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Does form meet function in the coronavirus replicative organelle?

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If we use the analogy of a virus as a living entity, then the replicative organelle is the part of the body where its metabolic and reproductive activities are concentrated. Recent studies have illuminated the intricately complex replicative organelles of coronaviruses, a group that includes the largest known RNA virus genomes. This review takes a virus-centric look at the coronavirus replication transcription complex organelle in the context of the wider world of positive sense RNA viruses, examining how the mechanisms of protein expression and function act to produce the factories that power the viral replication cycle.

Function of coronavirus organelles

The maxim that 'form follows function' is prominent in the field of design. However, in the context of the subcellular architectures being remodeled into viral replicative organelles, it is unclear how form and function are related. Following several excellent ultrastructural studies, the role played by replicative organelles in the replication cycle remains unclear [1]. For example, studies showing that viral RNA accumulates in and around the coronavirus organelles [2,3] and studies demonstrating that organelles are not formed when RNA synthesis is halted [4,5] show that the appearance of these organelles is tied to RNA synthesis. However, other studies demonstrated that only some organelles are sites of active RNA synthesis [6], and that RNA synthesis occurs before membrane rearrangements are detectable [7]. More recently, a study that used a panel of coronaviruses with mutations affecting the size and number of organelles showed that producing fewer or smaller organelles did not necessarily decrease RNA synthesis or lead to a detectable competitive fitness disadvantage [8], although it is not yet clear whether this is the case *in vivo* or in immunologically active cells such as primary macrophages. These studies are difficult to reconcile with an interpretation of the organelle as the obligate site of viral RNA synthesis.

For these reasons, along with the observations that RNA replication is detectable before the first appearance of organelles [7], we favor an interpretation in which the organelles are a delayed manifestation of amassed viral proteins resulting from abundant RNA expression. Whatever their purpose, it is clear that the coronavirus organelle is dynamic [9], closely tied to vesicular transport in the host cell [5,10], and consists mainly of paired membranes that form a variety of complex shapes including convoluted membranes and double-membrane vesicles (DMVs) [2,11].

Context of +RNA viruses

The catalytic domain of the coronavirus RNA polymerase is related to the RNA-dependent RNA polymerases (RdRp) from all of the other viruses that package a single strand of positive-sense RNA, collectively known as +RNA viruses. The +RNA virus *RdRp* is considered to be one of the signature genes that distinguish viruses from their hosts [12]. Because +RNA viruses share both the central component of the RNA-making machinery and a common replication strategy, it is useful to consider how coronaviruses fit into the wider world of +RNA viruses.

It is a good generalization to say that all +RNA viruses induce membrane-bound replicative organelles, but there are exceptions. Table 1 summarizes the evidence, or lack thereof, for membrane-bound replication factories in all of the currently recognized families of +RNA viruses. For many viruses, particularly those that infect plants, the presence of virus-induced inclusion bodies or 'viroplasms' has been long noted, but detailed ultrastructural data has been slow to appear. As Table 1 demonstrates, the evidence for membrane-bound viral organelles is widespread with a few notable exceptions. This table also serves to highlight areas in need of further research.

In some cases, homology can be used to infer that further investigation is likely to turn up evidence for replicative organelles. For example, members of the Dicistroviridae have proposed homologs of the *2B*, *2C*, and *3A* genes, which have been implicated in organelle formation for other members of the Picornavirales [13–15]. Likewise, the Permutotetraviridae encode a homolog of the conserved tetravirus replicase protein (Rep), and may therefore form similar organelles to related viruses of the Alphatetraviridae and Carmotetraviridae [16].

For other groups there is less evidence regarding whether further investigation will turn up viral organelles. For example, membrane-bound factories do not appear to be formed by Leviviridae, which are known to infect members

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Keywords: RNA virus replication; membrane rearrangement; replicative organelle; virus factory.

0966-842X/
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Table 1. Membrane rearrangements in +RNA virus families

Order	Family	Host[a]	Membrane[b]	Type[c]	Proteins[d]	Refs
Nidovirales	Arteriviridae	A	ER	DMV, PM	nsp2, 3	[21,29,30]
	Coronaviridae	A	ER	DMV, CM, spherule, PM	nsp3, 4, 6	[2,11,20]
	Mesoniviridae	A	ER	PM, tubule	nr[e]	[34]
	Roniviridae	A	nr	Vesicle	nr	[35]
Picornavirales	Dicistroviridae	A	nr	nr	nr	
	Iflaviridae	A	nr	Vesicle	nr	[36]
	Marnaviridae	Alg, Pro	nr	Vesicle	nr	[37]
	Picornaviridae	A	ER	DMV	2BC, 3A	[13–15,38]
	Secoviridae	P	ER	Vesicle, CM	nr	[39]
Tymovirales	Alphaflexiviridae	P, F	ER	VP	TGB1	[40,41]
	Betaflexiviridae	P	ER	VP, DMV	nr	[42,43]
	Gammaflexiviridae	F	nr	VP	nr	[44]
	Tymoviridae	P	Chlor, Mito	DMV	nr	[45]
Unclassified	Alphatetraviridae	A	nr	VP	Rep	[16]
	Alvernaviridae	Din	nr	VP	nr	[46]
	Astroviridae	A	ER	DMV	nsP1a	[47,48]
	Barnaviridae	F	nr	nr	nr	
	Bromoviridae	P	ER	Spherule	Protein 1a	[49,50]
	Caliciviridae	A	ER	Vesicle	P30	[51]
	Carmotetraviridae	A	nr	VP	nr	[52]
	Closteroviridae	P	nr	VP, DMV	nr	[53]
	Flaviviridae	A	ER	Spherule	NS4A, 4B	[54–59]
	Hepeviridae	A	ER	Vesicle, PM	nr	[60]
	Leviviridae	B	Not present			[17]
	Luteoviridae	P	nr	SMV, tubule	nr	[61]
	Narnaviridae	F	nr	nr	nr	
	Nodaviridae	A	Mito	Spherule	Protein A, viral RNA	[62,63]
	Permutotetraviridae	A	nr	nr	nr	
	Potyviridae	P	ER	Vesicle	6K, 6K2	[64–66]
	Togaviridae	A	Lyso, ER	Spherule	P123	[67–69]
	Tombusviridae	P	Perox	Spherule	nr	[70,71]
	Virgaviridae	P	ER	Spherule	nr	[72,73]

[a]Viruses of Animalia (A), Plantae (P), Fungi (F), Bacteria (B), Algae (Alg), Dinoflagellates (Din), and other Protists (Pro).

[b]Proposed membrane donor site: endoplasmic reticulum (ER), chloroplast (Chlor), mitochondrion (Mito), lysosome (Lyso), and peroxisome (Perox).

[c]Type of replicative organelle: double-membrane vesicle (DMV), single-membrane vesicle (SMV), uncharacterized vesicle (vesicle), single or double membrane invagination (spherule), uncharacterized membrane-containing inclusion or viroplasm (VP), convoluted membrane (CM), paired membrane (PM), and tubular structures (tubule).

[d]Proteins implicated in forming viral replicative organelles.

[e]Not reported (nr).

of the Proteobacteria [17]. This is not surprising, because Proteobacteria typically lack the types of internal membranes that other +RNA viruses co-opt to form organelles, but it does suggest that it is theoretically possible for a +RNA polymerase to work in the absence of replicative organelles. Another example is the poorly characterized Narnaviridae, which infect fungi. The Narnaviridae appear to lack both capsid and nucleoprotein genes, but encode an RdRp that is closely related to those of the Leviviridae. Further work is also needed to investigate the function and detailed structure of other fungal viruses such as the Barnaviridae and fungus-infecting members of the Tymovirales. A final group that could shed light on the evolutionary origin of viral membrane-bound organelles is the uncharacterized hyperthermophilic +RNA virus group that was detected genetically in near-boiling, archaea-dominated acidic hot springs of Yellowstone National Park [18].

From Table 1 we can conclude that there is evidence of intracellular membrane-bound replicative organelles in most +RNA viruses of eukaryotes. Another point that can be taken from the table is that the architecture of the organelle can vary considerably within a family or order, as evidenced for the Nidovirales (Figure 1). Most of the viral proteins implicated in organelle formation are either non-enzymatic, or are large multi-domain proteins that also include the RdRp. This suggests that organelle formation is a derived characteristic that arose in +RNA viruses of eukaryotes after, and as an accessory to, RdRp function. The apparent lack of homology between viruses of different families (which will be discussed below), suggests that if organelle-making proteins did arise as replicative accessories, they were probably acquired independently in each virus lineage.

Replicative organelles have been reported for viruses that infect each of the kingdoms of cellular life, but are so far absent from +RNA viruses of prokaryotes. Although it is tempting to speculate that the appearance of membrane-bound organelles was an adaptation that made it possible

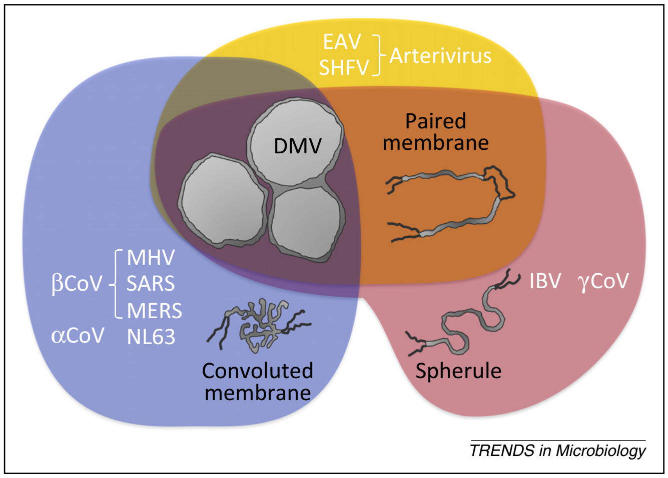

Figure 1. Membrane phenotypes associated with nidovirus replication. Types of membrane are shown as they would appear in cross-section. Examples of well-characterized nidovirus replicative organelles are shown, including the alpha-, beta-, and gammacoronavirus genera.

for primitive prokaryotic viruses to colonize eukaryotic hosts, further evidence from +RNA viruses of the archaea and eubacteria is needed to address this question.

Organelle-making proteins of the Nidovirales

Coronaviruses are grouped with arteriviruses, roniviruses, and mesoniviruses in the order Nidovirales. Together, the Nidovirales lineage has attained a genetic diversity comparable to that observed in the archaea, bacteria, and eukaryota combined [19]. The evidence for a common origin of the Nidovirales comes from the conserved RdRp, superfamily 1 helicase coupled to a metal-binding domain, and a serine proteinase flanked by hydrophobic domains, which occur in all members of Nidovirales.

It was recently demonstrated that only three proteins of the severe acute respiratory syndrome coronavirus (SARS-CoV) are needed to form structures that resemble the authentic viral organelles [20]. Of these proteins, SARS-CoV nonstructural protein 4 (nsp4) and nsp6 are highly conserved across the Nidovirales (Figure 2). For example, the equine arteritis virus nsp3 shows a similar organization and function to coronavirus nsp4 (Figure 2; [21]). These two multi-pass transmembrane proteins flank the conserved viral nsp5 serine proteinase, which cleaves at sites including the nsp4-5, 5-6, and 6-7 boundaries to release nsp4 and nsp6 [22].

The function of nsp4 and nsp6 is not well understood. Neither nsp4 nor nsp6 appears to carry enzymatic signatures, although both are necessary for SARS-CoV replicative organelle formation [20]. nsp4 also contains a widely conserved structural signature at the C terminus, which appears to be dispensable for replication in cell culture [23,24].

The third protein that is needed to form SARS-CoV organelles is nsp3. In its final processed form, nsp3 is the largest single protein encoded by the Coronaviridae, typically occupying about one-fifth of the coding capacity of each virus. nsp3 is the least securely conserved part of the organelle-making apparatus (Figure 2). Nidovirus proteins encoded in the same genomic position as SARS-CoV nsp3 have several hallmarks – most include a papain-related cysteine proteinase, hydrophobic regions flanking a cysteine-histidine cluster, and one or more RNA-binding macrodomains [25]. *nsp3* genes of the Coronaviridae also encode a poorly understood C-terminal Y domain and may contain two ubiquitin-related domains. Most of these features are unrecognizable in the arthropod-infecting Mesoniviridae and Roniviridae, making it less certain that they are true nsp3 homologs.

The enzymatic functions of coronavirus nsp3 are reasonably well understood, but less is known about the role of nsp3 in the viral replication cycle. The proteinase domain(s) of nsp3 process the polyprotein at sites including

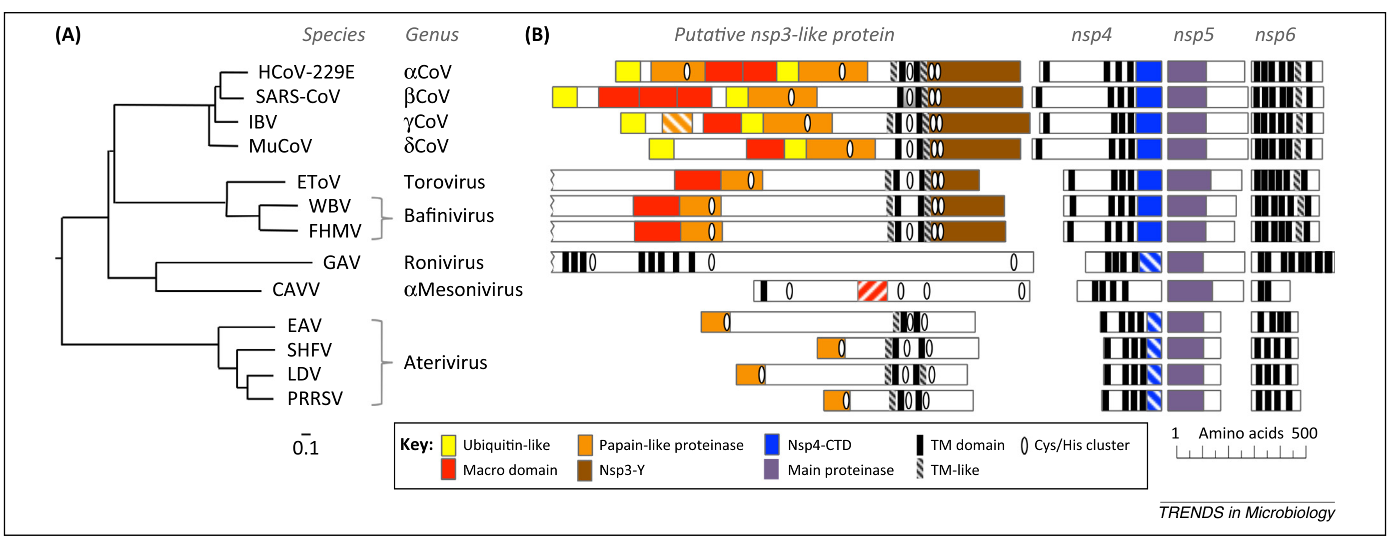

Figure 2. Conservation of double-membrane vesicle (DMV)-making proteins in the Nidovirales. **(A)** Phylogenetic tree of the Nidovirales, adapted from [19] with the approximate position of FHMV added from [74]. **(B)** Domain annotations were based on conserved amino acid sequences (solid colors) or secondary structure patterns (diagonal stripes). Positions of transmembrane and hydrophobic non-transmembrane regions were predicted by TMHMM 2.0 [75] and amended to reflect known topologies [76–78] 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). A jagged line denotes the uncertain position of the amino terminus.

the nsp2-3 and nsp3-4 boundaries to release nsp3 [22]. There is also one reported conditional-lethal mutant in nsp3 that interferes with RNA synthesis by somehow inhibiting the function of the nsp5 main proteinase [4].

In terms of both conservation and function, it appears likely that the genes from nsp3 to nsp6 represent an ancestral organelle-making functional unit. Each of the four genes participates in organelle formation (*nsp3*, *nsp4*, and *nsp6*) or processing of the organelle-forming genes (*nsp3* and *nsp5*). This organization of a protease bracketed by transmembrane proteins has so far only been observed in the Nidovirales. The clustering of organelle-making apparatus, and the fact that all four proteins seem to be necessary to form the authentic organelle, suggests that it may have been appropriated by an ancestral nidovirus en bloc through horizontal gene transfer. However, this interpretation raises the question of how other parts of the replicase protein were processed before the addition of nsp5.

Does form meet function?

It makes sense that replicative organelles would benefit the virus by creating an environment where viral proteins can interact with as little interference from host membrane protein traffic as possible. Nearly all coronavirus replicase proteins have been shown to form complexes – both as homo-oligomers [25] and in groups with complementary functions such as the RNA cap methylation complex of nsp10, nsp14, and nsp16 [26]. Concentrating replicative machinery in and around the DMV could provide economies of scale by integrating the processes of priming, capping, proofreading, and synthesizing viral genomes.

The purpose of these organelles remains uncertain, but it seems logical to predict that DMVs help to concentrate viral proteins and may offer some protection from the antiviral detection and elimination machinery of the cell.

At the peak of the infection, organelles of the coronaviruses mouse hepatitis virus (MHV), Middle Eastern respiratory syndrome (MERS) virus, and SARS-CoV appear similar, taking the form of paired membranes arranged in clusters of roughly 200 nm-wide DMVs, which are sometimes linked by a convoluted membrane [2,7,27]. In a more recent publication, the SARS-CoV convoluted membranes were resolved as paired membranes, with the same inter-membrane distance found in DMVs [20].

Organelle architecture in the other coronavirus genera has revealed some surprises. Alphacoronavirus NL63 formed clusters of betacoronavirus-like DMVs, suggesting that DMV architecture is highly conserved among coronaviruses [28]. A recent study of the gammacoronavirus infectious bronchitis virus (IBV) showed that in addition to the DMVs formed by other coronaviruses, IBV induced extensive paired membranes reminiscent of arterivirus organelles [21,29,30] and smaller 60–80 nm spherules [11]. This result was unexpected because IBV has clear homologs of SARS-CoV nsp3, 4, 5, and 6.

Combining phenotypes

Angelini and collaborators explored how SARS-CoV DMVs are made by expressing nsp3, 4, and 6 singly and in combination and found a possible explanation for how complex coronavirus organelles are formed [20]. Figure 3 shows a schematic representation of the findings from that study. They observed that nsp3 accumulated in perinuclear

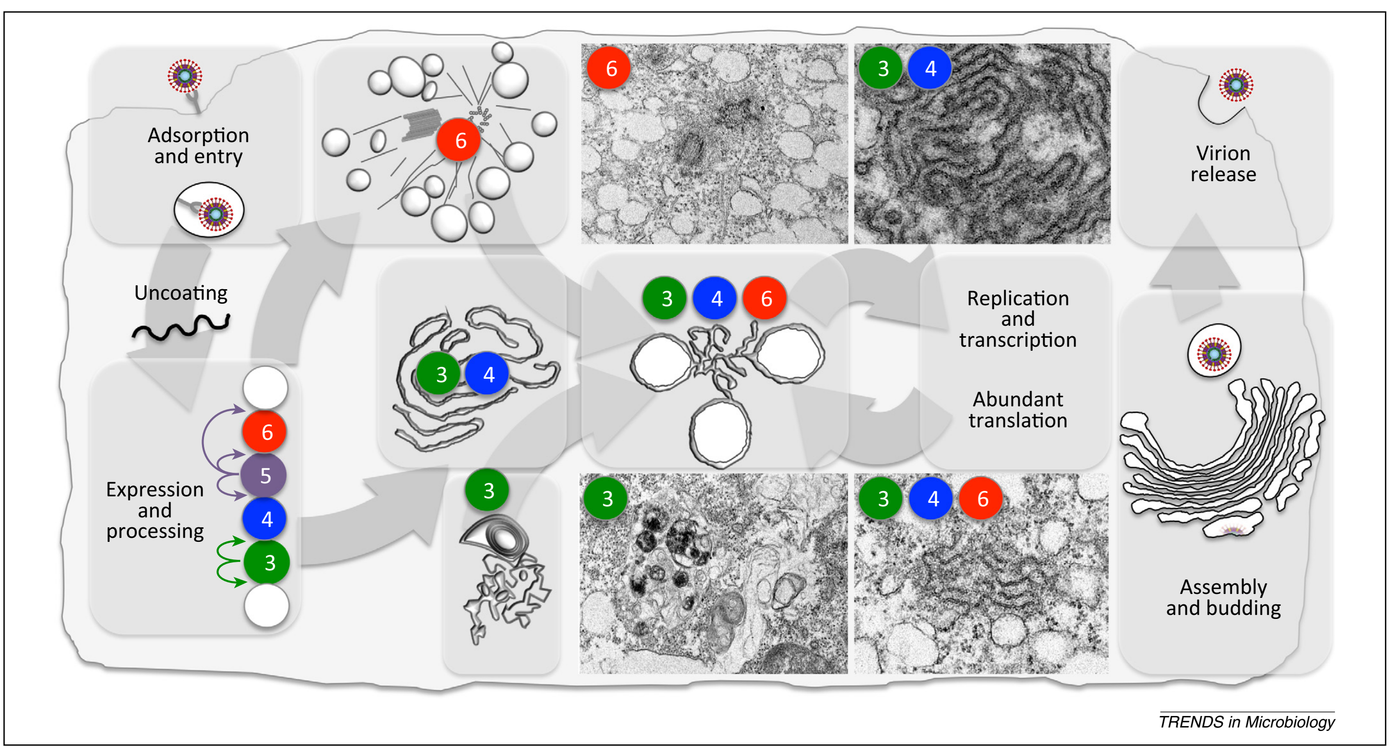

Figure 3. Schematic of severe acute respiratory syndrome coronavirus (SARS-CoV) replication highlighting organelle formation. The replication cycle proceeds from left to right. Nonstructural proteins (nsps) 3–6 are shown as colored circles and other nsps are indicated with white circles. Single nsp and combined membrane phenotypes are shown in schematic form and as electron micrographs of negatively stained ultrathin sections [20].

clusters consisting of large multilamellar vesicles and disordered membrane bundles. This membrane proliferation phenotype was also induced by expression of the C-terminal part of nsp3 starting from the first transmembrane and running to the end of the Y domain. Nsp4 also showed a reticular localization, but did not induce any detectable membrane rearrangements in the absence of nsp3. Coexpression of nsp3 and nsp4 induced extensive membrane pairing, in the form of tubular 'maze-like bodies'. These paired membranes showed the same spacing observed in both DMVs and convoluted membranes, suggesting that nsp3-4 interactions mediate the membrane pairing that is common to all the replicative structures of the Nidovirales (Figure 1).

The same study revealed that nsp6, which had previously been linked to structures involved in autophagy [31], induced an accumulation of single-membrane vesicles around the microtubule organization center [20]. However, it was not clear whether this phenotype resulted from aberrant vesicle formation or transport. The nsp6 phenotype disappeared in the presence of nsp4, suggesting an interaction between nsp4 and nsp6.

Previous studies had shown evidence for nsp3-4 and nsp4-6 interactions in MHV [32,33]. The new wrinkle from the Angelini study showed how the combination of nsp3 membrane proliferation, nsp3-4 membrane-pairing, and nsp6 vesicle-inducing phenotypes resulted in formation of DMV clusters, consisting of paired membranes leading to terminal double vesicles [20]. That study noted that in each of the cells where DMV-like membranes were found, both nsp3-4 maze-like bodies and nsp6 vesiculation were also apparent. Our interpretation of these findings is that nsp6 disturbs the paired membranes, reshaping maze-like bodies into DMVs and convoluted membranes. This raises the question whether differences in nsp6 homologs are at least partly responsible for the observed differences in nidovirus replicative organelles.

Concluding remarks

The studies of separately expressed proteins described above have been useful in illuminating the process of coronavirus replicative organelle formation, but much remains to be learned. The mechanisms leading to membrane proliferation and vesicle accumulation still need to be explored in detail, including which host cell factors are involved (Box 1). Also the protein interactions involved in membrane pairing and protein–membrane interactions that define DMV and spherule size remain to be explored. These studies will undoubtedly reveal fascinating new aspects of coronavirus organelle biology while shedding light on the processes that shape intracellular membranes.

Box 1. Outstanding questions

- Does nidovirus RNA synthesis take place anywhere except inside DMVs?
- What are the differences in protein composition and conformation in spherules, convoluted membranes, and DMVs?
- How do viral and host proteins interact as replicative organelles are formed?
- What happens to the RNA that accumulates inside DMVs?

References

1 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

2 Knoops, K. *et al.* (2008) SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. *PLoS Biol.* 6, e226

3 Gosert, R. *et al.* (2002) RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. *J. Virol.* 76, 3697–3708

4 Stokes, H.L. *et al.* (2010) A new cistron in the murine hepatitis virus replicase gene. *J. Virol.* 84, 10148–10158

5 Verheije, M.H. *et al.* (2008) Mouse hepatitis coronavirus RNA replication depends on GBF1-mediated ARF1 activation. *PLoS Pathog.* 4, e1000088

6 Hagemeijer, M.C. *et al.* (2012) Visualizing coronavirus RNA synthesis in time by using click chemistry. *J. Virol.* 86, 5808–5816

7 Ulasli, M. *et al.* (2010) Qualitative and quantitative ultrastructural analysis of the membrane rearrangements induced by coronavirus. *Cell. Microbiol.* 12, 844–861

8 Al-Mulla, H.M.N. *et al.* (2014) Competitive fitness in coronaviruses is not correlated with size or number of double-membrane vesicles under reduced-temperature growth conditions. *mBio* 5, e01107–e01113

9 Hagemeijer, M.C. *et al.* (2010) Dynamics of coronavirus replication-transcription complexes. *J. Virol.* 84, 2134–2149

10 de Haan, C.A. *et al.* (2010) Autophagy-independent LC3 function in vesicular traffic. *Autophagy* 6, 994–996

11 Maier, H.J. *et al.* (2013) Infectious bronchitis virus generates spherules from zippered endoplasmic reticulum membranes. *mBio* 4, e00801–e00813

12 Koonin, E.V. *et al.* (2006) The ancient virus world and evolution of cells. *Biol. Direct* 1, 29, http://dx.doi.org/10.1186/1745-6150-1-29

13 Richards, A.L. *et al.* (2014) Generation of unique poliovirus RNA replication organelles. *MBio* 5, e00833–00813, http://dx.doi.org/10.1128/mBio.00833-13

14 Teterina, N.L. *et al.* (1997) Induction of intracellular membrane rearrangements by HAV proteins 2C and 2BC. *Virology* 237, 66–77

15 Suhy, D.A. *et al.* (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

16 Short, J.R. and Dorrington, R.A. (2012) Membrane targeting of an alpha-like tetravirus replicase is directed by a region within the RNA-dependent RNA polymerase domain. *J. Gen. Virol.* 93, 1706–1716

17 Nishihara, T. (2003) Various morphological aspects of *Escherichia coli* lysis by RNA bacteriophage MS2 observed by transmission and scanning electron microscopes. *New Microbiol.* 26, 163–168

18 Bolduc, B. *et al.* (2012) Identification of novel positive-strand RNA viruses by metagenomic analysis of archaea-dominated Yellowstone hot springs. *J. Virol.* 86, 5562–5573

19 Lauber, C. *et al.* (2013) The footprint of genome architecture in the largest genome expansion in RNA viruses. *PLoS Pathog.* 9, e1003500

20 Angelini, M.M. *et al.* (2013) Severe acute respiratory syndrome coronavirus nonstructural proteins 3, 4, and 6 induce double-membrane vesicles. *MBio* 4, e00524–00513, http://dx.doi.org/10.1128/mBio.00524-13

21 Posthuma, C.C. *et al.* (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

22 Ziebuhr, J. *et al.* (2000) Virus-encoded proteinases and proteolytic processing in the *Nidovirales*. *J. Gen. Virol.* 81, 853–879

23 Manolaridis, I. *et al.* (2009) Structure of the C-terminal domain of nsp4 from feline coronavirus. *Acta Crystallogr. D: Biol. Crystallogr.* 65, 839–846

24 Sparks, J.S. *et al.* (2007) Genetic analysis of murine hepatitis virus nsp4 in virus replication. *J. Virol.* 81, 12554–12563

25 Neuman, B.W. *et al.* (2008) Proteomics analysis unravels the functional repertoire of coronavirus nonstructural protein 3. *J. Virol.* 82, 5279–5294

26 Bouvet, M. *et al.* (2010) *In vitro* reconstitution of SARS-coronavirus mRNA cap methylation. *PLoS Pathog.* 6, e1000863

27 de Wilde, A.H. *et al.* (2013) MERS-coronavirus replication induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon-alpha treatment. *J. Gen. Virol.* 94, 1749–1760

28 Orenstein, J.M. *et al.* (2008) Morphogenesis of coronavirus HCoV-NL63 in cell culture: a transmission electron microscopic study. *Open Infect. Dis. J.* 2, 52–58

29 Snijder, E.J. *et al.* (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
30 Wood, O. *et al.* (1970) Electron microscopic study of tissue cultures infected with simian haemorrhagic fever virus. *J. Gen. Virol.* 7, 129–136
31 Cottam, E.M. *et al.* (2011) Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum via an omegasome intermediate. *Autophagy* 7, 1335–1347
32 Hagemeijer, M.C. *et al.* (2011) Mobility and interactions of coronavirus nonstructural protein 4. *J. Virol.* 85, 4572–4577
33 Hagemeijer, M.C. *et al.* (2012) Biogenesis and dynamics of the coronavirus replicative structures. *Viruses* 4, 3245–3269
34 Zirkel, F. *et al.* (2011) An insect nidovirus emerging from a primary tropical rainforest. *MBio* 2, e00077–00011, http://dx.doi.org/10.1128/mBio.00077-11
35 Spann, K.M. *et al.* (1995) Lymphoid organ virus of *Penaeus monodon* from Australia. *Dis. Aquat. Organ.* 23, 127–134
36 Gauthier, L. *et al.* (2011) Viruses associated with ovarian degeneration in *Apis mellifera* L. queens. *PLoS ONE* 6, e16217
37 Takao, Y. *et al.* (2005) Isolation and characterization of a novel single-stranded RNA virus infectious to a marine fungoid protist, *Schizochytrium* sp. (Thraustochytriaceae, Labyrinthulea). *Appl. Environ. Microbiol.* 71, 4516–4522
38 Hsu, N.Y. *et al.* (2010) Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. *Cell* 141, 799–811
39 Roberts, I.M. and Harrison, B.D. (1970) Inclusion bodies and tubular structures in *Chenopodium amaranticolor* plants infected with strawberry latent ringspot virus. *J. Gen. Virol.* 7, 47–54
40 Tilsner, J. *et al.* (2012) The TGB1 movement protein of Potato virus X reorganizes actin and endomembranes into the X-body, a viral replication factory. *Plant Physiol.* 158, 1359–1370
41 Linnik, O. *et al.* (2013) Unraveling the structure of viral replication complexes at super-resolution. *Front. Plant Sci.* 4, 6, http://dx.doi.org/10.3389/fpls.2013.00006
42 Edwardson, J.R. and Christie, R.G. (1978) Use of virus-induced inclusions in classification and diagnosis. *Ann. Rev. Phytopathol.* 16, 31–55
43 Rudzinska-Langwald, A. (1990) Cytological changes in phloem parenchyma cells of *Solanum rostratum* (Dunal.) related to the replication of potato virus M (PVM). *Acta Societatis Botanicorum Poloniae* 59, 45–53
44 Boine, B. *et al.* (2012) Recombinant expression of the coat protein of *Botrytis* virus X and development of an immunofluorescence detection method to study its intracellular distribution in *Botrytis cinerea*. *J. Gen. Virol.* 93, 2502–2511
45 Lesemann, D.E. (1977) Virus group-specific and virus-specific cytological alterations induced by members of the tymovirus group. *J. Phytopathol.* 90, 315–336
46 Tomaru, Y. *et al.* (2004) Isolation and characterization of two distinct types of HcRNAV, a single-stranded RNA virus infecting the bivalve-killing microalga *Heterocapsa circularisquama*. *Aquat. Microb. Ecol.* 34, 207–218
47 Guix, S. *et al.* (2004) C-terminal nsP1a protein of human astrovirus colocalizes with the endoplasmic reticulum and viral RNA. *J. Virol.* 78, 13627–13636
48 Mendez, E. *et al.* (2007) Association of the astrovirus structural protein VP90 with membranes plays a role in virus morphogenesis. *J. Virol.* 81, 10649–10658
49 Moreira, A.G. *et al.* (2010) Identification and partial characterization of a *Carica papaya*-infecting isolate of *Alfalfa mosaic virus* in Brazil. *J. Gen. Plant Pathol.* 76, 172–175
50 Schwartz, M. *et al.* (2002) A positive-strand RNA virus replication complex parallels form and function of retrovirus capsids. *Mol. Cell* 9, 505–514
51 Bailey, D. *et al.* (2010) Feline calicivirus p32, p39 and p30 proteins localize to the endoplasmic reticulum to initiate replication complex formation. *J. Gen. Virol.* 91, 739–749
52 Pringle, F.M. *et al.* (2003) Providence virus: a new member of the *Tetraviridae* that infects cultured insect cells. *Virology* 306, 359–370
53 Medina, V. *et al.* (1998) Specific inclusion bodies are associated with replication of lettuce infectious yellows virus RNAs in *Nicotiana benthamiana* protoplasts. *J. Gen. Virol.* 79 (Pt 10), 2325–2329
54 Gillespie, L.K. *et al.* (2010) The endoplasmic reticulum provides the membrane platform for biogenesis of the flavivirus replication complex. *J. Virol.* 84, 10438–10447
55 Welsch, S. *et al.* (2009) Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell Host Microbe* 5, 365–375
56 Romero-Brey, I. *et al.* (2012) Three-dimensional architecture and biogenesis of membrane structures associated with hepatitis C virus replication. *PLoS Pathog.* 8, e1003056
57 Miller, S. *et al.* (2007) The non-structural protein 4A of dengue virus is an integral membrane protein inducing membrane alterations in a 2K-regulated manner. *J. Biol. Chem.* 282, 8873–8882
58 Roosendaal, J. *et al.* (2006) Regulated cleavages at the West Nile virus NS4A-2K-NS4B junctions play a major role in rearranging cytoplasmic membranes and Golgi trafficking of the NS4A protein. *J. Virol.* 80, 4623–4632
59 Egger, D. *et al.* (2002) Expression of hepatitis C virus proteins induces distinct membrane alterations including a candidate viral replication complex. *J. Virol.* 76, 5974–5984
60 Rehman, S. *et al.* (2008) Subcellular localization of hepatitis E virus (HEV) replicase. *Virology* 370, 77–92
61 Gill, C.C. and Chong, J. (1979) Cytopathological evidence for the division of barley yellow dwarf virus isolates into two subgroups. *Virology* 95, 59–69
62 Kopek, B.G. *et al.* (2010) Nodavirus-induced membrane rearrangement in replication complex assembly requires replicase protein a, RNA templates, and polymerase activity. *J. Virol.* 84, 12492–12503
63 Kopek, B.G. *et al.* (2007) Three-dimensional analysis of a viral RNA replication complex reveals a virus-induced mini-organelle. *PLoS Biol.* 5, e220
64 Schaad, M.C. *et al.* (1997) Formation of plant RNA virus replication complexes on membranes: role of an endoplasmic reticulum-targeted viral protein. *EMBO J.* 16, 4049–4059
65 Grangeon, R. *et al.* (2012) Impact on the endoplasmic reticulum and Golgi apparatus of turnip mosaic virus infection. *J. Virol.* 86, 9255–9265
66 Wei, T. and Wang, A. (2008) Biogenesis of cytoplasmic membranous vesicles for plant potyvirus replication occurs at endoplasmic reticulum exit sites in a COPI- and COPII-dependent manner. *J. Virol.* 82, 12252–12264
67 Magliano, D. *et al.* (1998) Rubella virus replication complexes are virus-modified lysosomes. *Virology* 240, 57–63
68 Fontana, J. *et al.* (2010) Three-dimensional structure of rubella virus factories. *Virology* 405, 579–591
69 Salonen, A. *et al.* (2003) Properly folded nonstructural polyprotein directs the Semliki Forest virus replication complex to the endosomal compartment. *J. Virol.* 77, 1691–1702
70 Sharma, M. *et al.* (2011) Inhibition of phospholipid biosynthesis decreases the activity of the tombusvirus replicase and alters the subcellular localization of replication proteins. *Virology* 415, 141–152
71 Barajas, D. *et al.* (2009) A unique role for the host ESCRT proteins in replication of Tomato bushy stunt virus. *PLoS Pathog.* 5, e1000705
72 Kawakami, S. *et al.* (2004) Tobacco mosaic virus infection spreads cell to cell as intact replication complexes. *Proc. Natl. Acad. Sci. U.S.A.* 101, 6291–6296
73 Reichel, C. *et al.* (1999) The role of the ER and cytoskeleton in plant viral trafficking. *Trends Plant Sci.* 4, 458–462
74 Batts, W.N. *et al.* (2012) Genetic analysis of a novel nidovirus from fathead minnows. *J. Gen. Virol.* 93, 1247–1252
75 Krogh, A. *et al.* (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J. Mol. Biol.* 305, 567–580
76 Kanjanahaluethai, A. *et al.* (2007) Membrane topology of murine coronavirus replicase nonstructural protein 3. *Virology* 361, 391–401
77 Oostra, M. *et al.* (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
78 Oostra, M. *et al.* (2007) Localization and membrane topology of coronavirus nonstructural protein 4: involvement of the early secretory pathway in replication. *J. Virol.* 81, 12323–12336