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Biogenesis and architecture of arterivirus replication organelles

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ABSTRACT

All eukaryotic positive-stranded RNA (+RNA) viruses appropriate host cell membranes and transform them into replication organelles, specialized micro-environments that are thought to support viral RNA synthesis. Arteriviruses (order *Nidovirales)* belong to the subset of +RNA viruses that induce double-membrane vesicles (DMVs), similar to the structures induced by e.g. coronaviruses, picornaviruses and hepatitis C virus. In the last years, electron tomography has revealed substantial differences between the structures induced by these different virus groups. Arterivirus-induced DMVs appear to be closed compartments that are continuous with endoplasmic reticulum membranes, thus forming an extensive reticulovesicular network (RVN) of intriguing complexity. This RVN is remarkably similar to that described for the distantly related coronaviruses (also order *Nidovirales*) and sets them apart from other DMV-inducing viruses analysed to date. We review here the current knowledge and open questions on arterivirus replication organelles and discuss them in the light of the latest studies on other DMV-inducing viruses, particularly coronaviruses. Using the equine arteritis virus (EAV) model system and electron tomography, we present new data regarding the biogenesis of arterivirus-induced DMVs and uncover numerous putative intermediates in DMV formation. We generated cell lines that can be induced to express specific EAV replicase proteins and showed that DMVs induced by the transmembrane proteins nsp2 and nsp3 form an RVN and are comparable in topology and architecture to those formed during viral infection. Co-expression of the third EAV transmembrane protein (nsp5), expressed as part of a self-cleaving polypeptide that mimics viral polyprotein processing in infected cells, led to the formation of DMVs whose size was more homogenous and closer to what is observed upon EAV infection, suggesting a regulatory role for nsp5 in modulating membrane curvature and DMV formation.

Contents

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1. Membrane modifications to accommodate +RNA virus replication

All positive-stranded RNA (+RNA) viruses of eukaryotes replicate their genome in the cytoplasm of the host cell using a common strategy characterized by the modification of host membranes into organelle-like structures that, for many +RNA viruses, have been directly implicated in viral RNA synthesis (reviewed in Miller and Krijnse-Locker, 2008; Paul and Bartenschlager, 2013; Romero-Brey and Bartenschlager, 2014). Although these virus-induced membrane modifications, often referred to as viral replication organelles or replication structures, have been known for decades, their exact purpose remains enigmatic. Three major advantages of associating viral RNA synthesis with dedicated membranes have been proposed. Firstly, confining viral RNA synthesis in designated compartments could generate optimally suited micro-environments by concentrating the viral proteins and precursors necessary for the process. Furthermore, the anchoring of viral replication complexes to membranes creates a planar geometry for diffusion of metabolites and macromolecules, which can also increase the efficacy of the enzymatic processes. Secondly, compartmentalization could provide a means to spatially separate and coordinate the different stages of the infectious cycle, such as genome translation, replication and packaging. Finally, during viral RNA synthesis several intermediate nucleic acid species such as double-stranded RNA (dsRNA) and 5′ triphosphate-containing RNAs are presumed to be formed, which are potent activators of the innate immune response (Bowie and Unterholzner, 2008; Gurtler and Bowie, 2013). Insulation of these intermediates could therefore prevent or delay detection by the defence systems of the host cell (Neufeldt et al., 2016). Clearly, these proposed functions are not mutually exclusive and, likely, +RNA viruses take advantage of multiple benefits associated with the compartmentalization of their replicative process.

Both viral and cellular factors are thought to be important for the biogenesis of +RNA viral replication organelles, although the specific players and processes involved remain in most cases poorly understood. Multiple +RNA viruses encode non-structural proteins (nsps) that have proven or predicted transmembrane domains and, for some of them, a combination of membrane-associated viral nsps has been shown to be necessary and sufficient for the induction of membrane modifications resembling those found in infected cells (Angelini et al., 2013; Egger et al., 2002; Romero-Brey et al., 2015; Romero-Brey et al., 2012; Salonen et al., 2003; Schwartz et al., 2002; Snijder et al., 2001; Suhy et al., 2000). Besides these viral proteins, a wide variety of host factors have been implicated in the formation and functioning of these structures (reviewed in Belov and van Kuppeveld, 2012; Nagy and Pogany, 2012). Their roles range from the recruitment of viral replication proteins to the induction of membrane modifications. Not surprisingly, the list of identified host factors includes proteins involved in lipid metabolism (e.g. phosphatidylinositol-4-phosphate (PI4P) kinases, fatty acid synthase), specific lipids (e.g. PI4P, sterols), and membrane-shaping proteins (e.g. reticulons and endosomal sorting complexes required for transport (ESCRT) proteins).

A large number of +RNA virus replication organelles have been characterized by electron microscopy (EM) over the last decades. It has become apparent that, despite the large evolutionary distances between these viruses and the different cellular organelles they manipulate, all +RNA viruses seem to induce one of two basic morphotypes of membrane modifications: invaginations or double-membrane vesicles. In the last decade, the study of the architecture of viral replication organelles has been stimulated further by the increasing use of electron tomography (ET). ET enables the three-dimensional (3D) characterization of biological specimens at nanometer resolution by computationally combining images collected at different tilt angles in a transmission electron microscope (Barcena and Koster, 2009). The first +RNA virus replication organelles characterized by ET were those induced by the nodavirus Flock House virus (FHV) (Kopek et al., 2007). FHV induces invaginations in the outer mitochondrial membrane and therefore belong to the first morphotype of +RNA virus replication organelles. Using immuno electron microscopy (IEM) to detect BrUTP incorporated into viral RNA, the interior of these spherules was shown to contain newly-synthesized viral RNA that is thought to be exported to the cytosol through a neck-like channel of ~10 nm in diameter, which could be clearly visualized in the 3D reconstruction. Since this first study, the membrane modifications induced by, for example, coronaviruses (Knoops et al., 2008; Maier et al., 2013a), arteriviruses (Knoops et al., 2012), flaviviruses (Gillespie et al., 2010; Junjhon et al., 2014; Miorin et al., 2013; Offerdahl et al., 2012; Welsch et al., 2009), hepaciviruses (Romero-Brey and Bartenschlager, 2014), togaviruses (Fontana et al., 2010), picornaviruses (Belov and van Kuppeveld, 2012; Limpens et al., 2011) and tombusviruses (Cao et al., 2015) have been characterized using ET.

The flavivirus dengue virus (DENV) provides another well-characterized example of virus-induced invaginations of cellular membranes (Welsch et al., 2009) that, in this case, occur at the rough endoplasmic reticulum (ER). Despite the different membrane donor organelle, the DENV-induced vesicles share several characteristics with those generated during FHV infection. DENV invaginations are also open to the cytosol through a neck-like connection (~10 nm in diameter) and contain dsRNA. Apart from these invaginations, DENV also induces the formation of so-called convoluted membranes, which are continuous with the vesicles through ER membranes and are speculated to be a reservoir of proteins and lipids used for DENV replication (Welsch et al., 2009). The formation of ER invaginations that retain a connection to the cytosol has also been shown for other flaviviruses, like tick-borne encephalitis virus (Miorin et al., 2013), West Nile virus (Gillespie et al., 2010), and Langat virus (Offerdahl et al., 2012).

The formation of cytopathic vacuoles (CPVs), which are modified endosomes and lysosomes of about 600–2000 nm that accommodate invaginations on their membranes, is a hallmark for togavirus infection. ET applied to cells infected with rubella virus (RUBV) showed that the CPVs also contained interconnected small vesicles, vacuoles and stacked membranes (Fontana et al., 2010). Furthermore, RUBV recruits rough ER, mitochondria, and Golgi membranes close to the CPVs, presumably to make use of the resources present