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Atomic model of an infectious rotavirus particle

Ethan C Settembre, James Z Chen, Philip R Dormitzer, Nikolaus Grigorieff and Stephen C Harrison

Corresponding author: Stephen Harrison, Children's Hospital -Member

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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 02 September 2010

Thank you for submitting your manuscript for consideration by the EMBO Journal. We sent your manuscript to three referees, and have received comments from two of them. We are still awaiting the third report, but since the other two are in agreement, we are taking a decision now in order to save you from further loss of time. If and when the third report comes in, we will of course send it on to you, and may ask you to address any concerns raised.

As you will see, both referees express interest in your study and support publication of the manuscript pending satisfactory revision. Only relatively minor points are raised concerning presentation of the figures and some text changes. In particular, I would encourage you to follow the recommendation of referee 2 to provide a more detailed explanation as to the methods involved.

I would therefore like to invite you to submit a revised version of the manuscript, addressing all the comments of both reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision. Acceptance of your manuscript will thus 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 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

We generally allow three months as a standard revision time, and as a matter of policy, we do not consider any competing manuscripts published during this period as negatively impacting on the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you

foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

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

Best wishes,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1 (Remarks to the Author):

The authors present a 4.3 angstrom resolution cryoEM reconstruction of the full rotavirus virion, which has permitted atomic model building of the viral proteins. Although individual proteins of rotavirus capsid has been resolved by x-ray crystallography to atomic resolution and the subviral particles (include a double layer particle DLP and DLP with VP7 shell) have been reconstructed to atomic resolution cryoEM, the current structure represent the first time the entire virion has been visualized to atomic resolution. In particular, the in situ structure of VP4 is determined in the present study. Amazingly, based on the cryoEM data and trimeric densities at the shell domain of VP4, the authors present direct evidence that VP4 is a trimer, instead of the dimeric appearance at the distal end of VP4. The authors present molecular interactions based on these in situ structures. The paper is well written and should be of general interest to the readership of the EMBO Journal.
I have only some minor suggestions for the authors to address in a revised manuscript.
1. in the abstract, the authors claim a resolution of "about 3.8 A", but in the Results, the method and Figure 5 the resolution is actually 6.5A based on FSC=0.5 and 4.3 on FSC=0.143. The abstract should be corrected accordingly.
2. Figure 5: the labels of "Before CTF correction" and "After CTF correction" are misleading. It should be CTF refinement.

Referee #2 (Remarks to the Author):

Settembre and colleagues describe the structure of a rotavirus particle determined by cryoEM and single particle analysis. The resolution of the electron density map extends to ~ 3.8 Å, which allowed tracing the complete polypeptide chain. This structure provides important new insight into the native conformation of VP4 and its interaction with the capsid. Comparison of this native-like activated structure of VP4 with a previously determined structure of isolated highlights the molecular transitions that occur upon membrane penetration required for rotavirus infection.

Overall this is an important manuscript providing novel insight into a structural motif catalyzing membrane destabilization. Furthermore the technique used to obtain "high" resolution information by cryo EM is cutting edge and novel.

Since the procedure to obtain almost atomic resolution by cryo EM is not routine, the authors should describe their procedure in more detail. It would be also helpful to include representative maps of all the important interactions described in the main text as supplementary figures.

Specific point:

1. The authors refer in the abstract to the resolution of 3.8 Å; this is not described in the main text

2. Page 4: In the VP5* most of the beta strand backbone .. were clear, and the resolution is ~ 4.5-5Å. In the VP8* lectin domain, most of the beta strand backbone was not clearly resolved ... effective resolution of 7-8 Å.

Since these domains could not be traced based on the EM density alone it should be indicated that the images represented in figure 1 are hybrid models.

3. Page 5: There is no FigS2 included in the current version?

4. Page 5; In the final model it became clear that key residues in the hydrophobic loops are buried in a hydrophobic surface cavity in VP8*.

These features should be shown in a figure, because it seems to be a central structural motif required for membrane interaction.

5. A list of potential interactions between VP5* and VP7 and VP5* and VP6 should be included as supplementary table to help non structural biologist to exploit the information for further studies.

6. Representative maps of a few important interaction sites (VP5* and VP7 and VP5* and VP6) should be included as supplementary figures.

7. Page 9: There is no Figure 4C in the current version?

8. The position of the missing lectin domain should be indicated in figure 1.

Minor point:

The C-C helix in figure 2C should be shown in the same color as indicated in figure 1B.

Additional Correspondence 14 September 2010

Last week (while I was away on holiday) we received the third referee's report on your manuscript, which I am attaching below. As you will see, this referee is also positive about the study and has only relatively minor suggestions for revision. One point I would in particular draw your attention to: in point 4, the referee asks whether you can gather biochemical support for your model of the VP4 structure - specifically the presence/absence of the C subunit lectin domain. Clearly, any data you can obtain in this regard would be very valuable. Otherwise, his/her points should be addressable by revision to the text: in this regard, extending the discussion as suggested in point 7 would be important.

I hope you find these comments helpful, and sorry for the delay in getting them to you!

Best wishes,
Editor

REFEREE REPORT

In this manuscript, the authors describe a structural model of an infectious rotavirus, determined by three-dimensional cryoelectron microscopy using hybrid methods that combine x-ray crystallography and single-particle analyses, and propose a rotavirus entry mechanism. This is a very interesting study; the complete VP4 protein (the viral spike), which for years has eluded high-resolution structural analysis, is traced for the first time and puzzling aspects related to initial events of virus penetration are solved. Quasi-atomic resolution structures have only been achieved by two or three research groups to date, but this study is the first demonstration of a drastic structural polymorphism of a protein, VP4, without use of x-ray data. These findings are significant in the field and should be of interest to all virologists and the structural biology community. I recommend publication of this manuscript after a few changes are introduced.

1. The abstract explains well the context of this study, the nature of the problem addressed, and the authors' experimental strategy. It nonetheless lacks a sentence or two explaining the major findings for VP4 spike structure.

2. The head, body, stalk and foot regions of VP4 spikes are morphological features that make sense when indicated on the viral spike, but are misleading when indicated on the VP4 sequence (as in Fig. 1B). For example, the VP5Ag domain of A and B subunits build the body domain, but the VP5Ag domain of C subunit is located on the stalk region. This item must be clarified throughout the main text to avoid confusing readers not intimately familiar with the rotaviruses. Fig. 1A-B should be shown as an introductory figure addressing this confusing item, and Fig. 1 C-E as the first results figure, i.e., as Fig. 2. Since VP4 structure is the major point of this study, I would recommend including a view of VP4 subunit C alone to be directly compared by visual inspection with VP4 subunits A or B (already included in parts D and E). In addition, the alpha helix that forms the three-chain coiled-coil in the post-entry conformation should be highlighted for A, B and C subunits (as in Fig. 3).

3. In the Results section "atomic model of VP4", Fig. S2 is cited, apparently as a supplementary figure, but there is no Fig. S2.

4. Last paragraph of the "atomic model of VP4" Section. Regarding the lectin domain of VP4 subunit C, which is missed in the structure, is there any biochemical evidence in support of any of the suggested explanations? It seems feasible to distinguish between the two possibilities suggested by simple mass spectrometry analysis of purified rotavirus particles.

5. In section "VP4 interactions with VP6", the authors clearly demonstrate that VP4 spikes only insert into the cavities closer to the icosahedral fivefold axis. It is not clear whether there are steric hindrances that prevent insertion of VP4 spikes on the cavities closer to the icosahedral threefold axis or, alternatively, any kind of biochemical explanation such as selective modification, etc.

6. Fig. 4 is poorly designed. To avoid confusion, VP4 structural domains should be shown without variation; e.g., VP5* body domains are shown as ribbons (left and right) and as ovals (middle). All participating structural elements (hydrophobic loops, the alpha helix of the three chain coiled-coil, etc.) should be indicated in each of the sequential conformational intermediates. Another idea that must be explained is related to the participation of the three VP5* body domains. Would a fusion event be possible in which only two VP5* domains are directly involved?

7. Finally, the discussion is excessively restricted to experimental data from the authors' groups in recent years, and there is no comparison with other non- enveloped viruses. Specifically, a recent paper by Zhang et al. (Cell 141:472, 2010), centered on another dsRNA virus, should be cited and the models (or partial fusion steps) compared. There are a number of reports on different aspects of the cell entry mechanism for viruses such as nodavirus, reovirus and birnavirus that should be reviewed to determine whether there is a general cell entry mechanism or steps for these viruses.

Minor comments:

8. Reference for Kim et al., 2010 is incomplete.

9. Fig. 3B. Chains A, B and C are represented with the same color instead of different colors.

1st Revision - authors' response 18 October 2010

Referee #1:

1. In the abstract, the authors claim a resolution of "about 3.8 A", but in the Results, the method and Figure 5 the resolution is actually 6.5A based on FSC=0.5 and 4.3 on FSC=0.143. The abstract should be corrected accordingly.

We have done so.

2. Figure 5: the labels of "Before CTF correction" and "After CTF correction" are misleading. It should be CTF refinement.

We have corrected the label. Thanks.

Referee #2:

Since the procedure to obtain almost atomic resolution by cryo EM is not routine, the authors should describe their procedure in more detail. It would be also helpful to include representative maps of all the important interactions described in the main text as supplementary figures.

We have added a few details to the Methods and explicitly pointed to references where all further details have been published.

Specific points:

1. The authors refer in the abstract to the resolution of 3.8 Å; this is not described in the main text

Changed to 4.3 Å throughout. We point out in the text that because of threefold averaging, the "effective resolution" is enhanced in the foot, but we agree that there is at present no firm number to describe it.

2. Page 4: In the VP5 most of the beta strand backbone .. were clear, and the resolution is ~ 4.5-5Å.*
In the VP8 lectin domain, most of the beta strand backbone was not clearly resolved ... effective resolution of 7-8 Å.*
Since these domains could not be traced based on the EM density alone it should be indicated that the images represented in figure 1 are hybrid models.

We have done so in the caption to Fig. 2 (which is the relevant part of the old Figure 1 -- see response to reviewer 3), although we prefer to be explicit about the docking procedure, rather than use the phrase "hybrid model".

3. Page 5: There is no FigS2 included in the current version?

This was a typo, now fixed.

4. Page 5; In the final model it became clear that key residues in the hydrophobic loops are buried in a hydrophobic surface cavity in VP8.*

These features should be shown in a figure, because it seems to be a central structural motif required for membrane interaction.

We have done so in a new panel B in Fig. 3 (old Fig. 2).

5. A list of potential interactions between VP5 and VP7 and VP5* and VP6 should be included as supplementary table to help non structural biologist to exploit the information for further studies.*

We understand the reason for this suggestion, but because we would need to assign confidence limits to different levels of interaction and in general provide the kinds of details that would not be directly useful to "non structural biologists", we have decided not to include such a table. We have, of course, deposited the coordinates, and we will be happy to respond to inquiries about specific interfaces or contacts.

6. Representative maps of a few important interaction sites (VP5 and VP7 and VP5* and VP6) should be included as supplementary figures.*

We have included some additional maps as Supplementary figures. Some of the contacts specified by the reviewer are too hard to show in a two-dimensional figure, however; the maps have been deposited in the EM data bank.

7. Page 9: There is no Figure 4C in the current version?

Figure numbering and references to figures have been changed throughout.

8. The position of the missing lectin domain should be indicated in figure 1.

We have done so (in what is now Fig. 2), both with an asterisk in the upper panel, left, and with inclusion of subunit C in the lower panel, right.

Minor point:

The C-C helix in figure 2C should be shown in the same color as indicated in figure 1B.

Doing so would interrupt the color ramping. We have instead used asterisks to show the beginning and end of the relevant polypeptide-chain segment.

Referee #3:

1. The abstract explains well the context of this study, the nature of the problem addressed, and the authors' experimental strategy. It nonetheless lacks a sentence or two explaining the major findings for VP4 spike structure.

Sentence added.

2. The head, body, stalk and foot regions of VP4 spikes are morphological features that make sense when indicated on the viral spike, but are misleading when indicated on the VP4 sequence (as in Fig. 1B). For example, the VP5Ag domain of A and B subunits build the body domain, but the VP5Ag domain of C subunit is located on the stalk region. This item must be clarified throughout the main text to avoid confusing readers not intimately familiar with the rotaviruses.
Fig. 1A-B should be shown as an introductory figure addressing this confusing item, and Fig. 1 C-E as the first results figure, i.e., as Fig. 2. Since VP4 structure is the major point of this study, I would recommend including a view of VP4 subunit C alone to be directly compared by visual inspection with VP4 subunits A or B (already included in parts D and E). In addition, the alpha helix that forms the three-chain coiled-coil in the post-entry conformation should be highlighted for A, B and C subunits (as in Fig. 3).

We have separated the former Fig. 1 into two figures, as suggested. We have redrawn Fig. 1B to remedy the terminology confusion pointed out by the reviewer, and carefully edited the text to remove confusing phrases such as "body domain" (which is, indeed, misleading, now that we know that the stalk is also the -barrel).

3. In the Results section "atomic model of VP4", Fig. S2 is cited, apparently as a supplementary figure, but there is no Fig. S2.

See response to point 3, of referee 2.

4. Last paragraph of the "atomic model of VP4" Section. Regarding the lectin domain of VP4 subunit C, which is missed in the structure, is there any biochemical evidence in support of any of the suggested explanations? It seems feasible to distinguish between the two possibilities suggested by simple mass spectrometry analysis of purified rotavirus particles.

The mass spectrometry suggestion is a good one (and we thank the reviewer for suggesting it), but it would take some effort and time to do the experiment correctly. As mass spectrometry is not straightforwardly quantitative, we would need to look for specific species (e.g., residues 1-29), and even were they present, we would not be confident, without more complex experiments, whether they were present in the expected amounts. We plan to do this experiment, but not on the timescale appropriate for this revision. As the point is not a major one (and we are careful to say that whether the proposed excision has occurred, or whether instead the C subunit is merely very flexible beyond residue 29 does not affect any of our major conclusions), we do not believe that these data are

essential for publication.

5. In section "VP4 interactions with VP6", the authors clearly demonstrate that VP4 spikes only insert into the cavities closer to the icosahedral fivefold axis. It is not clear whether there are steric hindrances that prevent insertion of VP4 spikes on the cavities closer to the icosahedral threefold axis or, alternatively, any kind of biochemical explanation such as selective modification, etc.

See new last sentence in the section on "VP4 interactions with VP6". The selectivity depends on differences between the two sites in the DLP; these differences diminish (although do not vanish) when VP7 binds. We illustrated these changes in a figure in the earlier paper on VP7-recoated DLPs, which is referenced in the revised manuscript.

6. Fig. 4 is poorly designed. To avoid confusion, VP4 structural domains should be shown without variation; e.g., VP5 body domains are shown as ribbons (left and right) and as ovals (middle). All participating structural elements (hydrophobic loops, the alpha helix of the three chain coiled-coil, etc.) should be indicated in each of the sequential conformational intermediates. Another idea that must be explained is related to the participation of the three VP5* body domains. Would a fusion event be possible in which only two VP5* domains are directly involved?*

We have partly redrawn the figure (now Fig. 6). We retain the ovals in the representation of the intermediate, because it is still a hypothetical structure, and we believe that there should be a clear, visual distinction in figures between experimentally determined structures and hypothetical or modeled ones. The "realistic" aspects of the figure are those for which we have direct experimental evidence; the "schematic" aspects are those for which there is some degree of indirect inference. As described in the section ìVP4 conformational transitions,î the entry-associated rearrangement inferred by comparing VP4 structures includes the zipping up of a triple coiled-coil and other three-fold interactions. It therefore requires three VP5* domains.

7. Finally, the discussion is excessively restricted to experimental data from the authors' groups in recent years, and there is no comparison with other non-enveloped viruses. Specifically, a recent paper by Zhang et al. (Cell 141:472, 2010), centered on another dsRNA virus, should be cited and the models (or partial fusion steps) compared. There are a number of reports on different aspects of the cell entry mechanism for viruses such as nodavirus, reovirus and birnavirus that should be reviewed to determine whether there is a general cell entry mechanism or steps for these viruses.

See two new paragraphs in the Discussion (the two preceding the concluding one).

Minor comments:

8. Reference for Kim et al., 2010 is incomplete.

Fixed.

9. Fig. 3B. Chains A, B and C are represented with the same color instead of different colors.

Caption corrected.

2nd Editorial Decision 27 October 2010

Many thanks for submitting the revised version of your manuscript EMBOJ-2010-75603R to the EMBO Journal. It has now been seen again by the original referee 3, whose comments you are appended below. As you will see, he/she is happy with the revision and I am therefore pleased to be able to tell you that we will be able to accept your manuscript for publication in EMBOJ.

However, I do have a couple of comments from an editorial point of view that I need to ask you to

deal with. Firstly, I note that Figure 7 is only referred to in the Materials and Methods, and not at all in the results section. Particularly since the question of resolution is an important one here, I think it would be important to mention this figure in the results - this may entail some reorganisation of the figure order. Secondly, we are currently implementing a policy of requesting an author contributions statement for all accepted manuscripts, and I would therefore ask you to include such a statement after the Acknowledgments section.

Once we receive a final version of your manuscript incorporating these minor changes, we should be able to accept the paper without further delay.

Editor

REFEREE REPORT

Referee #3 (Remarks to the Author):

The paper has been improved and is much more enjoyable to read than the first version. The authors have done a nice job of emphasizing the experimental procedure as well as the biological implications.

2nd Revision - authors' response 12 November 2010

1. Figures have been reordered as requested, so that the (new) Fig. 3 is referenced in the Results section.

2. Author contribution statement added.

