and the map. Furthermore, if the resolution in the most relevant part is significantly lower than the overall resolution, this should be clearly stated in the main text.

More information should also be provided about the refinement and classification. Page 8 and the legend of Fig. S1 state that the data were classified into five classes. However, the supplementary methods section mentions two consecutive classification rounds: one with 15 classes and a second one with 3 classes. Then it should be clarified which classes are described in Fig. S1 and the main text. Furthermore, for the final maps, how many particles were used, how were the resolution estimates obtained, to what resolution were alignments performed, and how do FSC curves between half-reconstructions look like?

For clarity, all figures should adopt the standard color schemes in the ribosome field (blue and yellow subunits, etc).

Page 4 states that the atomic model was fitted using rigid-body refinement, but the citations involve methods that use MD simulations for flexible fitting. (In the supplementary information it should be clarified how many rigid bodies were used to fit the map.)

In Fig. 2a the 23S rRNA just appears to be a big pile of spaghetti in the background, and therefore only makes the figure harder to understand. Perhaps only the relevant parts could be shown?

In Fig. S2 the black structure is hard to distinguish from the red and blue structures.