Review of: "Nucleocytoviricota Viral Factories Are Transient Organelles Made by Phase Separation"

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Potential competing interests: No potential competing interests to declare.

Rigou *et al.* present a highly relevant article revealing that Mimivirus viral factories are formed by phase separation and that at least two scaffold proteins are involved in this process. They also demonstrate client proteins to show the sub-compartmentalization of functions within the Mimivirus viral factory. In addition, they computationally identified and experimentally verified other scaffold proteins from viruses in the same phylum (*Nucleocytoviricota*). Since the molecular grammar for viruses within the *Nucleocytoviricota* is conserved, the authors make the case that the common ancestor of this phylum already possessed a viral factory.

It is clear that a lot of work has gone into this manuscript to provide the evidence for the conclusions. While indeed the evidence is strong, the manuscript is also written for experts on *Nucleocytoviricota*, phase separation, and genetic modification. For the text itself, I have the following general comments/suggestions:

- Many of the terms used are not explained, making this manuscript complicated for readers unfamiliar with them. Additional explanations throughout the manuscript would be beneficial to reach a wider audience.
- The introduction is very short, and I had the impression I was reading a second abstract. A clear introduction section with additional information on the topic will aid readers in navigating the manuscript.
- Include the aim of the study in the abstract and introduction.
- Highlight the study's novelty, as its findings are exciting!
- The manuscript uses too many acronyms, which makes it confusing to read. For clarity, the authors could try to reduce the number of acronyms by limiting them to only those that are often used (*e.g.* VF, OL, IL, PS) and using the full words for those that are only used in subsections of the manuscript (*e.g.* IDR, BMC, ...)
- To follow the ICTV nomenclature for viruses (https://ictv.global/faq/names)

Are high-resolution images and/or raw data available for the fluorescent microscopy images? Many of these images are small and blurry, so even though the description explains what is to be seen, it is not clearly visible (e.g., Figs 1B, 1F, 2B, 4A, 4B). It is often hard to distinguish between the cell, the viral factory, and the viral particles. I would like to zoom in on the images to see them better.

Minor comments:

Introduction and abstract: Please revise the following phrase: “Members of the phylum*Nucleocytoviricota* include the *Poxviridae*, the climate-modulating *Emiliania huxleyi virus*, and the previously termed Nucleocytoplasmic large DNA viruses (NCLDV)”. Viruses within the phylum *Nucleocytoviricota* were previously referred to as NCLDVs. Therefore, the statement that the *Nucleocytoviricota* include the previously termed NCLDVs is incorrect; both names refer to the same group of viruses. https://ictv.global/taxonomy/etymology?page=7

Page 4:

- Mollivirus is mentioned here, but there is no previous explanation that Mollivirus replicates in the nucleus instead of forming its own VF. The reader has to deduce this from the last phrase of this paragraph. When introducing the viruses used, it would be helpful to directly indicate which of the viruses is nuclear and which is cytoplasmic.
- Briefly explain what 1,6-hexanediol does.
- State which proteins were fluorescently labelled to identify the sub-compartments. This only becomes clear later in the manuscript. Perhaps changing the order in which the results are presented (first identification of scaffold proteins, and then all other experiments using these two proteins) will help to clarify this.

Fig 1C: the legend does not explain what the dark blue and light blue coloured bars mean.

Fig 1E legend and main text: explain what the proposed “Virion Factory” means.

Fig. 6I is truncated and cannot be fully seen.

Typos:

- Page 5 subtitle: correct “viral factory’s” to “viral factories”.
- Page 6: add space between generated and Acanthamoeba.
- Figure 3B: amoebae instead of amoebas (this error occurs multiple times in the manuscript text as well)
- Figure 3 legend line 1: extra spaces in the text.