ONOMY Science

American Journal of Microbiology & Biochemistry
ISSN 2688-5174

Research Article Open Access

Atomic Force Microscopy Images of Coronavirus in the Course of Replication

Yuri, G. Kuznetsov and Alexander McPherson*

[1] Department of Molecular Biology and Biochemistry, 3205 McGaugh Hall, University of California, Irvine, Irvine, California 92697-3900

***Corresponding Author:** **Alexander McPherson,** Department of Molecular Biology and Biochemistry, 3205 McGaugh Hall, University of California, Irvine, Irvine, California 92697-3900; Email: amcphers@uci.edu

Citation: Atomic Force Microscopy Images of Coronavirus in the Course of Replication: Am J Micro and Bioche. 2019; 3(1): 001-005.

Submitted: 12 May 2020; **Approved:** 14 May 2020; **Published:** 16 May 2020

ABSTRACT

Murine hepatitis virus (M-CoV, MHV), which closely resembles human pathogenic coronaviruses such as SARS-CoV, MERS-CoV and SARS-CoV -2, were produced in DBT cells in culture, purified, and imaged by atomic force microscopy (AFM). Images contain virus in various structural states assumed during their replication cycle, from disrobing their S1 spike decorated membrane, through their opening as a pinwheel shaped protein – RNA complex, formation of a circular complex, to the synthesis of progeny RNA. A sample of AFM images of the virus at a series of structural stages is presented here.

Keywords: replication intermediates, protein – RNA complex, COVID-19, pandemic, virus structure

INTRODUCTION

Murine coronavirus (Mouse hepatitis virus, M-CoV, MHV) is a specie of coronavirus enveloped by a lipid membrane and containing as its genome one molecule of a positive-sense, single stranded RNA (Bang and Warwick 1960, Holmes and Lai 1996, Maclachlan and Dubovi 2016). It associates with target cells through its S1 spike protein with the CEACAM1 receptor on cells (Fehr and Perlman 2015). The S1 spike is a large (precursor) glycoprotein that is cleaved by a specific protease into two subunits, the head that confers receptor recognition, and the stalk, which is responsible for entering into the host cell (Collins, Knobler et al. 1982, White 1990, Nash and Buchmeier 1997, Gallagher and Buchmeier 2001).

Entry may proceed through an endocytic pathway, or through fusion of the viral and host cell membranes (Nash and Buchmeier 1997). Events that occur subsequent to entry are less well understood, but the virus is prolific and multiplies rapidly in living organisms and in cell culture. Two strains predominate, the polytropic strain that primarily infects the respiratory system, and the enterotropic strain that mainly infects the intestine and liver. Infections are commonly asymptomatic and generally lethal only in immunocompromised mice (Compton, Ball-Goodrich et al. 2004). The virus is highly contagious.

MHV is probably the most studied of all coronaviruses in the laboratory, as it poses no threat to investigators and is a good, representative specie for studying coronaviruses in both animals and in cell culture. MHV is very similar in most respects to coronaviruses that do infect humans and that are major health hazards. These include for examples SARS-CoV, MERS-CoV and the more recently recognized SARS-CoV-2, which is responsible for the current world pandemic of COVID-19.

In 2012 -2013 we undertook a study of MHV, produced in cell culture, using the technique of atomic force microscopy (AFM) (Binning, Quate et al. 1986, Bustamante and

Keller 1995, Kuznetsov, Malkin et al, 2001). The virus was produced and purified by Dr. Megan Angelini working in the laboratory of Prof. Michael J. Buchmeier at UC, Irvine. We recorded many AFM images of the virus and its intracellular intermediates. Many of these were of poor quality and marginally interpretable, but some showed quite clearly the intermediate structures assumed-by the virus as it replicated.

We have not previously published any of the AFM images, for what reasons we (YK and AM) can no longer recall. These are, however, so far as we are aware, the only AFM images of a corona-virus in existence, or at least images that reveal virus intermediates. Because of the current interest in coronaviruses as a consequence of the current COVID-19 pandemic, we thought it might be useful to other researchers and interested observers.

RESULTS

Several hundred AFM images were collected in 2012-2013 over a period of about six months using techniques and procedures that were proven with a host of other viruses including plant icosahedral viruses, HIV, MuLV, Mason Pfizer Monkey virus, vaccinia, Mimivirus and others (Kuznetsov, Malkin et al. 2001, Kuznetsov, Datta et al. 2002, Kuznetsov, Victoria et al. 2003, Malkin,McPherson et al. 2003, Malkin, Plomp et al. 2004). We present here only five of those that we feel are representative of some stages in the replication cycle of the coronavirus. While these may be the only existent AFM images of coronavirus, there are of course numerous studies using electron microscopy (Holmes and Lai 1996; Maclachlan and Dubovi 2016). The resolution of these images is difficult to ascertain but is probably in the range of three to five nanometers based on comparisons with earlier studies.

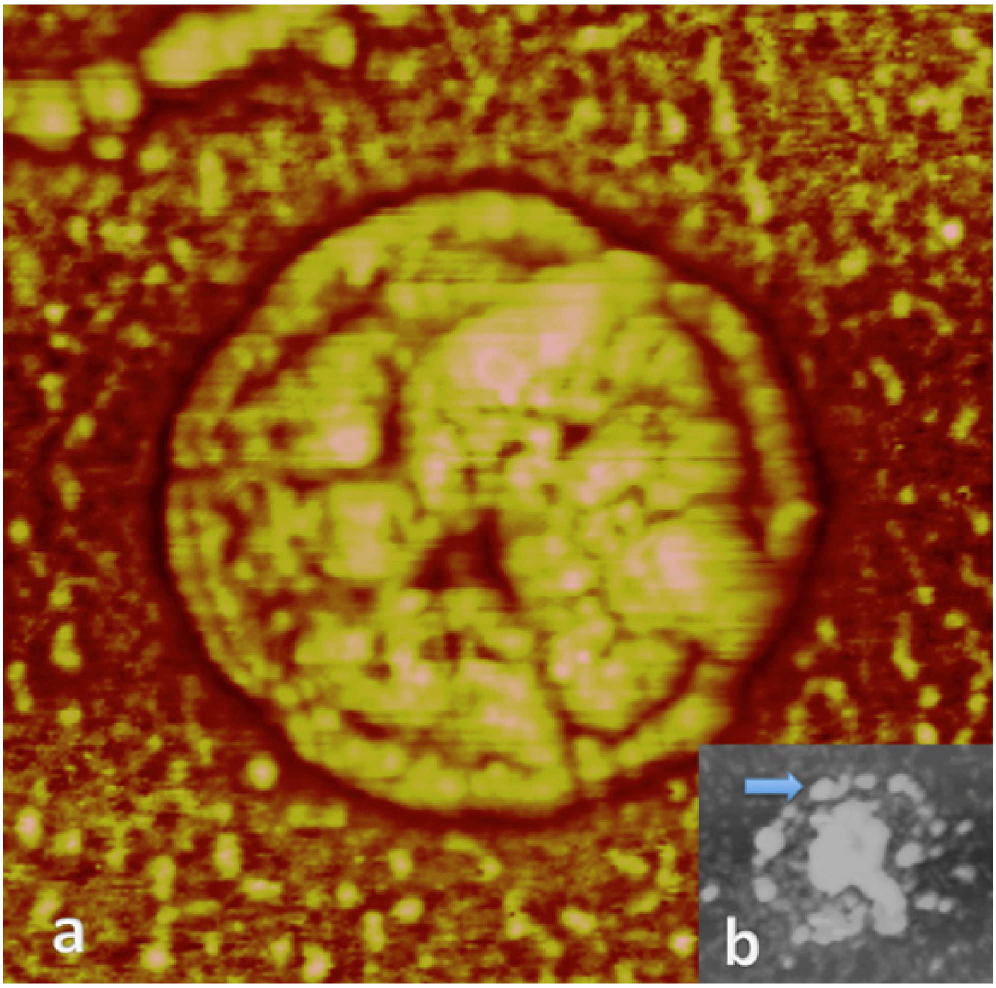

Figure 1 AFM image (250 nm x 250 nm) of a single, native MHV still enveloped in its

lipid membrane. The protein-RNA complex, the lighter (higher above the substrate) fills the interior. The S1 spike proteins that form the "corona", for which the virus is named, are not resolved due to its flexible nature. Flexible entities are seldom seen by AFM because as the AFM tip passes over a flexible structure, such as a spike protein, the subject moves rather than holding firm and displacing the AFM tip. Fixed objects, on the other hand, do displace the AFM tip upward, producing a signal indicative of height above the substrate plane. In these images, a dark tone represents material close to the substrate plane, brightest material height above the substrate plane. In (b) is a virus particle that has burst when it was fixed to the AFM substrate and the corona of spike proteins is displayed in a circle about the core (indicated by arrow). This image is 320 nm x 320 nm.

In Figure 1, is an intact coronavirus on the AFM substrate. The image is a mapping of the surface of the virus, i.e. it is a topographical representation with areas close to the substrate darkest and those projecting highest from the substrate light. One may think of such an image as simply similar to one obtained from a common photographic camera. The core of the virus is bounded by a distinctive band that is the layer of surface spike proteins, the corona, that gives the virus its name. Individual spikes, which are embedded in the very soft lipid membrane are not resolved, or only marginally in some regions. The interior of the virion shows the dense complex of protein coating the RNA genome. There is also a void at the center of the particle suggesting the circular arrangement of the complex.

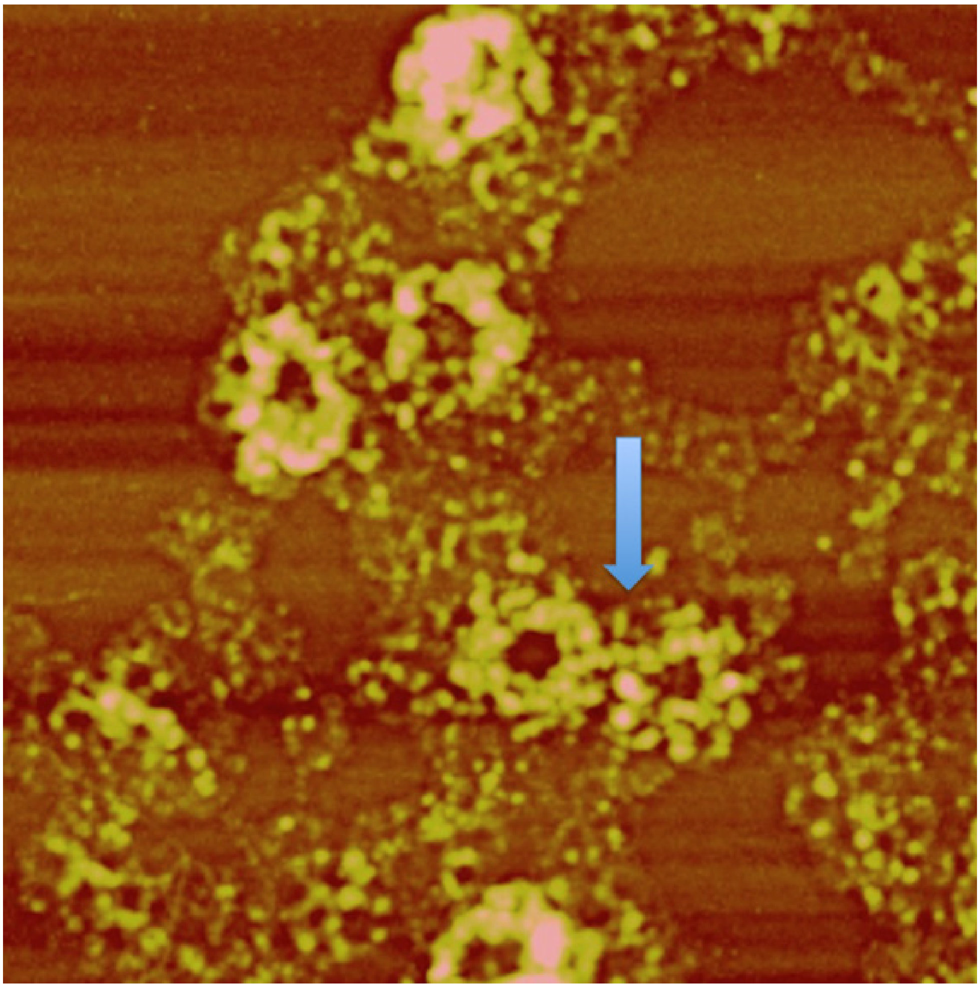

Figure 2a In (a) are virus particles that have entered the host cell and are in the process of disassembly in preparation for replication. The "greasy" or "jelly-like" material that appears smeared around the particles is the lipid envelope that has been cast aside and the white objects, appearing only as irregular grains, are probably the spike proteins, or

Cite this article: Atomic Force Microscopy Images of Coronavirus in the Course of Replication: Am J Micro and Bioche. 2019; 3(1): 001-005.

their heads, also cast off. They are visible here because they are fixed, though poorly, to the AFM substrate. In many cases the protein-RNA complexes are beginning to open up and expand. They form distinctive pinwheel structures (indicated by arrow). The image is 1um x 1um. In (b) is a high magnification AFM image of a pinwheel structure showing the genomic RNA molecule totally covered and protected by proteins. The protein shields are likely composed of both replicative enzymes and structural, protective proteins.

Figure 2(a) shows several virions from infected cells that are in the process of shedding their coronas of surface spikes and the membranes in which they are embedded. This is the grainy but gelatinous material spreading away from the unraveling protein – RNA complexes making up the cores. The hole at the center of the virion has become greatly enlarged and near the center of the image the two complexes have opened up and produced pinwheel structures. These pinwheels are characteristic of coronavirus and we have not observed anything like them in other viruses.

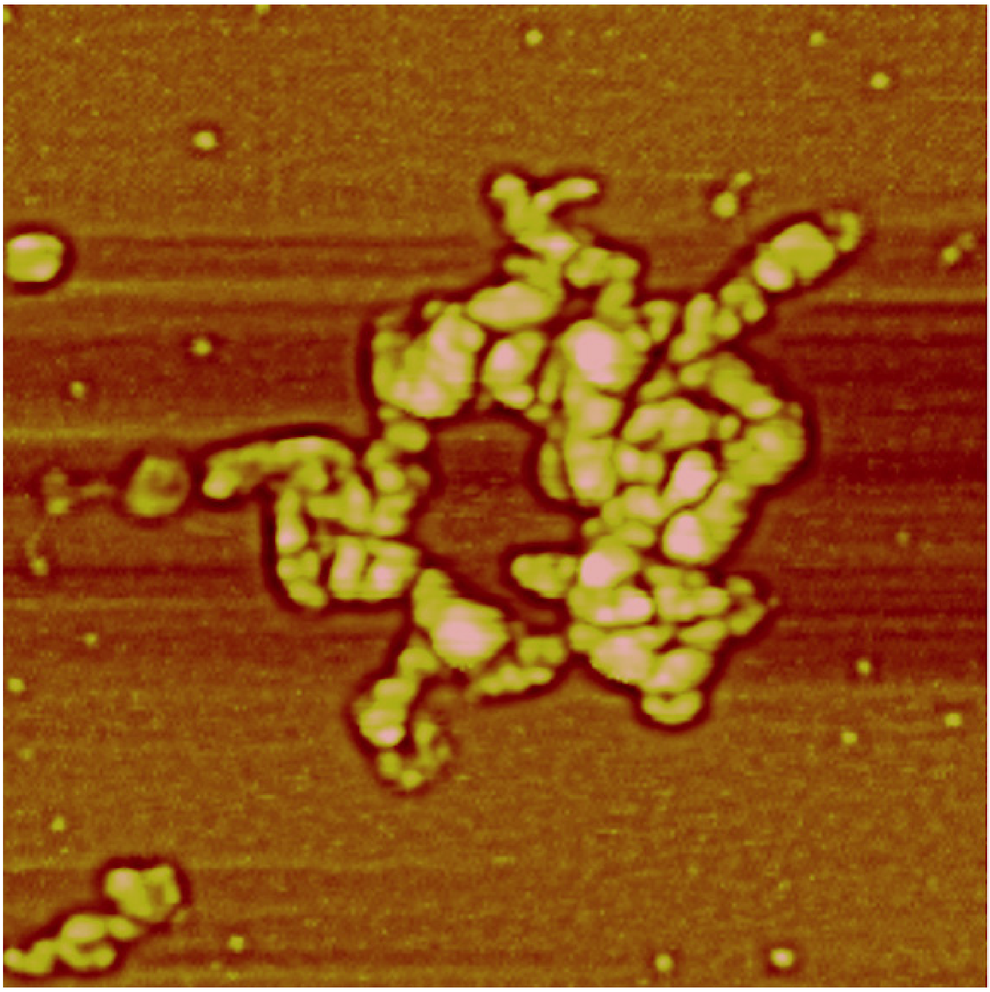

Figure 2(b) In the pinwheel complex of Figure 2(b) the RNA can be seen to be completely encased in large protein aggregates, as no single stranded RNA can be seen. The large central hole is clearly evident, and the linear nature of the protein – RNA complex as well. The image is 500 nm x 500 nm.

In Figure 2(b) is a high-resolution AFM image of a single pinwheel representing the protein – RNA genome complex.

Cite this article: Atomic Force Microscopy Images of Coronavirus in the Course of Replication: Am J Micro and Bioche. 2019; 3(1): 001-005.

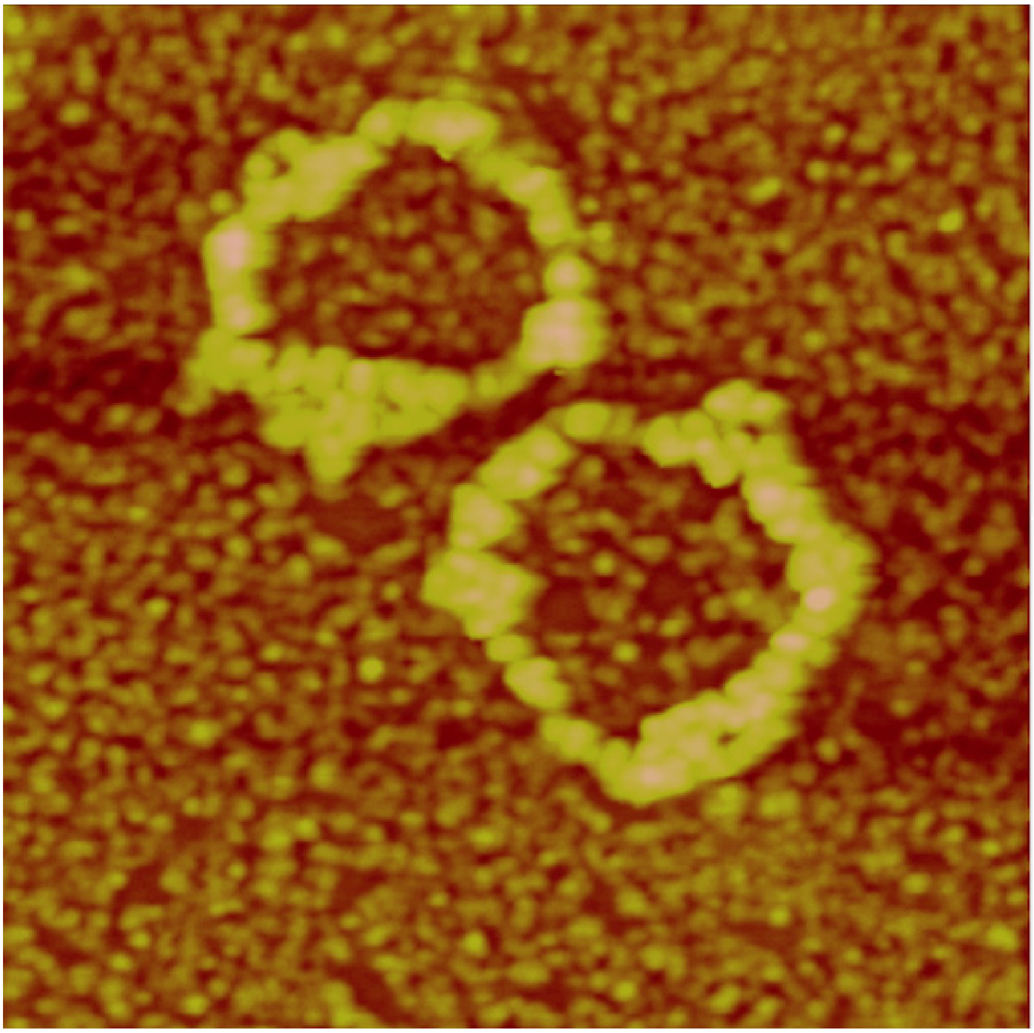

Figure 3 Once the pinwheels of Figure 2 have completely opened up, the ring structures seen here appear. The rings are viral, genomic RNA molecules coated with protein. The heavy background of small objects is evidence of other proteins that have evidently been ejected from the replication complex. The image is 500 nm x 500 nm.

In Figure 3 are two protein – RNA genomic complexes where it can be seen that the pinwheels have now fully expanded, resulting in circular complexes. The protein at this stage still coats and protects the RNA. In addition, the proteins associated with the RNA must include those enzymes responsible for the translation of the RNA and ultimately its replication. In the background, the substrate is littered with thousands of protein molecules that have been cast off during the transition from intact virion to circular complex. It may be noteworthy that the protein – RNA is indeed a closed circle with no free ends, not linear, as illustrated in many models.

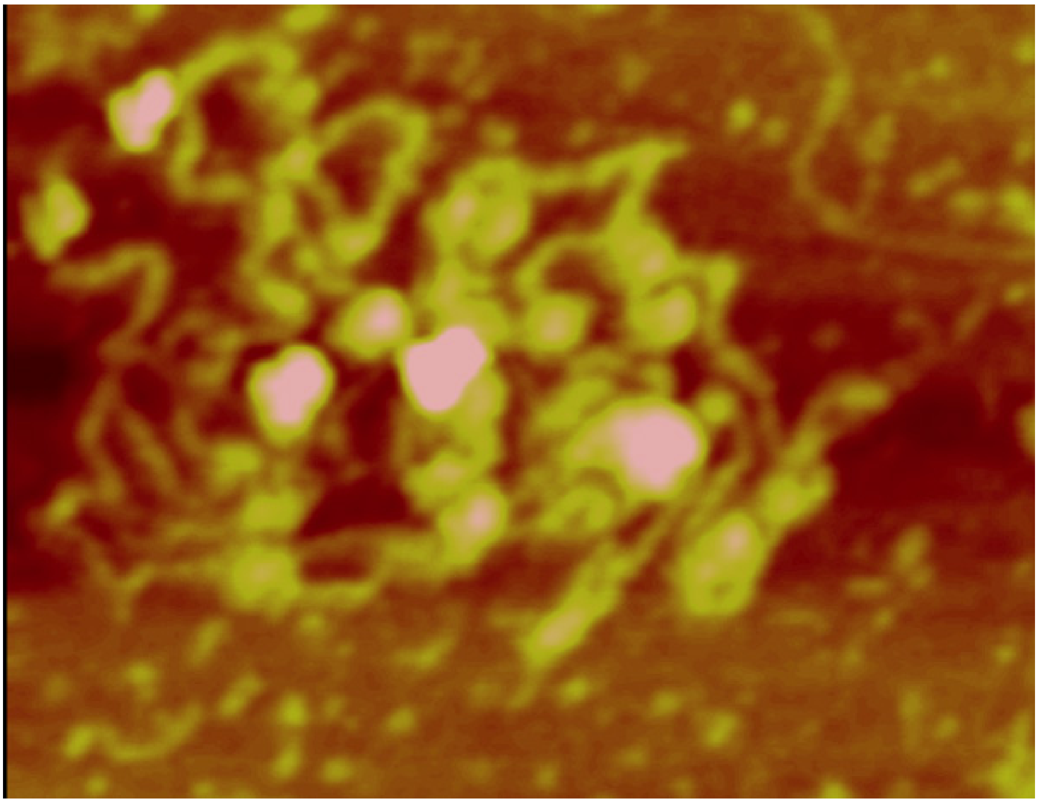

Figure 4 Seen here is a replication complex in the act of producing daughter nucleic acid strands, and probably mRNA to code for essential progeny proteins. The new RNA strands are evident as the tangled mass of threads still in complex with large protein assemblies. The image is 230 nm x 230 nm.

In Figure 4, much of the protein coating the RNA in the circular complexes has dissociated from the nucleic acid, which appears as the thick tangle of threads that pervades the complex. It is apparent, however, that many large protein aggregates are, at this stage, still closely associated with the RNA. At this stage, processes directly involving the RNA can presumably initiate.

CONCLUSION

The AFM images presented here show that once the coronavirus enters the cell it sheds its lipid membrane and the S1 proteins embedded in it. It then passes through a series of structural intermediates that include the pinwheel structures as the protein – nucleic acid complex begins to expand, and the closed, circular structures form in preparation for replication. From these circular complexes, additional proteins, but by no means all, are discarded or ejected, and segments of genomic RNA become visible. The AFM technique, as demonstrated here, provides a useful complement to conventional electron microscopic analyses of viruses.

MATERIALS AND METHODS

Sample preparation

MHV coronavirus strain A59 was infected and propagated in DBT cells in culture as previously described (Hirono, Goto et al. 1978, Nash and Buchmeier 1997), and subsequently purified from detergent lysed cells by centrifugation for 24 hours at 4°C on a 20% - 60% continuous sucrose gradient at 112,500 g. The virus was transferred to physiological saline containing 0.10 M HEPES buffer at pH 7. AFM samples were prepared in a variety of ways including direct drying on cleaved mica AFM substrate, treatment with detergent and drying, or direct imaging in liquid of the virus after thorough washing with distilled water.

AFM analysis

For in situ experiments, 1.5 ul of Corona virus in suspension in PBS was deposited on freshly cleaved mica coated with poly-l-lysine for 30 minutes at 23^{o}c . The substrate was pretreated with poly-l-lysine to ensure adherence of the virus and any other molecules that were present. The sample was then rinsed with PBS several times and excess PBS removed with filter paper. The sample was fixed with 5% glutaraldehyde in water for 15 minutes and rinsed with distilled water. Fixation with glutaraldehyde has been shown in previous studies not, to the resolution of the AFM technique, to perturb the surface structure of viral particles (Kuznetsov, Day et al. 2000, Kuznetsov, Malkin et al. 2001, Kuznetsov, Victoria et al. 2003, Malkin, McPherson et al. 2003, Kuznetsov, Victoria et al. 2004, Kuznetsov, Daijogo et al. 2005). Virus particles were imaged in liquid using silicon nitride tips. For imaging in air, glutaraldehyde fixed samples were dried in a stream of nitrogen gas before imaging.

AFM analysis was carried out using a Nanoscope III multimode instrument (Veeco Instruments, Santa Barbara, CA). Imaging procedures were fundamentally the same as de-scribed for previous investigations of viruses (Kuznetsov, Malkin et al. 2001, Kuznetsov, Datta et al. 2002, Kuznetsov, Victoria et al. 2003, Malkin, McPherson et al. 2003, Kuz-netsov, Victoria et al. 2004, Malkin, Plomp et al. 2004, McPherson and Kuznetsov 2011, Kuznetsov and McPherson 2012). For scanning in air, silicon tips were employed. The images were collected in tapping mode (Hansma and Hoh 1994, Hansma and Pietrasanta 1998) with an oscillation frequency of 300 kH with a scan frequency of 1 Hz. In the AFM images presented here, height above substrate is indicated by increasingly lighter color. Thus, points very close to the substrate are dark and those well above the substrate bright. Because lateral distances are distorted due to an AFM image being the convolution of the cantilever tip shape with the surface features scanned, quantitative measures of size were based either on heights above the substrate, or on center to center distances on par-ticle surfaces. The AFM instrument was calibrated to the small lateral distances by imaging the 111 face of a thaumatin protein crystal and using the known lattice spacings (Ko, Day et al. 1994, Kuznetsov Yu, Malkin et al. 1999) as standard.

For detailed descriptions of the AFM technique and its application to cells and particularly viruses, please see references (Kuznetsov, Malkin et al. 2001, Malkin, Plomp et al. 2004, Kuznetsov, Gurnon et al. 2005, Kuznetsov, Gershon et al. 2008, McPherson and Kuznetsov 2011, Kuznetsov and McPherson 2012).

REFERENCES

1. Bang, F. B. and A. Warwick (1960). "Mouse Macrophages as Host Cells for the Mouse Hepati-tis Virus and the Genetic Basis of Their Susceptibility." Proc Natl Acad Sci U S A 46(8): 1065-1075.
2. Binning, G., C. F. Quate and C. Gerber (1986). "Atomic force microscope." Phys. Rev. Lett 56: 930-933.
3. Bustamante, C. and D. Keller (1995). "Scanning force microscopy in biology." Phys. Today 48(12): 32-38.
4. Collins, A. R., R. L. Knobler, H. Powell and M. J. Buchmeier (1982). "Monoclonal antibodies to murine hepatitis virus-4 (strain JHM) define the viral glycoprotein responsible for attachment and cell--cell fusion." Virology 119(2): 358-371.
5. Compton, S. R., L. J. Ball-Goodrich, L. K. Johnson, E. A. Johnson, F. X. Paturzo and J. D. Macy (2004). "Pathogenesis of enterotropic mouse hepatitis virus in immunocompetent and immuno-de-ficient mice. ." Comp. Med. 54(6): 681-689.
6. Fehr, A. R. and S. Perlman (2015). "Coronaviruses: an overview of their replication and

pathogenesis." Methods Mol Biol 1282: 1-23. Gallagher, T. M. and M. J. Buchmeier (2001). "Coronavirus spike proteins in viral entry and patho-genesis." Virology 279(2): 371-374.
7. Hansma, H. G. and J. H. Hoh (1994). "Biomolecular imaging with the atomic force micro-scope." Annu Rev Biophys Biomol Struct 23: 115-139.
Hansma, H. G. and L. Pietrasanta (1998). "Atomic force microscopy and other scanning probe mi-croscopies." Curr Opin Chem Biol 2(5): 579-584.
8. Hirono, N., N. Goto, S. Makino and K. Fujiwara (1978). "Utility of mouse cell line DBT for propagation and assay of mouse hepatitis virus." Jpn. J. Exp. Med 48: 71-75.
9. Holmes, K. V. and M. M. C. Lai (1996). Coronaviridae: The viruses and their replica-tion. Virology. B. A. Fields. New York, Lippencott-Raven.
Ko, T. P., J. Day, A. Greenwood and A. McPherson (1994). "Structures of three crystal forms of the sweet protein thaumatin." Acta Crystallogr D Biol
10. Kuznetsov, Y., P. D. Gershon and A. McPherson (2008). "Atomic Force Microscopy Investigation of Vaccinia Virus Structure. ." Journal of Virology 85: 7551-7566.
11. Kuznetsov, Y. G., S. Daijogo, J. Zhou, B. L. Semler and A. McPherson (2005). "Atomic force microscopy analysis of icosahedral virus RNA." J Mol Biol 347(1): 41-52.
12. Kuznetsov, Y. G., S. Datta, N. H. Kothari, A. Greenwood, H. Fan and A. McPherson (2002). "Atomic force microscopy investigation of fibroblasts
infected with wild-type and mutant murine leukemia virus (MuLV)." Biophys J 83(6): 3665-3674.
13. Kuznetsov, Y. G., J. Day, R. Newman and A. McPherson (2000). "Chimeric human-simian anti-CD4 antibodies from crystalline high symmetry particles." J. Struc. Biol. 131: 108-115.
14. Kuznetsov, Y. G., J. R. Gurnon, J. L. Van Etten and A. McPherson (2005). "Atomic force microscopy investigation of a chlorella virus, PBCV-1." J Struct Biol 149(3): 256-263.
15. Kuznetsov, Y. G., A. J. Malkin, R. W. Lucas, M. Plomp and A. McPherson (2001). "Imag-ing of viruses by atomic force microscopy." J Gen Virol 82(Pt 9): 2025-2034.
16. Kuznetsov, Y. G. and A. McPherson (2012). Imaging of Cells, Viruses, and Virus – In-fected Cells by Atomic Force Microscopy Current Microscopy Contributions to Advances in Science and Technology. A. Mendez-Vilas. Badajoz, Spain, Formatex Resc. Center. 1: 540-548.
17. Kuznetsov, Y. G., J. G. Victoria, A. Low, W. E. Robinson, Jr., H. Fan and A. McPherson (2004). "Atomic force microscopy imaging of retroviruses: human immunodeficiency virus and murine leukemia virus." Scanning 26(5): 209-216.
18. Kuznetsov, Y. G., J. G. Victoria, W. E. J. Robinson and A. McPherson (2003). "Atomic force microscopy investigation of HIV and HIV-infected lymphocytes." J. of Virology 77(22): 11896-11909.
19. Kuznetsov Yu, G., A. J. Malkin and A. McPherson (1999). "AFM studies on the mecha-nisms of nucleation and growth of macromolecular crystals." J. Cryst. Growth 196: 489-502.
20. Maclachlan, N. J. and E. J. Dubovi (2016). Coronaviridae. Fenner's Veterinary Virolo-gy. New York, Academic Press: 435-461.
21. Malkin, A. J., A. McPherson and P. D. Gershon (2003). "Structure of intracellular ma-ture vaccinia virus visualized by in situ AFM." J. Viol. 77(11): 6332-6340.
24. McPherson, A. and Y. G. Kuznetsov (2011). "Atomic force microscopy investigation of viruses." Methods Mol Biol 736: 171-195.

25. Nash, T. C. and M. J. Buchmeier (1997). "Entry of mouse hepatitis virus into cells by endosomal and nonendosomal pathways." Virology 233(1): 1-8.
White, J. M. (1990). "Viral and cellular membrane fusion proteins." Annu Rev Physiol 52: 675-697.

Cite this article: Atomic Force Microscopy Images of Coronavirus in the Course of Replication: Am J Micro and Bioche. 2019; 3(1): 001-005.