nature portfolio

Peer Review File

Cryo-EM of human rhinovirus reveals capsid-RNA duplex interactions that provide insights into virus assembly and genome uncoating

Corresponding Author: Dr Jose Caston

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Human rhinoviruses (HRVs) are important pathogens that cause significant economic losses and missed work/school days. Currently, there are no approved vaccines or drugs for HRV infections. The authors have done an excellent job in determining the cryo-EM structures of the mature virion and empty viral particles of HRV14. The mature virion structure reveals the presence of RNA duplexes, each composed of 13 base pairs with two pendant bases at each end. These duplexes are bound to the capsid protein layer at particle two-fold axes. The genome-free capsids exhibit asymmetric opening of apertures. Comparison of the cryo-EM structures of RNA-filled virions with the genome-free capsids also shows conformational differences in the capsid proteins. This structural information provides insights into mechanisms of viral assembly, capsid stabilization, and genome uncoating. These findings will be of significant interest to virologists and structural biologists.
The manuscript is well-prepared. I have only a few minor concerns:
Line 154: Fig. S1B is cited, but no panel B is included in Fig. S1.
Lines 158-161: The occupancy of the RNA appears to be high. However, classification focusing on the RNA region may provide a more precise number. This may be achieved using procedures similar to those the authors used to identify different conformational states around the two-fold axis in the genome-free capsids.
Line 174: Table S2 mentions that 29 strains of the HRV B species were used for the conservation analysis. It would be helpful to clarify if these 29 strains represent all known HRV B strains. If not, including the strain name information would be beneficial.
Lines 194-195: The sentence, “Picornavirus capsid assembly is mediated by the viral RNA, which can be readily released from the HRV virion by mild heating,” should include a reference.
Fig. 5 has been cited before Fig. 4. Please ensure that figures are cited in sequential order.

Reviewer #2

(Remarks to the Author)
Gil-Cantero and colleagues determined, by cryo-EM from the same grids the 3D-structures of native RV-B14 at 2.9 Å, and of its empty capsid, at 3.8 Å. Based on its characteristics, the latter was identified as the uncoating product and not the provirion. They thereby confirm and extend the RV-B14 3D-structures previously obtained by X-ray crystallography and cryo-EM. In essence, and not surprisingly, the viral capsid proteins are arranged as previously observed for many rhinovirus types and other members of the ENTEROVIRUS genus. However, here they put the emphasis on the conformation of the RNA genome within the virus particle; in particular, they show how RNA stems of 13 base pairs interact with the viral capsid proteins at the 2-fold axes of icosahedral symmetry. From their results they deduce that about 30 such stems bind to amino acid residues at each 2-fold axis and illustrate how the conformation of these residues changes upon exit of the RNA. They also show the result of a folding prediction of the viral RNA and identify 31 stems as possible interactors at the inner wall of the capsid.
Using focused 3D-classification of the region at the 2-fold axes of empty capsids they find various degrees of opening of the Z-shaped pore as a result of the structural change occurring during uncoating. They interpret these results as indicating that not all 30 orifices are created simultaneously and/or open to the same degree; there might rather be larger and smaller holes

with the former preferentially serving as portal for RNA exit.
These are interesting findings teaching us more about possible pathways of assembly and uncoating of RV-B14 and the ENTEROVIRUS genus in general.

I have several questions and suggestions:
1) They should mention and discuss the possibility of uncoating occurring via holes in the viral capsid appearing upon loss of one or more pentamers. Such a mechanism was described by the Plevka group in several papers on viral uncoating. It should be considered that particles lacking one or more pentamers might be missed in icosahedral averaging as the density of would just be roughly 8% less.
2) Thirty-one candidate RNA stems for electrostatic binding (via the phosphate groups) to basic amino acid residues were identified by RNA secondary structure prediction. If this is a general phenomenon it should also be seen in other rhino- and enteroviruses. It would be interesting to compare such RNA folding predictions of several RV-genomes to see whether the stems are conserved despite differences in their sequences. By the same token they should run the same predictions on a random sequence of the same length. Would similar stems also be present? Is specificity of encapsidation only based upon the vicinity of the capsid proteins and the de novo synthesized RNA emerging from the replication complex or is the RNA sequence also implicated?
3) Fig. S1: Panel B is missing
4) Fig. S3 and Methods: they should describe the details of how the genome was 'computationally removed' and more clearly explain what is A, B, and C.

Discussion:
The RNA assisting in the capsid assembly was previously discussed by Jason D Perlmutter et al. (eLife 2:e00632. 2013. https://doi.org/10.7554/eLife.00632) and should be included in the discussion.
The model with 13bp stems interacting with residues at the 2-fold axes is illustrative but is in variance with the short- and long-range base pairing demonstrated experimentally in another enterovirus (Zhang, M. et al. Nat Commun 12, 2344 (2021). https://doi.org/10.1038/s41467-021-22552-y). The different models regarding ssRNA genome folding in enteroviruses should be mentioned and briefly discussed. By the same token, work by Hrebík et al., presented evidence for the presence of octanucleotide stems below the 2-fold axis. Similarities/differences from this previous finding and the current one should be discussed.

I noticed the following typos:
L31: complexnetwork > complex network
The RNA folding prediction server is in Vienna (English) not Viena (Spanish)

Minor point:
The current nomenclature of human rhinoviruses is 'RV-A2', 'RV-B14', etc. I recommend adhering to this rule for consistency although 'HRV14' was used in earlier papers.

I recommend acceptance after minor changes

Reviewer #3

(Remarks to the Author)
The manuscript by Gil-Cantero et.al., describes a 2.9A cryo-EM structure of human rhinovirus and the ordered RNA fragments attached to the internal capsid surface. The authors have also determined a 3.8A structure of empty capsids for comparison. Structures of rhinoviruses are well-described and have been known, but the new discovery here is the local ordering of genome in the rhinovirus capsid which sheds light on molecular interactions between virus capsid and RNA. The authors also report asynchronous opening in the virus capsids, providing data to suggest likely mechanism of genome release. Overall, the manuscript is well-written, and results are clearly described, but few points need to be addressed, as given below:
1. From the cryo-EM map at local resolution of 3.8A, the authors suggest that the flanking nucleotide are purines. The resolution of the map cannot confirm whether the nucleotide is purine or pyrimidine. The authors support this claim based on previous HRV14 structures in the results section, which is fine. However, in the discussion of the paper (line 261 and 276), the authors claim definitively that they identified unpaired purines in their current structures. This is a claim not supported by the resolution of the RNA segments and should be tempered down. Or the authors should add supplementary figures showing the fitting of the purine in density compared to a pyrimidine to bolster their claim. As such from the figures of the density maps, this cannot be confirmed.
2. In the discussion, the 2 paragraphs (lines 313-319 and lines 373-382) feel repetitive as they summarize the already described discussion in the previous paragraphs and may be removed or trimmed down.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

My concerns have been fully addressed.

Reviewer #3

(Remarks to the Author)
The authors have answered my queries satisfactorily. I have no further comments.

Reviewer #4

(Remarks to the Author)
The current acronym and nomenclatures for human rhinoviruses are 'RV', ‘RV-A2’, ‘RV-B14’, etc. I recommend adhering to this rule for consistency throughout the manuscript and figures.

Manuscript: COMMSBIO-24-4693

Point-by-point reply to Reviewer #1

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The manuscript is well-prepared. I have only a few minor concerns:

1. Line 154: Fig. S1B is cited, but no panel B is included in Fig. S1.
We apologize for this omission. Panels B (and C) has been included in a revised Fig. S1 relative to local resolution assessment for full (B) and empty (C) RV-B14 maps (l. 128-131).

2. Lines 158-161: The occupancy of the RNA appears to be high. However, classification focusing on the RNA region may provide a more precise number. This may be achieved using procedures similar to those the authors used to identify different conformational states around the two-fold axis in the genome-free capsids.
As suggested by the reviewer, we have analyzed the virion interior at the two-fold axes by extracting the region corresponding to the location of the dsRNA segments from the original cryo-EM images. For that, a mask with the size and morphology of the dsRNA duplexes was used, and the independent subparticles were then subjected to a three-dimensional classification without alignment. The five resulting classes contained a density that corresponded to a dsRNA segment, and there was no empty class indicative that dsRNA segment is absent. The occupancy of the RNA was nearly 100%. To clarify this point, a new paragraph is included in the main text (l. 166-168), and a new supplemental movie is provided (Movie S2).

3. Line 174: Table S2 mentions that 29 strains of the HRV B species were used for the conservation analysis. It would be helpful to clarify if these 29 strains represent all known HRV B strains. If not, including the strain name information would be beneficial.
Yes, the 29 serotypes (strains) of the HRV B species correspond to all known HRV B serotypes. This point has been clarified in the main text of the revised manuscript (l. 192).

4. Lines 194-195: The sentence, "Picornavirus capsid assembly is mediated by the viral RNA, which can be readily released from the HRV virion by mild heating," should include a reference.
A reference (#19) has been included in that sentence in the revised manuscript (l. 207).

5. Fig. 5 has been cited before Fig. 4. Please ensure that figures are cited in sequential order.
In the revised manuscript Fig. 5 and Fig. 4 have been renumbered and reordered so they are now cited and shown in sequential order.

Point-by-point reply to Reviewer #2

Gil-Cantero and colleagues determined, by cryo-EM from the same grids the 3D-structures of native RV-B14 at 2.9 Å, and of its empty capsid, at 3.8 Å. Based on its characteristics, the latter was identified as the uncoating product and not the provirion. They thereby confirm and extend the RV-B14 3D-structures previously obtained by X-ray crystallography and cryo-EM. In essence, and not surprisingly, the viral capsid proteins are arranged as previously observed for many rhinovirus types and other members of the ENTEROVIRUS genus. However, here they put the emphasis on the conformation of the RNA genome within the virus particle; in particular, they show how RNA stems of 13 base pairs interact with the viral capsid proteins at the 2-fold axes of icosahedral symmetry. From their results they deduce that about 30 such stems bind to amino acid residues at each 2-fold axis and illustrate how the conformation of these residues changes upon exit of the RNA. They also show the result of a folding prediction of the viral RNA and identify 31 stems as possible interactors at the inner wall of the capsid.
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These are interesting findings teaching us more about possible pathways of assembly and uncoating of RV-B14 and the ENTEROVIRUS genus in general.
I have several questions and suggestions:

1. They should mention and discuss the possibility of uncoating occurring via holes in the viral capsid appearing upon loss of one or more pentamers. Such a mechanism was described by the Plevka group in several papers on viral uncoating. It should be considered that particles lacking one or more pentamers might be missed in icosahedral averaging as the density of would just be roughly 8% less.

In the revised manuscript the possibility that RNA uncoating in rhinovirus via holes upon loss of one or more pentamers, as found for echovirus by Plevka and colleagues, has been discussed and referenced (l. 383-385, reference #49).

2. Thirty-one candidate RNA stems for electrostatic binding (via the phosphate groups) to basic amino acid residues were identified by RNA secondary structure prediction. If this is a general phenomenon it should also be seen in other rhino- and enteroviruses. It would be interesting to compare such RNA folding predictions of several RV-genomes to see whether the stems are conserved despite differences in their sequences. By the same token they should run the same predictions on a random sequence of the same length. Would similar stems also be present? Is specificity of encapsidation only based upon the vicinity of the capsid proteins and the de novo synthesized RNA emerging from the replication complex or is the RNA sequence also implicated?

We fully agree on the interest of comparing the RNA folding predictions for RV-B14 with other rhinoviruses, enteroviruses and picornaviruses in general, including a random sequence and heterologous RNAs as a control. The situation is quite complex as the possibility of interconverting structures and the influence of capsid-RNA interactions during packaging and in the assembled virion cannot be disregarded. We have already undertaken an in-depth, detailed study that attempts to address that question that is beyond the present study. Until that study is completed, the existence in the RV-B14 genome of the predicted RNA stems remains a tentative hypothesis. We have modified several sentences in the revised manuscript to make clear that the existence of the predicted stems is only a possibility that will be investigated in depth in a further study (l. 285-288).

3. Fig. S1: Panel B is missing
We apologize for this omission. Panels B (and C) has been included in a revised Fig. S1 relative to local resolution assessment for full (B) and empty (C) RV-B14 maps (l. 128-131).

4. Fig. S3 and Methods: they should describe the details of how the genome was 'computationally removed' and more clearly explain what is A, B, and C.
The genome is computationally removed from the original images by applying a mask based on the contour of the protein capsid. That is stated in the new version of the manuscript (l. 433-435). The procedure to calculate the asymmetric reconstructions shown in Fig. S3A, B and C is explained in detail in the section of Methods (l. 430-441).

5.Discussion:
The RNA assisting in the capsid assembly was previously discussed by Jason D Perlmutter et al. (eLife 2:e00632. 2013. https://doi.org/10.7554/eLife.00632) and should be included in the discussion.
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The detailed theoretical study by Perlmutter et al. on RNA-assisted virus assembly has been cited in the Discussion in relation to charge-charge capsid-RNA interactions and base pairing that help drive virus assembly (l. 317-319, reference #42).
The models regarding possible RNA folding in enteroviruses described by Zhang et al. have been briefly mentioned and referenced in the Discussion of the revised manuscript (l. 283-285; reference #36).
A discussion on the similarities and differences between the paired octanucleotide strands found by Hrebík et al. and the 13bp duplexes described in our work, and of the possibility of a dynamic connection between both, has been included in the Discussion section of the revised manuscript (l. 354-365).

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Those mistakes have been corrected in the revised manuscript.

7. Minor point:
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RV-14 has been used instead of HRV14 throughout the text of the revised manuscript

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Point-by-point reply to Reviewer #3

The manuscript by Gil-Cantero et.al., describes a 2.9A cryo-EM structure of human rhinovirus and the ordered RNA fragments attached to the internal capsid surface. The authors have also determined a 3.8A structure of empty capsids for comparison. Structures of rhinoviruses are well-described and have been known, but the new discovery here is the local ordering of genome in the rhinovirus capsid which sheds light on molecular interactions between virus capsid and RNA. The authors also report asynchronous opening in the virus capsids, providing data to suggest likely mechanism of genome release. Overall, the manuscript is well-written, and results are clearly described, but few points need to be addressed, as given below:

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We agree that, from the density shown in the original Fig. 2C, one cannot be certain that the flanking nucleotides are purines and not pyrimidines. To clarify this point and following the reviewer suggestion, we have fitted a purine or a pyrimidine in the density of the flanking nucleotide bases of the RNA duplexes. The fitting results show that a purine (R) has a higher occupancy than that of a pyrimidine (Y). In addition, the Trp38 was used as an internal control that confirms the correct density threshold selected for the cryo-EM map of full virions. A new supplementary figure, Fig. S5, is included in the new version of the manuscript together with a new version of Fig. 2C, in which we have changed the rectangle of the unpaired base by a purine (l. 184-185). We have modified the first sentence in the discussion and it now reads “In addition, unpaired flanking bases, most probably purines, were identified at both ends of each dsRNA segment” (l. 271-272)

2. In the discussion, the 2 paragraphs (lines 313-319 and lines 373-382) feel repetitive as they summarize the already described discussion in the previous paragraphs and may be removed or trimmed down.

The indicated two paragraphs have been removed and merged as a short paragraph at the end of the Discussion (l. 388-394).