26 February 2009

Dr. Stephen C. Harrison

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Ref: *******

Dear Dr. Harrison:

Thank you for submitting your manuscript "Molecular interactions in rotavirus assembly and uncoating seen by high-resolution cryo-EM." We have now received the detailed reviews of your paper. Unfortunately they are not positive enough to support publication of the paper in Science. Although we recognize that you could likely address many of these specific criticisms in a revised manuscript, the overall nature of the reviews is such that the paper would not be able to compete for our limited space. We are open to the possibility of including data from this manuscript into the co-submitted manuscript *******, but appreciate that this might be logistically difficult and would understand if you decide instead to send the full manuscript elsewhere.

We are grateful that you gave Science the opportunity to consider your work.

Sincerely,

Senior Editor

Review 1

The paper by Chen et al describes the three-dimensional structure of a synthetic rotavirus-related particle produced by coating the double-layered particle with trimers of the outer layer protein VP7. The structure was determined by electron cryomicroscopy and the authors claim an unusually high (for EM) resolution, about 4 Å, for it. Into their density map, they dock the crystal structure for most of VP7 that they determined separately by X-ray crystallography (accompanying manuscript) and from the map they assign a fold for the small N-terminal region that is not seen in the crystal structure.

In the last year, three EM structures at similar nominal resolutions have been published, two in Nature (refs 14 and 15) and one, by this group, in PNAS (ref 16).

I have no doubt that the authors are performing their EM at or very close to the state of the art. However, this paper appears better suited for the specialist literature.

The EM technology is impressive but the claim made in this Summary (“and illustrate that electron cryomicroscopy now affords greatly expanded possibilities for studying large-scale assemblies in molecular detail ”) has already been made in the three papers cited above and there is no further advance here.

Reasons:

1) The physiological status of the 7RP particle is unclear.

2) Differences are discussed between the VP7 structure in the 7RP particle and the crystal structure. However, it is not clear whether the conformation seen in the crystal is affected by binding the Fab with which it is complexed or by crystallization or crystal contact effects. The molecule in crystallization trials is a 1 : 1 complex of VP7 monomer with Fab. 4F8, not a trimer of VP7.

3) The claimed resolution (4 Å) looks to be exaggerated and more at the 6Å level. The authors only get to 4Å after averaging and symmetrizing the VP7 trimers and using the overly optimistic and little used threshold of 0.142 (0.5 is usual) in Supp Fig 1. That this is so is supported by the appearance of the blue net density on the right hand side of Fig 2C. If the map was at 4.5Å or better, the two strands should be clearly resolved in this slab of density.

4) It is interesting but not convincingly demonstrated that they can determine the CTF for individual particles with the precision and accuracy claimed. It would be better to present this work fully in a Methods paper that would include pictures of individual diffraction patterns and quantitative statistical analyses of the residual uncertainties in the parameters estimated.

Review 2

Rotaviruses are serious pathogens that are a significant cause of morbidity and mortality. In addition, they are also fascinating viral machines that still harbor many secrets. Some of these are now revealed in the two manuscripts jointly submitted by Chen et al. and Aoki et al. The two papers, one of which reporting the high-resolution analysis of a recoated rotavirus particle by electron microscopy and the other reporting the crystal structure of the VP7 coat protein in complex with an Fab, make important contributions to our understanding of rotavirus structure and function, and they complement each other nicely by revealing conformational changes in VP7, and by providing support for a mechanism of uncoating. In addition, the paper by Chen et al. is one of still very few that reports a structural analysis using cryo-electron microscopy at near-atomic resolution, which is in itself a remarkable achievement. Although previous electron microscopy and crystallographic studies of rotaviruses have already revealed many interesting features, the level of detail seen here (exemplified by the connector between VP7 and VP6) is a major step forward. The crystal structure of VP7 reported in the second paper also forms a promising basis for new approaches for anti-rotavirus therapy. Together, the two manuscripts also generate much support for a calcium-ion mediated uncoating mechanism that is blocked by Fab binding. Both manuscripts are therefore well suitable for publication in Science.

I do have some questions and comments for the authors to consider:

Chen et al.:

1. The authors used recoated particles for their reconstruction of 7RP, that is they incubated the double-layered virus particle with recombinantly produced VP7. I am a little unclear on the effects of recoating procedure described in the supplemental methods. As stated there, DLPs (which do not contain either VP7 nor VP4) were incubated with VP7, and a reconstruction of this recoated particle, called 7RP, was performed. In the paper on DLP recoating by Trask & Dormitzer, which is cited here in support, it is stated that “VP4 must be added before VP7 to obtain high level of infectivity”, and that “VP7 binds the particles and locks VP4 in place”. I did not read the Trask paper in detail, but I wonder if leaving out VP4 in the recoating would lead to altered VP7 structure or altered contacts with the rest of the particle?

2. Page 3: The authors cite a publication by McClain et al reporting a DLP

crystal structure (12). No journal or other information is given in the reference list. What is the state of this manuscript?

3. Figure 1A contains a mistake – the red protein labeled as "VP2" should be labeled "VP4". Also I found it odd (and confusing) that the lower-resolution triple-layered particle (and not the high-resolution 7RP structure) is shown here.

4. Figure 1B is never referred to in the text. It is nice to look at but it can probably be moved to the Supplemental Material section.

5. Figure S1 (the Fourier shell correlation plot) is missing its labels/units for the x- and y axes (resolution and correlation coefficient).

6. The authors discuss a glycan attached to Asn69 of VP7, and the terminal portion of this glycan is clearly visible in the density maps and was actually very helpful in tracing the N-terminal arms of VP7. On page 6 (line 6) they state that the carbohydrate moiety is recessed between VP6 trimers and not exposed to the surface. Is there enough space in the recessed space to accommodate a typical large, branched glycan structure? Would this glycan be able to mediate contacts between VP6 molecules and thus play a role in the conformational changes described in the paper (e.g. in Figure 3)? These questions are perhaps also of relevance as VP7 was produced in insect cells, which differ in their glycosylation pattern from the natural host.

7. The contacts between the VP7 and VP6 trimers are very interesting and also quite unusual. The N-terminal arms serve as anchors that hold VP7 above the VP6 trimers, and few other contacts between the two proteins are seen. The N-terminal arms seem to contain a region that is neither alpha-helical nor beta-strand, from residue 65 to residue 71, and that does not seem to contact any other protein chains. One would expect such a region to be quite flexible, which in this case it obviously is not as it has good density and serves to position the VP7 trimer. Maybe the authors can include a bit more detail on the thermal factors and conformational stability of this central region?

8. Figure 2 B shows differences between the viral-bound VP7 and the crystallized VP7 protein presented by Aoki et al. The description of these differences is somewhat unclear. How were these two trimers superimposed? From the lower panel of Figure 2B, it looks as if the VP7 trimer tilts to one side (red chain: left side moving up, right side not).

9. A sentence in the legend to Fig 2B is also a bit unclear: "The way in which the domain hinge displacement flattens the subunit when the trimer binds VP7…." Is the "trimer" the authors refer to the VP6 trimer? Or should "binds VP7" read "binds VP6"? I think the latter is meant here, but I am not sure.

Aoki et al. (accompanying manuscript):

1. The VP7 surface that faces VP6 is described as "somewhat negatively charged" (page 5). Do the authors think that this plays a role in the interaction with VP6 (accompanying paper)? What are the characteristics of the opposing surface of the VP6 trimer?

2. The Rsym value of 72% for the highest resolution bin (at 3.4 Å) would seem to be rather high. What were the criteria used by the authors to determine the maximum resolution? Given the limited resolution of the data, I think it would be helpful to include a figure showing the electron density, perhaps at the VP7-Fab interface, as a supplemental figure. The authors should also list the refinement Rfactors (Rwork, Rfree) for the highest resolution shell.

3. The authors state that the Fab heavy chain forms most of the contacts with VP7. Can they support this statement with numbers, such as the percentages of surface areas on VP7 buried by the different parts of the Fab?

4. The VP7-Fab complex was separated from unbound Fab using gel filtration. Why does Figure S1 show two peaks for the unbound Fab?

5. The paper refers to “Table 2” on page 5. Presumably “Table S1” is meant here?

6. The authors produced a disulfide-linked VP7 trimer that binds antibodies and could be a candidate for an immunogen. Are the disulfide bonds exposed, that is can they be reduced in solution?

Editorial Assistant

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