Cryo-EM cools down swine fever

DOI 10.1074/jbc.H119.012169

John R. Gallagher and Audray K. Harris

From the Laboratory of Infectious Diseases, NIAID, National Institutes of Health, Bethesda, Maryland 20892

Edited by Craig E. Cameron

African swine fever virus (ASFV) is among the most complex DNA viruses known. Outbreaks have killed millions of swine around the world, and there is currently no vaccine. Three recent papers report the cryo-EM structure of the complete ASFV virion, comprising a viral particle of multiple layers, and resolve the major outer-capsid protein p72 to higher resolution. Progress in these reports provides a further understanding of the structure-function relationships of large viruses and should aid in ASFV vaccine development.

African swine fever virus (ASFV)² is a terrifyingly complex DNA virus causing rampant disease in the world’s swine population. Millions of pigs are being culled to limit the spread of infection due to the lack of treatment or vaccination to protect from the disease (1). Beyond the supply of pork, concerns are mounting that other disruptions will occur, such as to the global supply of heparin (2). A safe and effective ASFV vaccine is badly needed. Efforts so far have focused on live-attenuated viruses, as well as subunit-based approaches using purified antigens. Whereas attenuated virus vaccine candidates have conferred protection from ASFV, side effects and safety concerns dictate that further development is required. Meanwhile, subunit-based vaccines, which yielded early results in antigenicity, thus far have failed to protect swine from infection (3). One possible explanation for these failures is that the fully assembled virion is thought to consist of five distinct layers, leading to multiple possible antigens. A detailed structural understanding of the ASFV virion may be just what is needed to reboot subunit-based vaccine design. A clear picture of the ASFV antigens as they are displayed on virus particles would point to vaccine targets capable of eliciting protective immune response and, potentially, neutralizing antibodies.

While the viruses we are most familiar with, such as influenza, are about 120 nm in diameter (4), ASFV virions are almost twice as big at 200 nm in diameter. Cryo-EM structure determination has played a critical role in solving the structure of other large viruses, such as herpesvirus (5), but the pure size of ASFV poses many challenges. For example, particles of this size span more than one focal plane when imaged by cryo-EM, causing image blurring, which must be corrected or selectively ignored. Moreover, large viruses are thicker than the typical layer of thin ice used to capture cryo-EM specimens, inviting either artifacts due to freezing in thin ice or loss of signal-to-noise in thicker ice. Compounding other challenges, few viral particles can be recorded per image simply due to the limited field of view of the camera, frustrating efforts to collect sufficient data for 3D reconstruction. Three recent reports, however, illustrate that these large particles are now within the grasp of cryo-EM structure determination thanks to biochemical or computational decomposition (6–8) and provide important new details of the ASFV virion structure.

In a brute-force effort to collect over 1,000 viral particles, in this issue Andrés et al. (6) are able to report the structure of the complete, intact ASFV virion. Starting from the inside, the structure includes an inner nucleoid region containing the dsDNA genome (170 – 190 kbp) enclosed by an inner capsid. This inner capsid is coated by an endoplasmic reticulum–derived membrane. Next there is an outer capsid layer, which is enveloped loosely by a plasma membrane–derived outer envelope (Fig. 1). The outer capsid primarily consists of the viral protein p72, which has been implicated as an antigen that induces ASFV-neutralizing antibodies (9).

Andrés et al. employed additional approaches to overcome the degradation of resolution at the outer viral capsid, which contains antigenically important protein p72. To circumvent problems posed by imaging overly large particles by cryo-EM, they deconstructed the virus biochemically and then purified and resolved the structure of p72 as individual homotrimeric free in solution (6). In a parallel effort, Wang et al. (7) have recently reported the structure of ASFV, but they employ a different approach to resolve the outer capsid. Wang et al. computationally extract patches of the virion outer capsid during cryo-EM image analysis to correct for the local defocus and arrive at a similar-resolution p72 structure by a complementary approach (7). Finally, Liu et al. (8) report similar structural results for the outer capsid using block-based reconstruction-processing programs.

Andrés et al. also resolve the inner capsid in fine structural detail. Built from polyprotein components pp220 and pp62, the inner capsid structure sheds light on critical steps in virion assembly (10). Although the inner capsid is unlikely to be the target of neutralizing antibodies, antiviral compounds may be able to interfere with the elaborate process of concomitant assembly of inner and outer capsids.

Elucidation of the structure of ASFV opens up new questions that can now be pursued. Because the ASFV genome sizes may vary by as much as 20 kbp, it will be interesting to discover what, if any, structural differences are present in any of the other
genotypes of ASFV. ASFV is a known arbovirus, meaning that it can infect arthropods (and ticks specifically). Because numerous host proteins are incorporated in the virion, is the virion different antigenically when ASFV replicates in tick cells? Cryo-EM work on ASFV will bring together the fields of structural biology and vaccinology to aid the understanding of ASFV structure-function, antigen localization, and epitope identification. Also, the cryo-EM work on ASFV not only defines strategies for determining the structures of large complex viruses but also advances our understanding of the structures of the nucleocytoplasmic large dsDNA virus family to which ASFV belongs.

Figure 1. Schematic of African swine fever virus depicting the multilayer architecture consisting two membrane and two capsids with genomic nucleoid at the center.

References
1. Mallapaty, S. (2019) Spread of deadly pig virus in China hastens vaccine research. Nature 569, 13–14 CrossRef Medline
2. Vilanova, E., Tovar, A. M. F., and Mourão, P. A. S. (2019) Imminent risk of a global shortage of heparin caused by the African swine fever afflicting the Chinese pig herd. J. Thromb. Haemost. 17, 254–256 CrossRef Medline
3. Revilla, Y., Pérez-Núñez, D., and Richt, J. A. (2018) African swine fever virus biology and vaccine approaches. Adv. Virus Res. 100, 41–74 CrossRef Medline
4. Gallagher, J. R., McCraw, D. M., Torian, U., Gulati, N. M., Myers, M. L., Conlon, M. T., and Harris, A. K. (2018) Characterization of hemagglutinin antigens on influenza virus and within vaccines using electron microscopy. Vaccines (Basel) 6, E31 CrossRef Medline
5. Dai, X., and Zhou, Z. H. (2018) Structure of the herpes simplex virus 1 capsid with associated tegument protein complexes. Science 360, eaao7298 CrossRef Medline
6. Andres, G., Charro, D., Matamoros, T., Dillard, R. S., and Abrescia, N. G. A. (2019) The cryo-EM structure of African swine fever virus unravels a unique architecture comprising two icosahedral protein capsids and two lipoprotein membranes. J. Biol. Chem. 294, 1–12 CrossRef Medline
7. Wang, N., Zhao, D., Wang, J., Zhang, Y., Wang, M., Gao, Y., Li, F., Wang, J., Bu, Z., Rao, Z., and Wang, X. (2019) Architecture of African swine fever virus and implications for viral assembly. Science 366, 640–644 CrossRef Medline
8. Liu, S., Luo, Y., Wang, Y., Li, S., Zhao, Z., Bi, Y., Sun, J., Peng, R., Song, H., Zhu, D., Sun, Y., Li, S., Zhang, L., Wang, W., Sun, Y., Qi, J., Yan, J., Shi, Y., Zhang, X., Wang, P., Qiu, H. J., and Gao, G. F. (2019) Cryo-EM structure of the African swine fever virus. Cell Host Microbe 26, 836–843 CrossRef Medline
9. Escribano, J. M., Galindo, I., and Alonso, C. (2013) Antibody-mediated neutralization of African swine fever virus: myths and facts. Virus Res. 173, 101–109 CrossRef Medline
10. Andrés, G., Simón-Mateo, C., and Viñuela, E. (1997) Assembly of African swine fever virus: role of polyprotein pp220. J. Virol. 71, 2331–2341 CrossRef Medline