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

Yves Gaudin[1]

1 Virology, Institute of Integrative Biology of the Cell, Gif-sur-Yvette, France

Potential competing interests: No potential competing interests to declare.

In their manuscript, Rigou and colleagues have characterized the viral factories (VF) of the Nucleocytoviricota and provide evidence that they are biomolecular condensates (BMCs) formed by phase separation.
They also generate a pipeline to bioinformatically identify putative scaffold proteins in all other Nucleocytoviricota.
Moreover, by immunoprecipitation and mass spectrometry analysis, they identify several client proteins of the viral factories of mimivirus, which reveals sub-compartmentalization of functions.
This is an important work that will be of interest to those working on giant DNA viruses. Furthermore, their bioinformatic pipeline could likely be adapted to several other BMCs conserved among organisms.

I have some remarks that could be taken into consideration to improve the manuscript and its conclusions.

1) In the paragraph entitled "*Nucleocytoviricota viral factories are biomolecular condensates*", the authors mention that they use 1,6-hexanediol (1,6-HD) to confirm the membrane-less organelle-like nature of these structures. The absence of a membrane is obvious just by looking at the micrographs in figure 1A and supplementary figure 1.

Generally, 1,6-HD is used to demonstrate that the structures are formed by liquid-liquid phase separation (LLPS). Its use is not without artifacts (see S. Kroschwald, S. Maharana, S. Alberti. Hexanediol: a chemical probe to investigate the material properties of membrane-less compartments. Matters (2017), 10.19185/matters.201702000010 that might be cited).

Similarly, in figure 1H, the authors show a fusion event between the outer layer of two VF. In general, such fusion events are used to demonstrate that the biomolecular condensates have liquid properties and are formed by LLPS.

Do the authors suggest that the VF (at least their outer layer) have liquid properties and are formed by LLPS? This must be clearly stated because in the text they only refer to PS.

That said, I am not completely convinced by the fusion event presented in figure 1H. Even after 330 seconds, I do not see a relaxation toward a spherical structure. If I am correct, the authors have used a Zeiss Axio Observer Z1 inverted microscope. So this is not confocal microscopy, and the Z depth is important. The two structures may not have merged, but one may have positioned itself partly below the other.

2) The condensates formed in vitro by OLS1 and ILS1 are very different. Those formed by OLS1 (panel 2D, S3A, S3B) are well individualized and spherical, suggesting that they are formed by LLPS. This is not the case for those formed by

ILS1 in the presence of DNA (panel 2C, S2E, S2F, S2G). They are not particularly spherical. Moreover, they are not homogeneously dispersed and rather form clusters. Also, the authors do not mention the size of the linear and circular DNA used. If the DNA is long enough, it may be locally compacted by ILS1 (giving this aggregated pearl necklace appearance)?

I do not know if the authors have access to the FRAP technique. If that is the case, it would be great to perform FRAP experiments in vitro and in vivo on the outer and inner layers of the VF using fluorescent versions of OLS1 and ILS1. This would probably reveal important differences in dynamics between these two proteins within their compartments and would allow us to see whether one or the other of these proteins shuttles between the VF and the cytoplasm.

In any case, the difference in behavior between the two proteins (in terms of condensate characteristics) should be mentioned and discussed.

3) Some sentences are somewhat intellectual shortcuts, and the authors should clarify what they mean. Here are a few examples:

The last sentence of the introduction: "*We demonstrated that mimivirus VF nuclear-like functions are accomplished by PS.*"
In fact, the functions are not accomplished by PS. Indeed, PS allows the formation of membrane-less organelles (the VFs) concentrating several proteins having nuclear-like functions.

They also often refer to the central dogma.
In the abstract: "*We demonstrate important sub-compartmentalization of functions including the central dogma*"
I would rather say "*including those related to the central dogma*."
Similarly, the caption of Figure 6 is a bit strange. "*Sub-cellular and sub-organelle compartmentalization of the central dogma.*"
I would replace it with: "*Sub-cellular and sub-organelle compartmentalization of proteins involved in the central dogma*"

The authors wrote:
"*The viral factories (VFs) of several viruses (DNA and RNA) are formed by PS including members of herpesvirus, adenovirus and the respiratory syncytial virus.*"
This seems to give a special status to the RSV. I would suggest writing:
"*The viral factories (VFs) of several viruses (DNA and RNA) are formed by PS including members of herpesvirus, adenovirus and reovirus families as well as of the mononegavirales order.*"
Indeed, RSV is a Mononegavirales among others, and the formation of viral factories by LLPS has been demonstrated for several viral families of this order, including Rhabdovidae (rabies virus and VSV), filoviridae (Ebola virus), Paramyxoviridae (measles virus and mumps virus), and Pneumoviridae (RSV).
Similarly, dsRNA viruses of the reoviridae family (including reoviruses and rotaviruses) also form their viral factories by LLPS.

Finally, regarding herpesvirus, there is a controversy over how their replication centers are formed. (See D.T. McSwiggen, A.S. Hansen, S.S. Teves, H. Marie-Nelly, Y. Hao, A.B. Heckert, et al. Evidence for DNA-mediated nuclear compartmentalization distinct from phase separation. eLife, 8 (2019), Article e47098, 10.7554/eLife.47098).