DNA AND CELL BIOLOGY
Volume 33, Number 3, 2014

Pp. 122–127
DOI: 10.1089/dna.2013.2304

Untangling Membrane Rearrangement in the *Nidovirales*

Megan Mary Angelini,[1] Benjamin William Neuman,[2] and Michael J. Buchmeier[1,3]

All known positive sense single-stranded RNA viruses induce host cell membrane rearrangement for purposes of aiding viral genome replication and transcription. Members of the *Nidovirales* order are no exception, inducing intricate regions of double membrane vesicles and convoluted membranes crucial for the production of viral progeny. Although these structures have been well studied for some members of this order, much remains unclear regarding the biogenesis of these rearranged membranes. Here, we discuss what is known about these structures and their formation, compare some of the driving viral proteins behind this process across the nidovirus order, and examine possible routes of mechanism by which membrane rearrangement may occur.

Membrane Rearrangement as a General Strategy of Positive Sense RNA Viruses

Host cell membrane remodeling is a tactic used by many viruses as a means of reaching their end game of using the host cell for viral production. Positive sense single-stranded RNA viruses make use of the host's internal membrane environment for viral genome replication and transcription (Ahlquist, 2006; Denison, 2008; Miller and Krijnse-Locker, 2008; Netherton and Wileman, 2011). The reasons for doing so include the need to concentrate, localize, and anchor the machinery and precursors required for transcription and aiding in shielding double-stranded RNA intermediates from activating an innate immune response. Membrane rearrangements involved in viral genome replication and transcription for some members of the positive sense RNA viruses have been well characterized and have a wide range of complexity, both of membrane involvement and numbers and types of proteins responsible for the remodeling (Kirkegaard and Jackson, 2005; Mackenzie, 2005; Novoa *et al.*, 2005; Salonen *et al.*, 2005; den Boon and Ahlquist, 2010). The typical method of designating which membranes are involved is by mapping the intracellular localization of the dsRNA replicative intermediates, nascent RNA, and replicase proteins. Once the membranous replicase structures have been identified, further characterization and ultrastructural description can occur, including the more recently applied technique of three-dimensional electron tomography (Subramaniam, 2005; Kopek *et al.*, 2007; Subramaniam *et al.*, 2007).

Although the strategy of membrane remodeling is thought to be conserved in all positive sense RNA viruses, the actual modifications and structures made and the means by which they are formed can drastically vary between viruses. Flaviviruses induce an organized network of interconnected double-walled endoplasmic reticulum (ER)-derived membranes termed ''vesicle packets'' and ''convoluted membranes'' (Welsch *et al.*, 2009; Gillespie *et al.*, 2010; Miorin *et al.*, 2013). Picornaviruses have been shown to reorganize ER, Golgi, and lysosomes into both single and double membrane vesicles (DMVs), the latter of which are similar to autophagosomes (Suhy *et al.*, 2000; Limpens *et al.*, 2011; Belov *et al.*, 2012). Alphaviruses induce $\sim$50 nm in diameter single membrane vesicles termed ''spherules,'' seen to be derived from invaginated ER, plasma membrane, and endosomes/lysosomes depending on the virus (Froshauer *et al.*, 1988; Schwartz *et al.*, 2002; Kopek *et al.*, 2007; Spuul *et al.*, 2010). Nodaviruses reorganize the mitochondrial membrane into small $\sim$50 nm vesicles. These vesicles are single membranes and are positioned between the inner and outer mitochondrial membrane (Miller *et al.*, 2001; Kopek *et al.*, 2010).

An Introduction to Nidoviruses

The order *Nidovirales* contains families of positive sense nonsegmented single-stranded RNA viruses featuring an envelope and, notably, a mechanism of discontinuous transcription to produce nested subgenomic mRNAs for which the order is named (Latin nidus means nest) (González *et al.*, 2003; Gorbalenya *et al.*, 2006; Pasternak *et al.*, 2006). This order contains families capable of infecting both vertebrates (*Coronaviridae*, *Arteriviridae*, and *Roniviridae*) and invertebrates (*Mesoniviridae*) (Cowley *et al.*, 2000; Lauber *et al.*, 2013). All nidoviruses share a genome with a similar layout with the first two overlapping open reading frames (ORFs1a and 1b) producing two large polyproteins (pp1a and pp1ab) that are co- and post-translationally cleaved free

[1]Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, California.
[2]School of Biological Sciences, University of Reading, Reading, Berkshire, United Kingdom.
[3]Division of Infectious Disease, Department of Medicine, University of California, Irvine, Irvine, California.

into the nonstructural proteins (nsps) (Brian and Baric, 2005; Britton and Cavanagh, 2008). Processing of these polyproteins is directed by nsp encoded proteinases, which vary among families and species of these viruses (Ziebuhr, 2006; Snijder *et al.*, 2013).

These nsps are part of the viral replication transcription complex machinery necessary for viral genome replication and transcription, in association with cellular membranes (van Hemert *et al.*, 2008; Hagemeijer *et al.*, 2010). ORFs downstream from ORF1a and 1b encode varying structural and accessory proteins. These structural and accessory proteins are produced from nested 3′ co-terminal subgenomic mRNAs via a discontinuous transcription process (Faaberg, 2008; Hogue and Machamer, 2008). While nidovirus genomes range significantly in sequence and size, from 12.7 kb for the smaller arteriviruses to 31.7 kb for the large coronaviruses, they share some commonalities (Gorbalenya *et al.*, 2006). Figure 1 highlights the conservation across the *Nidovirales* in the pp1a-based nsps involved in membrane rearrangement. Coronaviruses and arteriviruses remain the best studied and characterized nidoviruses.

Membrane Rearrangement in the Nidoviruses

Ateriviruses and coronaviruses both induce characteristic DMVs in host cells (David-Ferreira and Manaker, 1965; van der Meer *et al.*, 1998; Pedersen *et al.*, 1999; Gosert *et al.*, 2002; Snijder *et al.*, 2006; Knoops *et al.*, 2008, 2012; Ulasli *et al.*, 2010; de Wilde *et al.*, 2013). The DMVs, so named for their distinctive two-layer delineated membranes, are interconnected with regions of convoluted membranes (CMs). Despite the fact that DMVs and CMs often appear in close and/or continuous proximity to the ER and their evidenced partial colocalization with the ER maker protein disulfide isomerase, the precise progenitor pathway from which they are formed remains unclear (Goldsmith *et al.*, 2004; Snijder *et al.*, 2006; Knoops *et al.*, 2008).

For both the coronaviruses and arteriviruses, members of the cellular autophagy machinery have been implicated in DMV formation (Maier and Britton, 2012). The autophagosomes themselves are double-membrane vesicles, which initially suggested the possibility of this pathway's involvement. Multiple studies have shown activation of autophagy machinery upon coronavirus infection or in the presence of coronavirus proteins (Prentice *et al.*, 2004; de Haan and Reggiori, 2008; Cottam *et al.*, 2011). Additionally, microtubule-associated protein light chain 3 (LC3) has been shown to associate with the DMVs of both arteriviruses and coronaviruses and loss of LC3 had an overall negative effect on DMV formation (Prentice *et al.*, 2004; Reggiori *et al.*, 2010; Monastyrska *et al.*, 2013). During autophagy, cytoplasmic LC3-I becomes lipidated and studs the autophagosome, serving as a marker for these vesicles. This is in contrast to the LC3 that has been shown to decorate the DMVs, which is the nonlipidated LC3-I form, which has also been implicated in the ER-associated degradation (ERAD) pathway. Additionally, chaperone members of the ERAD machinery were shown to be present in the DMVs, suggesting a role for ERAD in DMV formation (Calì *et al.*, 2008; Reggiori *et al.*, 2010). Absence of Atg5, a critical member of the autophagy machinery, did not cause a defect in DMV formation in coronavirus-infected cells, showing that DMV formation does not require an intact autophagy pathway (Zhao *et al.*, 2007).

Nonstructural Proteins Involved in Membrane Rearrangement: Similarities and Differences

The identity of the specific viral proteins responsible for inducing membrane rearrangement in both arterivirus and coronavirus-infected cells has long been of interest. Both

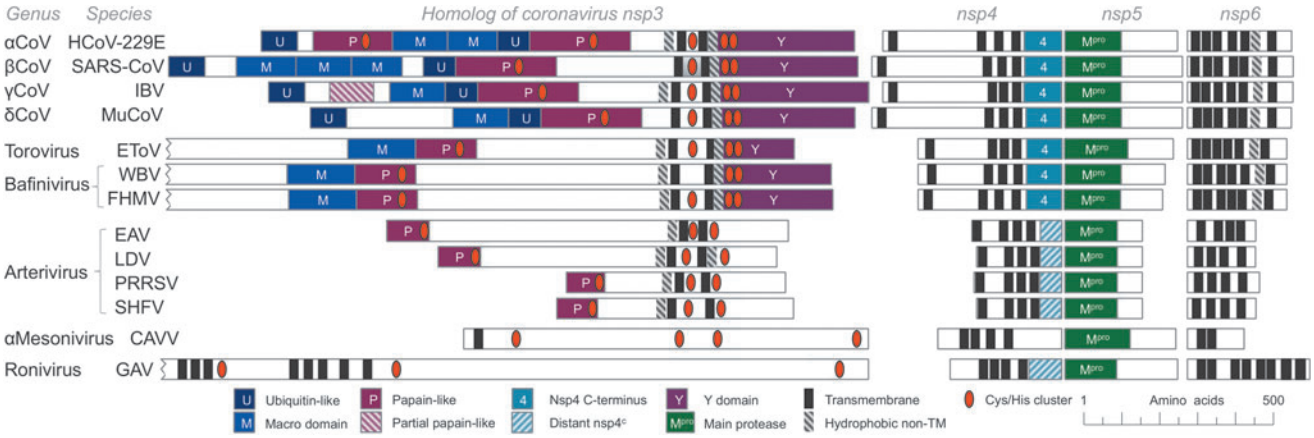

FIG. 1. Conservation of double membrane vesicle (DMV)-making proteins in the *Nidovirales*. Domain annotations were based on conserved amino acid sequences (solid colors) or secondary structure patterns (diagonal stripes). Positions of transmembrane and hydrophobic nontransmembrane regions were predicted by TMHMM 2.0 (Sonnhammer *et al.*, 1998; Krogh *et al.*, 2001) and amended to reflect known topologies (Baker nsp3, Oostra nsp4, Oostra nsp3+nsp6) wherever possible. Virus names are abbreviated as follows: Human coronavirus 229E (HCoV-229E), severe acute respiratory syndrome coronavirus (SARS-CoV), infectious bronchitis virus (IBV), munia coronavirus HKU13 (MuCoV), equine torovirus (EToV), white bream virus (WBV), fathead minnow virus (FHMV), equine arteritis virus (EAV), lactate dehydrogenase elevating virus (LDV), porcine reproductive and respiratory syndrome virus (PRRSV), simian hemorrhagic fever virus (SHFV), cavally virus (CAVV), and gill-associated virus (GAV). The amino-terminal region of the polyprotein is shown for CAVV and GAV because no obvious homolog of nonstructural protein (nsp)3 was detected. A jagged line denotes the uncertain position of the amino termini of EToV, WBV, FHMV, and GAV.

families have three nsps that feature putative transmembrane (TM) domains (Oostra *et al.*, 2007, 2008; Snijder *et al.*, 2013). Arteriviruses have TM domains within nsp2, nsp3, and nsp5, which appear to be homologous to coronavirus TM proteins nsp3, nsp4, and nsp6. These three proteins share conserved features between families and across the *Nidovirales*, as shown in Figure 1. Due to their presence at the DMVs and CMs and their TM domains, these three nsps were proposed as being important for membrane rearrangement. It was shown that the arterivirus equivalents of coronavirus nsp3 and nsp4 were sufficient for inducing membrane modifications similar to those seen in arterivirus-infected cells (Snijder *et al.*, 2001). Recently, we showed that coronavirus nsp3, nsp4, and nsp6 are all required to induce DMVs resembling those of virus-infected cells (Angelini *et al.*, 2013). Coronavirus nsp3 and nsp4 alone were incapable of forming full DMVs, but they were capable of creating maze-like patterns of paired membrane, agreeing with previously published immunofluorescence studies using expressed nsp4 and a truncated version of nsp3 (Hagemeijer *et al.*, 2011; Angelini *et al.*, 2013). Mutation in coronavirus nsp3 has been shown to lead to virus having a defect in DMV-formation ability, suggesting an important role for nsp5 and polyprotein processing in DMV formation (Stokes *et al.*, 2010). Coronavirus nsp4 and its arterivirus homolog have both been shown to be important for DMV formation through mutagenesis approaches (Posthuma *et al.*, 2008; Gadlage *et al.*, 2010). MHV nsp4 has been shown to be important for DMV formation. As mentioned earlier, nsp6 of coronavirus and nsp5–7 of arterivirus have been shown to induce autophagy when expressed in the absence of virus (Cottam *et al.*, 2011).

Theoretical Mechanism

As the identity of the viral proteins important for membrane rearrangement has become clear and the cellular membranes and pathways involved begin to be distinguished, the mechanism of membrane deformation can be deciphered. DMVs and CMs are comprised of highly curved membranes and presumably this curvature is induced via the TM domains located in coronavirus nsp3, nsp4, and nsp6 (nsp2, nsp3, and nsp5 for arteriviruses) and protein–protein interactions of these nsps with each other, with other viral proteins, and with host cellular proteins. TM domains that feature conical shapes, as a product of this shape, will induce membrane curvature by forcing the membrane to accommodate and deform around the conical region in a wedge-like manner (McMahon and Gallop, 2005; Shibata *et al.*, 2009). Additionally, suites of proteins may form together as a scaffold that has the ability to deform lipid bilayers and steric effects of these membrane-interacting proteins may also aid in deformation (Schley *et al.*, 2013). Since nsp3, nsp4, and nsp6 (and their *Nidovirales* homologs) contain TM domains and work together in a scaffold-like fashion, it is possible that both of these approaches work together to induce DMVs and CMs. As we propose in Figure 2, following initial genome polyprotein translation and proteolytic processing, nsp3 (dark blue), nsp4 (teal), and nsp6 (green) remain inserted in the lipid bilayer (Figure 2-upper left inset).

The intracellular phenotypes of cells expressing both nsp3 and nsp6 suggest that these proteins may promote membrane curvature, inducing proliferated membranes and vesiculation respectively on their own. Nsp4 alone appears incapable of inducing membrane curvature but, in conjunction with nsp3, is able to produce paired membranes, suggesting that some combination of homotypic and/or heterotypic interactions is driving this pairing (Figure 2-lower right inset). Nsp3–nsp3, nsp4–nsp4, and nsp3–nsp4 interactions have all been previously identified by mass spectrometry-based approaches, yeast two-hybrid assays, and co-immunoprecipitation studies (von Brunn *et al.*, 2007;

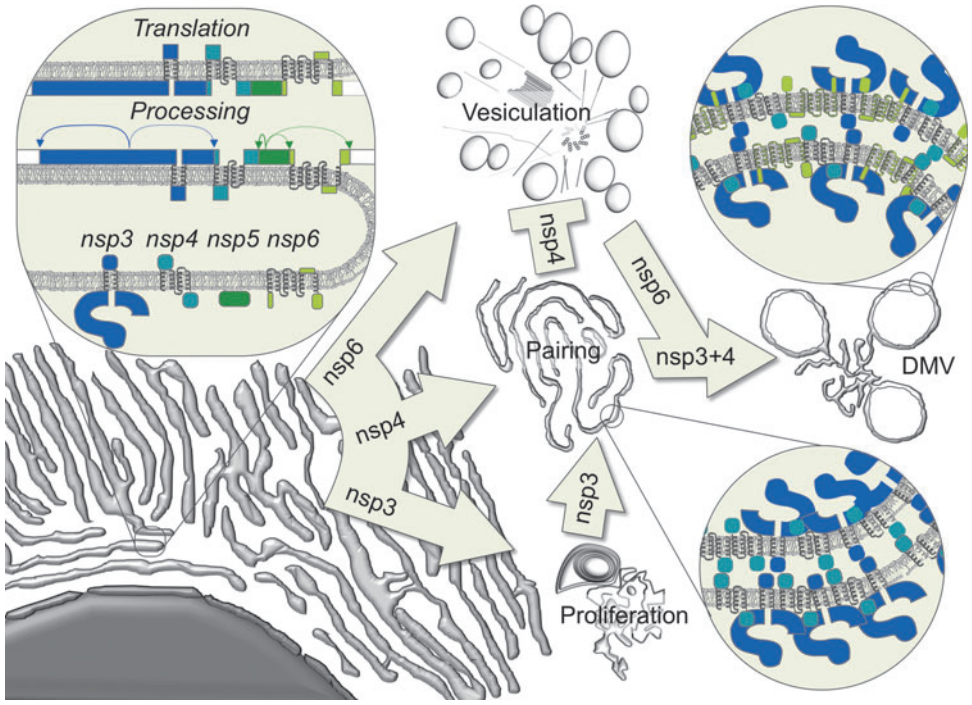

FIG. 2. Theoretical mechanism for DMV and convoluted membrane (CM) formation using coronavirus nsps as examples. Polyprotein translation occurs from genome, featuring co- and post-translational cleavage of nsps, including nsp3 (dark blue), nsp4 (teal), nsp5 (dark green), and nsp6 (light green). Nsp3 alone produces proliferated membrane, nsp4 alone has no membrane phenotype, nsp6 alone produces vesiculation. Nsp4 has a negative effect on nsp6's vesiculation. Nsp3 and nsp4 together induce paired membranes. Nsp3, nsp4, and nsp6 together induce DMVs.

Imbert *et al.*, 2008; Neuman *et al.*, 2008; Hagemeijer *et al.*, 2011). Since this membrane pairing was not observed when using a C-terminal region of nsp3, it is likely that the scaffolding function relies on interaction of nsp4 with some region of nsp3 N-terminal to its TM domain. Addition of nsp6 changes the organization of paired membranes from remarkably consistent maze-like swirls to a mixture of heterogeneous DMVs and CMs that resembles the viral replicative organelles found in infected cells. This suggests that nsp6 modifies the long-range organization of paired membranes without disrupting membrane pairing, possibly by inducing deep curvature into the nsp3 + nsp4 scaffold. Nsp4–nsp6 heterotypic interaction has been previously shown via co-immunoprecipitation and Venus reporter protein complementation assay (Hagemeijer *et al.*, 2011) (Figure 2-upper right inset). Due to the high homology of these three TM regions across the *Nidovirales*, this proposed mechanism may apply generally (Fig. 1).

The lipid environment may also be affected in nidovirus-infected cells for the purposes of membrane rearrangement. This is the case for dengue virus, another positive sense RNA virus featuring extensive membrane rearrangement (Perera *et al.*, 2012). The virus may induce changes in the lipid composition of the progenitor membranes to better facilitate DMV and CM formation as increased amounts of certain species of lipids may aid in certain types of membrane deformation (Baumgart *et al.*, 2003; Bacia *et al.*, 2005; McMahon and Gallop, 2005).

Overall, the study of membrane rearrangement in the *Nidovirales* still has many unanswered questions and a precise understanding of the mechanisms behind this process will aid in future research and potential therapies for these viruses and possibly for other positive sense RNA viruses as well.

Acknowledgments

Work leading to this review was supported by National Institutes of Health grant 5T32AI007319-23 as well as the California Center for Antiviral Drug Discovery MRPI #143226.

Disclosure Statement

The authors have no conflicts of interest.

References

Ahlquist, P. (2006). Parallels among positive-strand RNA viruses, reverse-transcribing viruses and double-stranded RNA viruses. Nat Rev Microbiol **4,** 371–382.

Angelini, M.M., Akhlaghpour, M., Neuman, B.W., and Buchmeier, M.J. (2013). Severe acute respiratory syndrome coronavirus nonstructural proteins 3, 4, and 6 induce double-membrane vesicles. MBio **4,** e00524–13.

Bacia, K., Schwille, P., and Kurzchalia, T. (2005). Sterol structure determines the separation of phases and the curvature of the liquid-ordered phase in model membranes. Proc Natl Acad Sci U S A **102,** 3272–3277.

Baumgart, T., Hess, S.T., and Webb, W.W. (2003). Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension. Nature **425,** 821–824.

Belov, G.A., Nair, V., Hansen, B.T., Hoyt, F.H., Fischer, E.R., and Ehrenfeld, E. (2012). Complex dynamic development of poliovirus membranous replication complexes. J Virol **86,** 302–312.

Brian, D.A., and Baric, R.S. (2005). Coronavirus genome structure and replication. Curr Top Microbiol Immunol **287,** 1–30.

Britton, P., and Cavanagh, D. (2008). Nidovirus genome organization and expression mechanisms. In *Nidoviruses*. S. Perlman, T. Gallagher, and E.J. Snijder, eds. (ASM Press, Washington, DC), pp. 29–46.

Calì, T., Galli, C., Olivari, S., and Molinari, M. (2008). Segregation and rapid turnover of EDEM1 by an autophagy-like mechanism modulates standard ERAD and folding activities. Biochem Biophys Res Commun **371,** 405–410.

Cottam, E.M., Maier, H.J., Manifava, M., Vaux, L.C., Chandra-Schoenfelder, P., Gerner, W., Britton, P., Ktistakis, N.T., and Wileman, T. (2011). Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum via an omegasome intermediate. Autophagy **7,** 1335–1347.

Cowley, J.A., Dimmock, C.M. Spann, K.M., and Walker, P.J. (2000). Gill-associated virus of Penaeus monodon prawns: an invertebrate virus with ORF1a and ORF1b genes related to arteri- and coronaviruses. J Gen Virol **81,** 1473–1484.

David-Ferreira, J.F., and Manaker, R.A. (1965). An electron microscope study of the development of a mouse hepatitis virus in tissue culture cells. J Cell Biol **24,** 57–78.

de Haan, C.A., and Reggiori, F. (2008). Are nidoviruses hijacking the autophagy machinery? Autophagy **4,** 276–279.

de Wilde, A.H., Raj, V.S., Oudshoorn, D., Bestebroer, T.M., van Nieuwkoop, S., Limpens, R.W., Posthuma, C.C., van der Meer, Y., Bárcena, M., Haagmans, B.L., Snijder, E.J., and van den Hoogen, B.G. (2013). MERS-coronavirus replication induces severe *in vitro* cytopathology and is strongly inhibited by cyclosporin A or interferon-α treatment. J Gen Virol **94,** 1749–1760.

den Boon, J.A., and Ahlquist, P. (2010). Organelle-like membrane compartmentalization of positive-strand RNA virus replication factories. Annu Rev Microbiol **64,** 241–256.

Denison, M.R. (2008). Seeking membranes: positive-strand RNA virus replication complexes. PLoS Biol **6,** e270.

Faaberg, K.S. (2008). Arterivirus structural proteins and assembly. In *Nidoviruses*. S. Perlman, T. Gallagher, and E.J. Snijder, eds. (ASM Press, Washington DC), pp. 211–234.

Froshauer, S., Kartenbeck, J., and Helenius, A. (1988). Alphavirus RNA replicase is located on the cytoplasmic surface of endosomes and lysosomes. J Cell Biol **107,** 2075–2086.

Gadlage, M.J., Sparks, J.S., Beachboard, D.C., Cox, R.G., Doyle, J.D., Stobart, C.C., and Denison, M.R. (2010). Murine hepatitis virus nonstructural protein 4 regulates virus-induced membrane modifications and replication complex function. J Virol **84,** 280–290.

Gillespie, L.K., Hoenen, A., Morgan, G., and Mackenzie, J.M. (2010). The endoplasmic reticulum provides the membrane platform for biogenesis of the flavivirus replication complex. J Virol **84,** 10438–10447.

Goldsmith, C.S., Tatti, K.M., Ksiazek, T.G., Rollin, P.E., Comer, J.A., Lee, W.W., Rota, P.A., Bankamp, B., Bellini, W.J., and Zaki, S.R. (2004). Ultrastructural characterization of SARS coronavirus. Emerg Infect Dis **10,** 320–326.

González, J.M., Gomez-Puertas, P., Cavanagh, D., Gorbalenya, A.E., and Enjuanes, L. (2003). A comparative sequence analysis to revise the current taxonomy of the family Coronaviridae. Arch Virol **148,** 2207–2235.

Gorbalenya, A.E., Enjuanes, L., Ziebuhr, J., and Snijder, E.J. (2006). Nidovirales: evolving the largest RNA virus genome. Virus Res **117,** 17–37.

Gosert, R., Kanjanahaluethai, A., Egger, D., Bienz, K., and Baker, S.C. (2002). RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. J Virol **76,** 3697–3708.

Hagemeijer, M.C., Ulasli, M., Vonk, A.M., Reggiori, F., Rottier, P.J., and de Haan, C.A. (2011). Mobility and interactions of coronavirus nonstructural protein 4. J Virol **85,** 4572–4577.

Hagemeijer, M.C., Verheije, M.H., Ulasli, M., Shaltiël, I.A., de Vries, L.A., Reggiori, F., Rottier, P.J., and de Haan, C.A. (2010). Dynamics of coronavirus replication-transcription complexes. J Virol **84,** 2134–2149.

Hogue, B.G., and Machamer, C.E. (2008). Coronavirus structural proteins and virus assembly. In *Nidoviruses*. S. Perlman, T. Gallagher, and E.J. Snijder, eds. (ASM Press, Washington DC), pp. 179–200.

Imbert, I., Snijder, E.J., Dimitrova, M., Guillemot, J.C., Lécine, P., and Canard, B. (2008). The SARS-Coronavirus PLnc domain of nsp3 as a replication/transcription scaffolding protein. Virus Res **133,** 136–148.

Kirkegaard, K., and Jackson, W.T. (2005). Topology of double-membraned vesicles and the opportunity for non-lytic release of cytoplasm. Autophagy **1,** 182–184.

Knoops, K., Bárcena, M., Limpens, R.W., Koster, A.J., Mommaas, A.M., and Snijder, E.J. (2012). Ultrastructural characterization of arterivirus replication structures: reshaping the endoplasmic reticulum to accommodate viral RNA synthesis. J Virol **86,** 2474–2487.

Knoops, K., Kikkert, M., Worm, S.H., Zevenhoven-Dobbe, J.C., van der Meer, Y., Koster, A.J., Mommaas, A.M., and Snijder, E.J. (2008). SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. PLoS Biol **6,** e226.

Kopek, B.G., Perkins, G., Miller, D.J., Ellisman, M.H., and Ahlquist, P. (2007). Three-dimensional analysis of a viral RNA replication complex reveals a virus-induced mini-organelle. PLoS Biol **5,** e220.

Kopek, B.G., Settles, E.W., Friesen, P.D., and Ahlquist, P. (2010). Nodavirus-induced membrane rearrangement in replication complex assembly requires replicase protein a, RNA templates, and polymerase activity. J Virol **84,** 12492–12503.

Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E.L. (2001). Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol **305,** 567–580.

Lauber, C., Goeman, J.J., Parquet, M.E.C., Thi Nga, P., Snijder, E.J., Morita, K., and Gorbalenya, A.E. (2013). The footprint of genome architecture in the largest genome expansion in RNA viruses. PLoS Pathog **9,** e1003500.

Limpens, R.W., van der Schaar, H.M., Kumar, D., Koster, A.J., Snijder, E.J., van Kuppeveld, F.J., and Bárcena, M. (2011). The transformation of enterovirus replication structures: a three-dimensional study of single- and double-membrane compartments. MBio **2,** e00166–11.

Mackenzie, J. (2005). Wrapping things up about virus RNA replication. Traffic **6,** 967–977.

Maier, H.J., and Britton, P. (2012). Involvement of autophagy in coronavirus replication. Viruses **4,** 3440–3451.

McMahon, H.T., and Gallop, J.L. (2005). Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature **438,** 590–596.

Miller, D.J., Schwartz, M.D., and Ahlquist, P. (2001). Flock house virus RNA replicates on outer mitochondrial membranes in *Drosophila* cells. J Virol **75,** 11664–11676.

Miller, S., and Krijnse-Locker, J. (2008). Modification of intracellular membrane structures for virus replication. Nat Rev Microbiol **6,** 363–374.

Miorin, L., Romero-Brey, I., Maiuri, P., Hoppe, S., Krijnse-Locker, J., Bartenschlager, R., and Marcello, A. (2013). Three-dimensional architecture of tick-borne encephalitis virus replication sites and trafficking of the replicated RNA. J Virol **87,** 6469–6481.

Monastyrska, I., Ulasli, M., Rottier, P.J., Guan, J.L., Reggiori, F., and de Haan, C.A. (2013). An autophagy-independent role for LC3 in equine arteritis virus replication. Autophagy **9,** 164–174.

Netherton, C.L., and Wileman, T. (2011). Virus factories, double membrane vesicles and viroplasm generated in animal cells. Curr Opin Virol **1,** 381–387.

Neuman, B.W., Joseph, J.S., Saikatendu, K.S., Serrano, P., Chatterjee, A., Johnson, M.A., Liao, L., Klaus, J.P., Yates, J.R., Wüthrich, K., Stevens, R.C., Buchmeier, M.J., and Kuhn, P. (2008). Proteomics analysis unravels the functional repertoire of coronavirus nonstructural protein 3. J Virol **82,** 5279–5294.

Novoa, R.R., Calderita, G., Arranz, R., Fontana, J., Granzow, H., and Risco, C. (2005). Virus factories: associations of cell organelles for viral replication and morphogenesis. Biol Cell **97,** 147–172.

Oostra, M., Hagemeijer, M.C., van Gent, M., Bekker, C.P., te Lintelo, E.G., Rottier, P.J., and de Haan, C.A. (2008). Topology and membrane anchoring of the coronavirus replication complex: not all hydrophobic domains of nsp3 and nsp6 are membrane spanning. J Virol **82,** 12392–12405.

Oostra, M., te Lintelo, E.G., Deijs, M., Verheije, M.H., Rottier, P.J., and de Haan, C.A. (2007). Localization and membrane topology of coronavirus nonstructural protein 4: involvement of the early secretory pathway in replication. J Virol **81,** 12323–12336.

Pasternak, A.O., Spaan, W.J., and Snijder, E.J. (2006). Nidovirus transcription: how to make sense…? J Gen Virol **87,** 1403–1421.

Pedersen, K.W., van der Meer, Y., Roos, N., and Snijder, E.J. (1999). Open reading frame 1a-encoded subunits of the arterivirus replicase induce endoplasmic reticulum-derived double-membrane vesicles which carry the viral replication complex. J Virol **73,** 2016–2026.

Perera, R., Riley, C., Isaac, G., Hopf-Jannasch, A.S., Moore, R.J., Weitz, K.W., Pasa-Tolic, L., Metz, T.O., Adamec, J., and Kuhn, R.J. (2012). Dengue virus infection perturbs lipid homeostasis in infected mosquito cells. PLoS Pathog **8,** e1002584.

Posthuma, C.C., Pedersen, K.W., Lu, Z., Joosten, R.G., Roos, N., Zevenhoven-Dobbe, J.C., and Snijder, E.J. (2008). Formation of the arterivirus replication/transcription complex: a key role for nonstructural protein 3 in the remodeling of intracellular membranes. J Virol **82,** 4480–4491.

Prentice, E., Jerome, W.G., Yoshimori, T., Mizushima, N., and Denison, M.R. (2004). Coronavirus replication complex formation utilizes components of cellular autophagy. J Biol Chem **279,** 10136–10141.

Reggiori, F., Monastyrska, I., Verheije, M.H., Calì, T., Ulasli, M., Bianchi, S., Bernasconi, R., de Haan, C.A., and Molinari, M. (2010). Coronaviruses hijack the LC3-I-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication. Cell Host Microbe **7,** 500–508.

Salonen, A., Ahola, T., and Kääriäinen, L. (2005). Viral RNA replication in association with cellular membranes. Curr Top Microbiol Immunol **285,** 139–173.

Schley, D., Whittaker, R.J., and Neuman, B.W. (2013). Arenavirus budding resulting from viral-protein-associated cell membrane curvature. J R Soc Interface **10,** 20130403.

Schwartz, M., Chen, J., Janda, M., Sullivan, M., den Boon, J., and Ahlquist, P. (2002). A positive-strand RNA virus replication complex parallels form and function of retrovirus capsids. Mol Cell **9,** 505–514.

Shibata, Y., Hu, J., Kozlov, M.M., and Rapoport, T.A. (2009). Mechanisms shaping the membranes of cellular organelles. Annu Rev Cell Dev Biol **25,** 329–354.

Snijder, E.J., Kikkert, M., and Fang, Y. (2013). Arterivirus molecular biology and pathogenesis. J Gen Virol **94(Pt 10),** 2141–2163.

Snijder, E.J., van der Meer, Y., Zevenhoven-Dobbe, J., Onderwater, J.J., van der Meulen, J., Koerten, H.K., and Mommaas, A.M. (2006). Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. J Virol **80,** 5927–5940.

Snijder, E.J., van Tol, H., Roos, N., and Pedersen, K.W. (2001). Non-structural proteins 2 and 3 interact to modify host cell membranes during the formation of the arterivirus replication complex. J Gen Virol **82,** 985–994.

Sonnhammer, E.L., von Heijne, G., and Krogh, A. (1998). A hidden Markov model for predicting transmembrane helices in protein sequences. Proc Int Conf Intell Syst Mol Biol **6,** 175–182.

Spuul, P., Balistreri, G., Kääriäinen, L., and Ahola, T. (2010). Phosphatidylinositol 3-kinase-, actin-, and microtubule-dependent transport of Semliki Forest Virus replication complexes from the plasma membrane to modified lysosomes. J Virol **84,** 7543–7557.

Stokes, H.L., Baliji, S., Hui, C.G., Sawicki, S.G., Baker, S.C., and Siddell, S.G. (2010). A new cistron in the murine hepatitis virus replicase gene. J Virol **84,** 10148–10158.

Subramaniam, S. (2005). Bridging the imaging gap: visualizing subcellular architecture with electron tomography. Curr Opin Microbiol **8,** 316–322.

Subramaniam, S., Bartesaghi, A., Liu, J., Bennett, A.E., and Sougrat, R. (2007). Electron tomography of viruses. Curr Opin Struct Biol **17,** 596–602.

Suhy, D.A., Giddings, T.H., and Kirkegaard, K. (2000). Remodeling the endoplasmic reticulum by poliovirus infection and by individual viral proteins: an autophagy-like origin for virus-induced vesicles. J Virol **74,** 8953–8965.

Ulasli, M., Verheije, M.H., de Haan, C.A., and Reggiori, F. (2010). Qualitative and quantitative ultrastructural analysis of the membrane rearrangements induced by coronavirus. Cell Microbiol **12,** 844–861.

van der Meer, Y., van Tol, H., Locker, J.K., and Snijder, E.J. (1998). ORF1a-encoded replicase subunits are involved in the membrane association of the arterivirus replication complex. J Virol **72,** 6689–6698.

van Hemert, M.J., de Wilde, A.H., Gorbalenya, A.E., and Snijder, E.J. (2008). The *in vitro* RNA synthesizing activity of the isolated arterivirus replication/transcription complex is dependent on a host factor. J Biol Chem **283,** 16525–16536.

von Brunn, A., Teepe, C., Simpson, J.C., Pepperkok, R., Friedel, C.C., Zimmer, R., Roberts, R., Baric, R., and Haas, J. (2007). Analysis of intraviral protein-protein interactions of the SARS coronavirus ORFeome. PLoS One **2,** e459.

Welsch, S., Miller, S., Romero-Brey, I., Merz, A., Bleck, C.K., Walther, P., Fuller, S.D., Antony, C., Krijnse-Locker, J., and Bartenschlager, R. (2009). Composition and three-dimensional architecture of the dengue virus replication and assembly sites. Cell Host Microbe **5,** 365–375.

Zhao, Z., Thackray, L.B., Miller, B.C., Lynn, T.M., Becker, M.M., Ward, E., Mizushima, N.N., Denison, M.R., and Virgin, H.W. (2007). Coronavirus replication does not require the autophagy gene ATG5. Autophagy **3,** 581–585.

Ziebuhr, J. (2006). The coronavirus replicase: insights into a sophisticated enzyme machinery. Adv Exp Med Biol **581,** 3–11.

Address correspondence to:
Michael J. Buchmeier, PhD
Division of Infectious Disease
Department of Medicine
University of California, Irvine
3205 McGaugh Hall
Irvine, CA 92697-3900

E-mail: m.buchmeier@uci.edu

Received for publication December 3, 2013; accepted December 3, 2013.