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New insights on the role of paired membrane structures in coronavirus replication

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ABSTRACT

The replication of coronaviruses, as in other positive-strand RNA viruses, is closely tied to the formation of membrane-bound replicative organelles inside infected cells. The proteins responsible for rearranging cellular membranes to form the organelles are conserved not just among the *Coronaviridae* family members, but across the order *Nidovirales*. Taken together, these observations suggest that the coronavirus replicative organelle plays an important role in viral replication, perhaps facilitating the production or protection of viral RNA. However, the exact nature of this role, and the specific contexts under which it is important have not been fully elucidated. Here, we collect and interpret the recent experimental evidence about the role and importance of membrane-bound organelles in coronavirus replication.

1. Paired membranes associated with viral RNA

All positive-stranded RNA viruses (+RNA) that infect eukaryotes are believed to form membrane-bound replicative organelles, though this remains to be formally tested for several families of viruses (Neuman et al., 2014). One of the most widespread membrane modifications caused by +RNA viruses results in the formation of paired membranes, i.e. two closely apposed lipid bilayers. A growing body of evidence, presented in Table 1 indicates that the paired membrane structures are induced by the expression of viral proteins – most typically by parts of the viral replicase. Table 1 lists the virus lineages for which there is evidence that some form of virus-induced paired-membrane structure is associated with viral replication. The wide distribution of membrane pairing in +RNA viruses suggests that this is an effective strategy for successfully producing new viruses, and that membrane pairing may somehow increase the competitive fitness of these viruses.

While we can speculate that +RNA viruses may gain a fitness advantage by replicating on the membranes of dedicated viral organelles, this has been difficult to test experimentally. However, there are several lines of experimental and genetic evidence that suggest that RNA synthesis is tied to the formation of replicative organelles. Viral RNA accumulates in the coronavirus organelles, suggesting that the organelles may be a site of RNA synthesis (Knoops et al., 2008, 2012; Gosert et al., 2002; Hagemeijer et al., 2012). Furthermore, viral organelles are not formed when RNA synthesis is stopped (Stokes et al., 2010; Verheije et al., 2008). While it is clear that RNA synthesis is linked with the organelles, it has proved difficult to directly test whether or to what extent the process of organelle formation is necessary for the process of RNA synthesis, because of the practical difficulty in separating the two processes in an experimental setting.

2. Structure of the organelles

Electron tomography studies have revealed that the replicative organelles of different nidoviruses are drawn from a repertoire of paired-membrane structures, including (paired) convoluted membranes, pouch-like double-membrane spherules, long paired membranes and double-membrane vesicles (Knoops et al., 2008, 2012; Maier et al., 2013a), though studies of the more recently discovered mesoniviruses and roniviruses remain poorly characterized (Zirkel et al., 2011; Spann et al., 1995). The nidoviruses that have been studied to date all induced a combination

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Table 1
Evidence paired membrane structures in +RNA virus infection.

Order	Family	Host[a]	Origin[b]	Type[c]	Proteins[d]	References
Nidovirales	*Arteriviridae*	A	ER	V,Z	nsp2, 3	Snijder et al. (2001), Posthuma et al. (2008), Wood et al. (1970), Pedersen et al. (1999), Pol et al. (1997)
	Coronaviridae	A	ER	V,Z,S,C	nsp3+4+6	Knoops et al. (2008), Maier et al. (2013a), Angelini et al. (2013b)
	Mesoniviridae	A	ER	V?	*nr*[e]	Thuy et al. (2013)
Picornavirales	*Picornaviridae*	A	ER	V	2BC, 3A	Hsu et al. (2010), Richards et al. (2014), Teterina et al. (1997), Suhy et al. (2000)
	Secoviridae	P	ER	V?	*nr*	Roberts and Harrison (1970)
Tymovirales	*Betaflexiviridae*	P	ER	V	*nr*	Edwardson and Christie (1978), Rudzinska-Langwald (1990)
	Tymoviridae	P	Cp, Mt	V	*nr*	Lesemann (1977)
Unclassified	*Astroviridae*	A	ER	V	nsp1a	Guix et al. (2004), Mendez et al. (2007)
	Bromoviridae	P	ER	Z,S	1a+2a^{pol}	Moreira et al. (2010), Schwartz et al. (2002), Schwartz et al. (2004)
	Closteroviridae	P	*nr*	V	*nr*	Medina et al. (1998)
	Flaviviridae	A	ER	V,S,C	NS4A+4B	Gillespie et al. (2010), Welsch et al. (2009), Romero-Brey et al. (2012), Miller et al. (2007), Roosendaal et al. (2006), Egger et al. (2002)
	Nodaviridae	A	Mito	S	pA+RNA	Kopek et al. (2010), Kopek et al. (2007)
	Togaviridae	A	Ly, ER	V,S?	P123	Magliano et al. (1998), Fontana et al. (2010), Salonen et al. (2003)
	Tombusviridae	P	Px	S	*nr*	Barajas et al. (2009)

[a] Animals (A) or Plants (P).
[b] Membranes from the endoplasmic reticulum (ER), chloroplast (Cp), mitochondria (Mt), lysosome (Ly) or peroxisome (Px).
[c] Paired membranes in the form of double-membrane vesicles (V), zippered ER (Z), open-necked spherules (S), or convoluted membranes (C).
[d] Proteins implicated in membrane rearrangements.
[e] Not reported (*nr*).

of paired-membrane features, the precise function of which have not been elucidated. It is also important to point out that to some extent the distinctions between paired membrane structures are open to interpretation and may only be fully accessible when three-dimensional imaging methods are used. A catalog of the virus-induced membrane structures that have been observed for each coronavirus is shown at right in Fig. 1.

The common element in nidovirus-like membrane rearrangement is that the membranes are paired, usually maintaining a consistent-sized gap between the two membranes (reviewed here, Angelini et al., 2014). Since protein-induced membrane

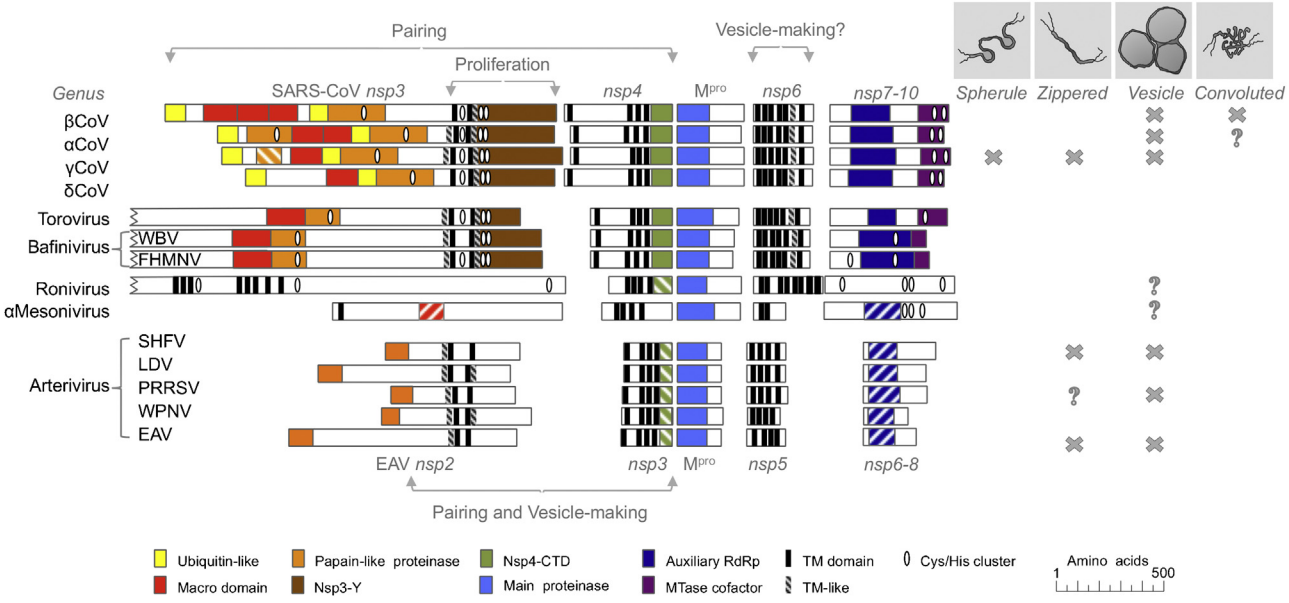

Fig. 1. Conservation and functional organization of the carboxyl-terminal region of nidovirus polyprotein 1a. Domains that are homologous at the amino acid level are shown at left in solid colors. More distantly related potential homologs identified by genome position and comparison of predicted secondary structures are marked with stripes. Positions of transmembrane regions (black bars) and hydrophobic non-transmembrane regions (striped bars) were predicted by TMHMM 2.0 (Krogh et al., 2001) and amended to reflect known topologies (Kanjanahaluethai et al., 2007; Oostra et al., 2007, 2008) wherever possible. Clusters of conserved cysteine and histidine residues that may bind metal ions are marked with white ovals. A jagged line denotes the uncertain position of the amino terminus. Regions that induce membrane pairing, proliferation or vesiculation in betacoronavirus SARS-CoV and arterivirus EAV are shown above and below the domain annotation, respectively, and all annotations come from the references listed for Table 1. Double-membrane organelles observed (x) or uncertainly observed (?) in infected cells are marked at right. Virus names are abbreviated as follows: white bream virus (WBV), fathead minnow nidovirus (FHMNV), equine arteritis virus (EAV), lactate dehydrogenase elevating virus (LDV), porcine reproductive and respiratory syndrome virus (PRRSV), simian hemorrhagic fever virus (SHFV) and wobbly possum nidovirus (WPNV).