Universiteit
Leiden
The Netherlands

Endomembrane mutiny: how picornaviruses hijack host organelles to support their replication

Melia, C.E.

Citation
Melia, C. E. (2019, May 21). *Endomembrane mutiny: how picornaviruses hijack host organelles to support their replication*. Retrieved from https://hdl.handle.net/1887/73640

Version:	Not Applicable (or Unknown)
License:	Leiden University Non-exclusive license
Downloaded from:	https://hdl.handle.net/1887/73640

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/73640 holds various files of this Leiden University dissertation.

Author: Melia, C.E.
Title: Endomembrane mutiny: how picornaviruses hijack host organelles to support their replication
Issue Date: 2019-05-21

CHAPTER 6

GENERAL DISCUSSION

Charlotte E. Melia[1,2]

[1] Department of Cell and Chemical biology, Section Electron Microscopy, Leiden University Medical Center, 2300 RC Leiden, The Netherlands.

[2] Sir William Dunn School of Pathology, University of Oxford, Oxford, OX1 3RE, United Kingdom.

THE MULTIFACETED ROLE OF PI4P IN PICORNAVIRUS INFECTION

As obligate intracellular parasites, viruses are dependent on the cells they infect for their propagation. In addition to hijacking cellular systems like endocytosis for virus entry and the ribosomal machinery for translation, individual host factors are utilised to support different processes throughout the virus life cycle.

In uninfected cells PI4P is a lipid with diverse functions, including the mediation of cholesterol exchange at the ER-Golgi interface via OSBP. Many of the viruses that utilise PI4P, including enteroviruses and cardioviruses, recruit PI4Ks to sites of replication to increase local PI4P levels (1-4), where it facilitates local cholesterol exchange (2, 5-8). A role for PI4P in modulating proteolytic processing of the viral polyprotein has also emerged from experiments in poliovirus (9, 10), where PI4P appears to mediate cleavage at the 3AB junction. In **Chapter 3**, we establish that this role is conserved in another enterovirus, CVB3. The mechanism underlying this effect in either enterovirus species has yet to be determined, but may relate to the influence of lipid composition on the orientation of 3A precursors within the membrane. Indeed, PI4KB resistant mutations in both poliovirus (3A-A70T) and CVB3 (3A-H57Y) lie within or proximal to the putative membrane-spanning hydrophobic domain of the 3A protein (11, 12). Interestingly, OSBP inhibition also impairs 3A-3B cleavage (10), suggesting that this effect could be mediated by low membrane cholesterol levels rather than low PI4P levels directly. In **Chapter 3** we also outline a new function of PI4KB. Utilising a CVB3 resistant to PI4KB inhibition, low local concentrations of PI4P were found to delay the formation of CVB3 ROs to beyond the phase of exponential viral RNA synthesis. ROs that appear to be morphologically typical formed only late in infection under PI4KB inhibition, and these structures appeared to be devoid of the high levels of PI4P associated with ROs in wt CVB3 infection. Thus, high PI4P levels are not strictly required for the development of ROs, but do expedite their formation. Inefficient cleavage of viral proteins has been suggested to affect RO formation in HCV (13), as well as in coronaviruses (14). However, poor polyprotein processing under low-PI4P conditions did not explain the delay in CVB3 RO formation we observed, as polyprotein processing of the PI4KB resistant CVB3 3A-H57Y was not impaired under PI4KB inhibition. This suggests that the role of PI4P in polyprotein processing and its role in expediting RO formation are independent in enterovirus-infected cells.

While the mechanism that underlies the role of PI4P in RO formation remains uncertain, it is likely to be more nuanced than for other host factors that influence RO development, like the ESCRT proteins and reticulons that shape virus-induced membrane modifications during infections of other +RNA viruses (15, 16), and perhaps also of enteroviruses (17). PI4P may in fact have a semi-redundant function during RO formation, rather than an intrinsic structural role. This is supported by findings in **Chapter 5**, in which an analogous PI4KA-resistant cardiovirus, EMCV 3A-A32V, was found to produce ROs under PI4KA inhibition at a similar time point post-infection to uninhibited wild-type virus. One tantalising possibility is that the effect of PI4P on RO formation stems from its role in OSBP-mediated cholesterol recruitment, as cholesterol has a direct effect on the formation of lipid domains and on membrane morphology (18-20). Indeed, enteroviruses have been shown to utilise other routes for cholesterol recruitment, for instance via endosomal transfer (21). These alternative routes may be more effectively harnessed, when PI4P levels are suppressed. However, while cholesterol is essential for replication and, like PI4P, has been shown to be important for polyprotein processing (21, 22), a role for cholesterol in RO formation has yet to be directly demonstrated.

MEMBRANE UTILISATION FOR RO FORMATION IS REMARKABLY FLEXIBLE

While our understanding of the viral and host components that engender enterovirus ROs is broadening, fundamental questions about their origin remain unanswered. Putative donor organelles have been identified for many +RNA viruses using electron microscopy, but connections between a donor organelle and ROs have been elusive in ultrastructural studies of enterovirus infection. Other studies that explore co-localisations between viral and host factors, or hunt for the locations of early viral RNA synthesis, have generated a sprawling and at times contradictory picture of where the first enterovirus ROs are formed. Much of the available evidence suggests that membranes of the Golgi apparatus contribute to enterovirus RO biogenesis (1, 23-25). This idea is supported by the chronological correspondence between Golgi apparatus disintegration and RO formation observed in EM (26), and in live-cell imaging data (**Chapter 2**) of infected cells, and by the association of viral replication with the Golgi in the absence of ROs found in **Chapter 3**. However, alternative origins have also been suggested, including the ER and the autophagy pathway (1, 23, 27-29). Interpretation of data that suggest potentially conflicting origins is confounded by the differences in virus species, the times post-infection assessed, and the experimental methodologies used across these studies. In **Chapter 4** we present compelling evidence that membrane utilisation by enteroviruses is diverse, revealing connections between ROs and both ER and Golgi membranes in SBF-SEM and TEM data. In addition to uniting disparate ideas about the origins of enterovirus ROs, these data highlight the surprising flexibility with which viruses can exploit cellular membranes for RO formation. This flexibility may extend to other +RNA viruses, as suggested by key experiments using tombusviruses and nodaviruses, where RO formation could be directed to specific organelles using localisation signals, or was found to occur at alternative organelles in the absence of the typical membrane target (30, 31).

Our observations further demonstrate that morphologically similar ROs can be derived from different membrane sources. This suggests that the host factors that drive or contribute to enterovirus RO biogenesis, and those that have a role in the transformation of single- into double-membrane structures, are not specific to the membrane origin, or can be recruited. These criteria are well met by PI4P, which is ubiquitous in the cell and produced by kinases that are readily recruited to specific cellular compartments. In uninfected cells PI4KB is recruited primarily to the Golgi apparatus, while PI4KA is responsible for PI4P generation at the plasma membrane and ER (32, 33). While PI4KB recruitment to the Golgi apparatus is enhanced during enterovirus infection, where it contributes to rapid RO formation (**Chapter 3**), the role of PI4P in ER-derived RO formation is less clear. PI4P production may be stimulated by the selective recruitment of PI4KB to the ER, else existing ER pools of PI4P generated by PI4KA may be sufficient (e.g. at ER exit sites (34)). Another possibility is that ROs of ER origin form independently of PI4P. Live-cell imaging data (Chapter 3) suggest that, under PI4KB inhibition, the 3A protein accumulates almost exclusively in the Golgi region rather than first forming in peripheral/ER-associated clusters, pointing to a role for PI4KB in ER-derived RO formation.

In **Chapter 5** this idea that characteristic picornavirus RO morphologies are not tied to a specific membrane origin was reinforced following the characterisation of the 3D morphology and development of EMCV ROs. Despite significant differences in cardiovirus and enterovirus host factor utilisation during replication, EMCV was found to produce ROs that bore a striking resemblance to

those of enteroviruses, producing initially single-membrane structures that developed into DMVs. Connections between endomembranes and early ROs were readily found during EMCV infection, suggesting that the ER is the exclusive donor organelle for cardiovirus ROs. Together with the results in **Chapter 4**, these data suggest that the donor organelle is not deterministic of the resulting RO morphology for enteroviruses and cardioviruses, and perhaps picornaviruses more generally. While studies in tombusviruses (35, 36) provide evidence that the viral replication machinery can be successfully diverted to different organelles, it is unclear whether virus-induced membrane rearrangements form at these sites and, if so, whether these structures have typical morphologies.
While viruses appear to target specific membrane compartments upon infection, these findings altogether raise the question of what defines an appropriate membrane platform for replication, or an appropriate membrane donor for RO formation. While viruses have typical membrane targets from which they derive ROs for genome replication, it is clear that the core requirements for replication are not tied to specific membrane compartments in all cases. Membrane utilisation may instead be decided by the localisation and dynamics of early replication events, the requirements for protein folding and processing, or be the result of favourable positioning or proximity to auxiliary organelles utilised during but not essential for replication, like lipid droplets or endosomes.

THE SITES OF RNA SYNTHESIS REPRESENT A NEW RO PHENOTYPE

The archetypal RO morphologies produced by +RNA viruses are broadly characterised as forming secluded compartments; the spherules of FHV (37), Zika virus (38), and DENV (39, 40) and the DMVs of SARS- and MERS- coronaviruses (41, 42), EAV (43), HCV (44), CVB3 (26), PV (25) and EMCV (**Chapter 5**). However, our findings from studies of EMCV (**Chapter 5**) and similar data from enterovirus studies ((25, 26), **Chapter 3**) suggest that, for the picornaviruses, the primary sites of RNA synthesis are single-membrane ROs with positive curvature. While the 3D structures of other picornavirus ROs remain to be explored, the 2D profiles found in EM cell-sections of other genera, like the aphthoviruses, also resemble those of the enteroviruses and cardioviruses (45). While putative DMVs identified in FMDV-infected cells could be found at the mid-point of infection, the predominant ROs at early and intermediate points in infection were single-membrane structures. Rather than the DMVs then, it is single-membrane structures that appear to be the primary support for picornavirus RNA synthesis. For this reason, single-membrane structures with positive curvature must be considered a third archetypal RO morphology produced by +RNA viruses. Unlike the spherules and DMVs utilised for genome replication in other +RNA virus families, these single-membrane tubule ROs of picornaviruses do not enclose cytosolic space.

THE ADVANTAGES OF GENERATING ROS FOR PICORNAVIRUS INFECTION

In **Chapter 3** we present an example of +RNA virus replication at a morphologically intact cellular organelle. These data effectively demonstrate that ROs are not strictly required for sustained enterovirus RNA synthesis, raising questions over the advantages of RO formation during replication. While a causative relationship between the delayed enterovirus RO formation and reduced replicative efficiency observed under PI4KB inhibition has not been established, this observation aligns with one of the prevailing hypotheses regarding the role of ROs; that they contribute to

effective RNA synthesis. The advantages conferred by RO formation in this regard may include the expansion of suitable membranes for genome replication, or the provision of an optimal environment for the RNA replication machinery to assemble and function. For FHV (46), poliovirus (47) and SARS-coronavirus (48) there is evidence to suggest that intact membranes are required for efficient viral RNA synthesis, supporting this notion.

Intact membranes may also prove beneficial by protecting viral RNA from sensors of the innate immune system. In vitro studies of FHV (46), and poliovirus (49) show a link between (intact) RO membranes and RNAse access to RO-associated viral RNA. Given that enterovirus replication complexes are thought to reside on the cytosolic face of ROs (28), this apparent protective function could serve to shield viral replication intermediates from cellular innate immune sensors in vivo. Another +RNA virus, HCV, appears to have developed an alternative or additional mechanism to shield viral RNA species from cellular sensors, by integrating nucleoporins into its membranous web to regulate protein access (50). It should be noted that, while these studies show that intact membranes confer protection, it remains unknown whether this effect is dependent upon specific ROs morphologies. For spherule-forming viruses, the morphology of ROs may provide a straightforward shielding mechanism. Given that only a small, neck-like opening bridges the space between cytosol and spherule interior, these compartments are relatively isolated from the remainder of the cytosol, limiting the availability of viral products for detection by cellular sensors. Unlike spherules, the tubules and vesicles produced by picornaviruses, and (if their outer membrane is utilised for genome replication) the DMVs generated by nidoviruses and flaviviruses replication, represent a large, exposed surface area vulnerable to sensing. For these viruses, the concept that the morphology of their ROs is adapted to shield RNA is less intuitive. In fact, an absence of ROs at the peak of RNA synthesis in CVB3 3A-H57Y infection was found to have no significant impact on the measured IFN-b response across infection, or the state of the innate immune sensors MAVS and PKR, as described in **Chapter 3**. While it remains to be established whether viral RNA is also physically shielded (e.g. from RNases) in these conditions, these data could suggest that any protective function is unrelated to RO morphology, and that replication at a morphologically intact Golgi apparatus is similarly protective to replication at ROs. Protection could feasibly be conferred by the viral replication machinery within the replication membrane. Viral dsRNA must be available to the C terminus and helicase domain of the RIG-I-like receptor MDA5 for its activation (51), and the juxtaposition of membrane and replicating RNA, or the viral machinery surrounding it, may limit accessibility. It may also be that membranes, morphology-dependent or otherwise, serve at best a semi-redundant function in innate immune evasion, given the efficacy of viral proteases that cleave innate immunity recognition and signalling molecules (52, 53).

ROs may also facilitate other processes during infection, by co-ordinating different events during the viral life cycle. For enteroviruses, genome replication at ROs is directly coupled to genome encapsidation (54, 55). This mechanism may have developed as a means of increasing the efficacy of nascent virion production by eliminating the need for RNA diffusion ahead of packaging. If RO membranes represent a protective environment for viral products, then diffusion of ssRNA away from ROs would also expose the virus to cellular innate immune proteins like RNAse L (56) in viruses like CVB3 whose genomes are vulnerable to its endonuclease activity (57). The direct coupling of viral genome synthesis and encapsidation also ensures specificity of RNA packaging, and represents part of the 'higher order proof-reading' mechanism inherent in the successful translation,

replication and packaging of viral genome (58). That these processes are coupled implies that virus particles also assemble at ROs, and may in some way utilise the membrane platform they provide to co-ordinate assembly. Alternatively, particles may assemble in the cytosol and aggregate at ROs. In accordance with these possibilities, a striking spatial proximity between ROs and virus particles was observed in EMCV-infected cells in **Chapter 5**, which could reflect a similar association between RNA synthesis and virion packaging in cardioviruses.

THE ROLE OF DMVS IN PICORNAVIRUS REPLICATION

Enteroviruses DMVs have also been reported to serve as vehicles for the en bloc transfer of mature virions (59-61), which may increase the likelihood of a viable infection if complementary viral quasispecies are exported together. However, the analysis of EMCV virion distribution in **Chapter 5** demonstrated that only a small fraction of DMVs contain virus particles. While this does not exclude the possibility that these DMVs are exported, it suggests that en bloc transfer is not a prominent mechanism of viral export in cardioviruses.

A clear role for DMVs in picornavirus replication has yet to be identified. The peak of viral RNA synthesis in enteroviruses and cardioviruses (**Chapters 4** and **5**) occurs when single-membrane structures predominate, ahead of their transformation into DMVs, raising the question of why this transformation occurs. One possibility is that DMV formation, by enwrapping small volumes of cytosol, is a mechanism to shield a proportion of the RNA replication machinery or replicative products from innate immune sensors. This could be advantageous particularly at late stages of infection, when other viral mechanisms to evade the cellular innate immune system, like the viral protease-mediated cleavage of cellular sensors, may be overburdened. Evidence for the accumulation of viral RNA within DMVs has been found in nidoviruses (41, 43), although it remains unclear whether the inner membrane of nidovirus DMVs, which do not have openings connecting the DMV interior to the cytosol, are the primary site of nidovirus RNA synthesis. A small proportion of enterovirus and cardiovirus DMVs were found in a vase-like configuration that connected the DMV interior and the cytosol ((26), **Chapter 5**). It is unclear whether these openings represent (semi-) stable structures found in a low percentage of DMVs, or a transient late stage of DMV formation. Given that picornavirus RNA synthesis continues following the transformation of tubules into DMVs, open DMVs could represent a shielded environment for late-stage viral genome replication. Indeed, given the evidence for coupled genome replication and virion packaging, those DMVs that contain openings may correspond to those that accumulate nascent virions, perhaps ahead of pore fusion and en bloc transfer.

RETHINKING THE VIRAL REPLICATION MEMBRANE

While categorisations based on morphology are contextually useful, archetypal RO morphologies are an incomplete picture of how viral replication membranes develop throughout infection. Indeed, prior to the accumulation of RO-forming viral proteins, viral genome replication may occur at largely unmodified membranes.

It is also significant that, while the vast majority of viral RNA synthesis occurs at ROs under typical infection conditions, imposing specific modifications or conditions can produce exceptions to this.

In **Chapter 3,** extended enterovirus RNA synthesis was shown to occur at a morphologically intact Golgi apparatus under low PI4P conditions, without any apparent penalty to innate immune evasion, but with a corresponding drop in RNA replication efficiency. In this case it is unclear whether low replication efficiency results directly from a lack of archetypal enterovirus ROs, or indirectly from (the incomplete rescue of) defects in polyprotein processing. Other evidence suggests that RO morphology can be dispensed without a cost to fitness. Manipulating the relative levels of the BMV 1a and 2a polymerase shifted the BMV replication membrane morphology from archetypal spherules to flat membrane sheets, without a reduction to replicative efficiency (62). This highlights again the surprising flexibility with which viruses can use membranes, and emphasises the pitfalls of defining the membranes utilised for replication by their morphology. Indeed, the evidence thus far aligns with a scenario where specific membrane morphologies may be secondary for viral RNA synthesis. A holistic approach is required to understand the membrane composition that underlies the efficient, sustained viral RNA synthesis that is typically associated with ROs. Improved procedures for the isolation and lipidomic or proteomic analysis of replication membrane fractions will allow for a more comprehensive understanding of the changing compositions of replication membranes at different stages of infection. This information can be coupled with powerful techniques to visualise native-state RO structures by cryo-electron microscopy, which can inform us regarding the conformation of intact replication complexes, and demonstrate how these assemble and function on the surface of replication membranes with changing compositions. The mechanism that drives the development of archetypal RO morphologies may well emerge from this enhanced understanding of their composition. It is this mechanism that lies at the heart of the question of RO function, and whether the morphologies that arise during infection represent the result of, or a prerequisite to, effective replication.

REFERENCES

1. Hsu NY, Ilnytska O, Belov G, Santiana M, Chen YH, Takvorian PM, Pau C, van der Schaar H, Kaushik-Basu N, Balla T, Cameron CE, Ehrenfeld E, van Kuppeveld FJM, Altan-Bonnet N. 2010. Viral Reorganization of the Secretory Pathway Generates Distinct Organelles for RNA Replication. Cell 141:799-811.
2. Dorobantu CM, Albulescu L, Harak C, Feng Q, van Kampen M, Strating JRPM, Gorbalenya AE, Lohmann V, van der Schaar HM, van Kuppeveld FJM. 2015. Modulation of the Host Lipid Landscape to Promote RNA Virus Replication: The Picornavirus Encephalomyocarditis Virus Converges on the Pathway Used by Hepatitis C Virus. Plos Pathogens 11.
3. Sasaki J, Ishikawa K, Arita M, Taniguchi K. 2012. ACBD3-mediated recruitment of PI4KB to picornavirus RNA replication sites. EMBO J 31:754-66.
4. Reiss S, Rebhan I, Backes P, Romero-Brey I, Erfle H, Matula P, Kaderali L, Poenisch M, Blankenburg H, Hiet MS, Longerich T, Diehl S, Ramirez F, Balla T, Rohr K, Kaul A, Buhler S, Pepperkok R, Lengauer T, Albrecht M, Eils R, Schirmacher P, Lohmann V, Bartenschlager R. 2011. Recruitment and activation of a lipid kinase by hepatitis C virus NS5A is essential for integrity of the membranous replication compartment. Cell Host Microbe 9:32-45.

5. Arita M, Kojima H, Nagano T, Okabe T, Wakita T, Shimizu H. 2013. Oxysterol-Binding Protein Family I Is the Target of Minor Enviroxime-Like Compounds. Journal of Virology 87:4252-4260.
6. Strating JRPM, van der Linden L, Albulescu L, Bigay J, Arita M, Delang L, Leyssen P, van der Schaar HM, Lanke KHW, Thibaut HJ, Ulferts R, Drin G, Schlinck N, Wubbolts RW, Sever N, Head SA, Liu JO, Beachy PA, De Matteis MA, Shair MD, Olkkonen VM, Neyts J, van Kuppeveld FJM. 2015. Itraconazole Inhibits Enterovirus Replication by Targeting the Oxysterol-Binding Protein. Cell Reports 10:600-615.
7. Ishikawa-Sasaki K, Nagashima S, Taniguchi K, Sasaki J. 2018. Model of OSBP-Mediated Cholesterol Supply to Aichi Virus RNA Replication Sites Involving Protein-Protein Interactions among Viral Proteins, ACBD3, OSBP, VAP-A/B, and SAC1. J Virol 92.
8. Wang HL, Perry JW, Lauring AS, Neddermann P, De Francesco R, Tai AW. 2014. Oxysterol-Binding Protein Is a Phosphatidylinositol 4-Kinase Effector Required for HCV Replication Membrane Integrity and Cholesterol Trafficking. Gastroenterology 146:1373-+.
9. Arita M. 2016. Mechanism of Poliovirus Resistance to Host Phosphatidylinositol-4 Kinase III beta Inhibitor. Acs Infectious Diseases 2:140-148.
10. Lyoo H, Dorobantu CM, van der Schaar HM, van Kuppeveld FJM. 2017. Modulation of proteolytic polyprotein processing by coxsackievirus mutants resistant to inhibitors targeting phosphatidylinositol-4-kinase IIIbeta or oxysterol binding protein. Antiviral Res 147:86-90.
11. Towner JS, Ho TV, Semler BL. 1996. Determinants of membrane association for poliovirus protein 3AB. J Biol Chem 271:26810-8.
12. Wessels E, Notebaart RA, Duijsings D, Lanke K, Vergeer B, Melchers WJG, van Kuppeveld FJM. 2006. Structure-function analysis of the coxsackievirus protein 3A - Identification of residues important for dimerization, viral RNA replication, and transport inhibition. Journal of Biological Chemistry 281:28232-28243.
13. Romero-Brey I, Berger C, Kallis S, Kolovou A, Paul D, Lohmann V, Bartenschlager R. 2015. NS5A Domain 1 and Polyprotein Cleavage Kinetics Are Critical for Induction of Double-Membrane Vesicles Associated with Hepatitis C Virus Replication. MBio 6:e00759.
14. Oudshoorn D, Rijs K, Limpens R, Groen K, Koster AJ, Snijder EJ, Kikkert M, Barcena M. 2017. Expression and Cleavage of Middle East Respiratory Syndrome Coronavirus nsp3-4 Polyprotein Induce the Formation of Double-Membrane Vesicles That Mimic Those Associated with Coronaviral RNA Replication. MBio 8.
15. Diaz A, Zhang JT, Ollwerther A, Wang XF, Ahlquist P. 2015. Host ESCRT Proteins Are Required for Bromovirus RNA Replication Compartment Assembly and Function. Plos Pathogens 11.
16. Diaz A, Wang XF, Ahlquist P. 2010. Membrane-shaping host reticulon proteins play crucial roles in viral RNA replication compartment formation and function. Proceedings of the National Academy of Sciences of the United States of America 107:16291-16296.
17. Tang WF, Yang SY, Wu BW, Jheng JR, Chen YL, Shih CH, Lin KH, Lai HC, Tang P, Horng JT. 2007. Reticulon 3 binds the 2C protein of enterovirus 71 and is required for viral replication. J Biol Chem 282:5888-98.
18. Lippincott-Schwartz J, Phair RD. 2010. Lipids and cholesterol as regulators of traffic in the endomembrane system. Annu Rev Biophys 39:559-78.

19. Yesylevskyy SO, Demchenko AP, Kraszewski S, Ramseyer C. 2013. Cholesterol induces uneven curvature of asymmetric lipid bilayers. ScientificWorldJournal 2013:965230.
20. Kawakami LM, Yoon BK, Jackman JA, Knoll W, Weiss PS, Cho NJ. 2017. Understanding How Sterols Regulate Membrane Remodeling in Supported Lipid Bilayers. Langmuir 33:14756-14765.
21. Ilnytska O, Santiana M, Hsu NY, Du WL, Chen YH, Viktorova EG, Belov G, Brinker A, Storch J, Moore C, Dixon JL, Altan-Bonnet N. 2013. Enteroviruses Harness the Cellular Endocytic Machinery to Remodel the Host Cell Cholesterol Landscape for Effective Viral Replication. Cell Host & Microbe 14:281-293.
22. Strating JR, van der Schaar HM, van Kuppeveld FJ. 2013. Cholesterol: fa(s)t-food for enterovirus genome replication. Trends Microbiol 21:560-1.
23. Schlegel A, Giddings TH, Jr., Ladinsky MS, Kirkegaard K. 1996. Cellular origin and ultrastructure of membranes induced during poliovirus infection. J Virol 70:6576-88.
24. Richards AL, Soares-Martins JAP, Riddell GT, Jackson WT. 2014. Generation of Unique Poliovirus RNA Replication Organelles. Mbio 5.
25. Belov GA, Nair V, Hansen BT, Hoyt FH, Fischer ER, Ehrenfeld E. 2012. Complex Dynamic Development of Poliovirus Membranous Replication Complexes. Journal of Virology 86:302-312.
26. Limpens RWAL, van der Schaar HM, Kumar D, Koster AJ, Snijder EJ, van Kuppeveld FJM, Barcena M. 2011. The Transformation of Enterovirus Replication Structures: a Three-Dimensional Study of Single- and Double-Membrane Compartments. Mbio 2.
27. Lai JKF, Sam IC, Chan YF. 2016. The Autophagic Machinery in Enterovirus Infection. Viruses-Basel 8.
28. Bienz K, Egger D, Pasamontes L. 1987. Association of Polioviral Proteins of the P2-Genomic Region with the Viral Replication Complex and Virus-Induced Membrane Synthesis as Visualized by Electron-Microscopic Immunocytochemistry and Autoradiography. Virology 160:220-226.
29. Rust RC, Landmann L, Gosert R, Tang BL, Hong WJ, Hauri HP, Egger D, Bienz K. 2001. Cellular COPII proteins are involved in production of the vesicles that form the poliovirus replication complex. Journal of Virology 75:9808-9818.
30. Rubino L, Russo M. 1998. Membrane targeting sequences in tombusvirus infections. Virology 252:431-437.
31. Miller DJ, Schwartz MD, Dye BT, Ahlquist P. 2003. Engineered retargeting of viral RNA replication complexes to an alternative intracellular membrane. Journal of Virology 77:12193-12202.
32. Boura E, Nencka R. 2015. Phosphatidylinositol 4-kinases: Function, structure, and inhibition. Exp Cell Res 337:136-45.
33. Clayton EL, Minogue S, Waugh MG. 2013. Mammalian phosphatidylinositol 4-kinases as modulators of membrane trafficking and lipid signaling networks. Progress in Lipid Research 52:294-304.
34. Blumental-Perry A, Haney CJ, Weixel KM, Watkins SC, Weisz OA, Aridor M. 2006. Phosphatidylinositol 4-phosphate formation at ER exit sites regulates ER export. Developmental Cell 11:671-682.

35. Xu K, Huang TS, Nagy PD. 2012. Authentic In Vitro Replication of Two Tombusviruses in Isolated Mitochondrial and Endoplasmic Reticulum Membranes. Journal of Virology 86:12779-12794.
36. Jonczyk M, Pathak KB, Sharma M, Nagy PD. 2007. Exploiting alternative subcellular location for replication: Tombusvirus replication switches to the endoplasmic reticulum in the absence of peroxisomes. Virology 362:320-330.
37. Kopek BG, Perkins G, Miller DJ, Ellisman MH, Ahlquist P. 2007. Three-dimensional analysis of a viral RNA replication complex reveals a virus-induced mini-organelle. Plos Biology 5:2022-2034.
38. Cortese M, Goellner S, Acosta EG, Neufeldt CJ, Oleksiuk O, Lampe M, Haselmann U, Funaya C, Schieber N, Ronchi P, Schorb M, Pruunsild P, Schwab Y, Chatel-Chaix L, Ruggieri A, Bartenschlager R. 2017. Ultrastructural Characterization of Zika Virus Replication Factories. Cell Reports 18:2113-2123.
39. Gillespie LK, Hoenen A, Morgan G, Mackenzie JM. 2010. The endoplasmic reticulum provides the membrane platform for biogenesis of the flavivirus replication complex. J Virol 84:10438-47.
40. Welsch S, Miller S, Romero-Brey I, Merz A, Bleck CKE, Walther P, Fuller SD, Antony C, Krijnse-Locker J, Bartenschlager R. 2009. Composition and Three-Dimensional Architecture of the Dengue Virus Replication and Assembly Sites. Cell Host & Microbe 5:365-375.
41. Knoops K, Kikkert M, van den Worm SHE, Zevenhoven-Dobbe JC, van der Meer Y, Koster AJ, Mommaas AM, Snijder EJ. 2008. SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. Plos Biology 6:1957-1974.
42. de Wilde AH, Raj VS, Oudshoorn D, Bestebroer TM, van Nieuwkoop S, Limpens RWAL, Posthuma CC, van der Meer Y, Barcena M, Haagmans BL, Snijder EJ, van den Hoogen BG. 2013. MERS-coronavirus replication induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon-alpha treatment. Journal of General Virology 94:1749-1760.
43. Knoops K, Barcena M, Limpens RW, Koster AJ, Mommaas AM, Snijder EJ. 2012. Ultrastructural characterization of arterivirus replication structures: reshaping the endoplasmic reticulum to accommodate viral RNA synthesis. J Virol 86:2474-87.
44. Romero-Brey I, Merz A, Chiramel A, Lee JY, Chlanda P, Haselman U, Santarella-Mellwig R, Habermann A, Hoppe S, Kallis S, Walther P, Antony C, Krijnse-Locker J, Bartenschlager R. 2012. Three-Dimensional Architecture and Biogenesis of Membrane Structures Associated with Hepatitis C Virus Replication. Plos Pathogens 8.
45. Monaghan P, Cook H, Jackson T, Ryan M, Wileman T. 2004. The ultrastructure of the developing replication site in foot-and-mouth disease virus-infected BHK-38 cells. J Gen Virol 85:933-46.
46. Short JR, Speir JA, Gopal R, Pankratz LM, Lanman J, Schneemann A. 2016. Role of Mitochondrial Membrane Spherules in Flock House Virus Replication. Journal of Virology 90:3676-3683.
47. Fogg MH, Teterina NL, Ehrenfeld E. 2003. Membrane requirements for uridylylation of the poliovirus VPg protein and viral RNA synthesis in vitro. J Virol 77:11408-16.
48. van Hemert MJ, van den Worm SHE, Knoops K, Mommaas AM, Gorbalenya AE, Snijder EJ.

2008. SARS-coronavirus replication/transcription complexes are membrane-protected and need a host factor for activity in vitro. Plos Pathogens 4.
49. Bienz K, Egger D, Pfister T, Troxler M. 1992. Structural and Functional-Characterization of the Poliovirus Replication Complex. Journal of Virology 66:2740-2747.
50. Neufeldt CJ, Joyce MA, Van Buuren N, Levin A, Kirkegaard K, Gale Jr M, Tyrrell DLJ, Wozniak RW. 2016. The Hepatitis C Virus-Induced Membranous Web and Associated Nuclear Transport Machinery Limit Access of Pattern Recognition Receptors to Viral Replication Sites. PLOS Pathogens 12:e1005428.
51. Wu B, Peisley A, Richards C, Yao H, Zeng X, Lin C, Chu F, Walz T, Hur S. 2013. Structural basis for dsRNA recognition, filament formation, and antiviral signal activation by MDA5. Cell 152:276-89.
52. Feng Q, Langereis MA, Lork M, Nguyen M, Hato SV, Lanke K, Emdad L, Bhoopathi P, Fisher PB, Lloyd RE, van Kuppeveld FJ. 2014. Enterovirus 2Apro targets MDA5 and MAVS in infected cells. J Virol 88:3369-78.
53. Barral PM, Sarkar D, Fisher PB, Racaniello VR. 2009. RIG-I is cleaved during picornavirus infection. Virology 391:171-6.
54. Nugent CI, Johnson KL, Sarnow P, Kirkegaard K. 1999. Functional coupling between replication and packaging of poliovirus replicon RNA. Journal of Virology 73:427-435.
55. Liu Y, Wang CL, Mueller S, Paul AV, Wimmer E, Jiang P. 2010. Direct Interaction between Two Viral Proteins, the Nonstructural Protein 2C(ATPase) and the Capsid Protein VP3, Is Required for Enterovirus Morphogenesis. Plos Pathogens 6.
56. Malathi K, Dong B, Gale M, Jr., Silverman RH. 2007. Small self-RNA generated by RNase L amplifies antiviral innate immunity. Nature 448:816-9.
57. Han JQ, Townsend HL, Jha BK, Paranjape JM, Silverman RH, Barton DJ. 2007. A phylogenetically conserved RNA structure in the poliovirus open reading frame inhibits the antiviral endoribonuclease RNase L. J Virol 81:5561-72.
58. Jiang P, Liu Y, Ma HC, Paul AV, Wimmer E. 2014. Picornavirus morphogenesis. Microbiol Mol Biol Rev 78:418-37.
59. Chen YH, Du WL, Hagemeijer MC, Takvorian PM, Pau C, Cali A, Brantner CA, Stempinski ES, Connelly PS, Ma HC, Jiang P, Wimmer E, Altan-Bonnet G, Altan-Bonnet N. 2015. Phosphatidylserine Vesicles Enable Efficient En Bloc Transmission of Enteroviruses. Cell 160:619-630.
60. Bird SW, Maynard ND, Covert MW, Kirkegaard K. 2014. Nonlytic viral spread enhanced by autophagy components. Proc Natl Acad Sci U S A 111:13081-6.
61. Taylor MP, Burgon TB, Kirkegaard K, Jackson WT. 2009. Role of microtubules in extracellular release of poliovirus. J Virol 83:6599-609.
62. Schwartz M, Chen J, Lee WM, Janda M, Ahlquist P. 2004. Alternate, virus-induced membrane rearrangements support positive-strand RNA virus genome replication. Proc Natl Acad Sci U S A 101:11263-8.