Investigating the role of viral integral membrane proteins in promoting the assembly of nepovirus and comovirus replication factories

Hélène Sanfaçon*
Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada

Formation of plant virus membrane-associated replication factories requires the association of viral replication proteins and viral RNA with intracellular membranes, the recruitment of host factors and the modification of membranes to form novel structures that house the replication complex. Many viruses encode integral membrane proteins that act as anchors for the replication complex. These hydrophobic proteins contain transmembrane domains and/or amphipathic helices that associate with the membrane and modify its structure. The comovirus Co-Pro and NTP-binding (NTB, putative helicase) proteins and the cognate nepovirus X2 and NTB proteins are among the best characterized plant virus integral membrane replication proteins and are functionally related to the picornavirus 2B, 2C, and 3A membrane proteins. The identification of membrane association domains and analysis of the membrane topology of these proteins is discussed. The evidence suggesting that these proteins have the ability to induce membrane proliferation, alter the structure and integrity of intracellular membranes, and modulate the induction of symptoms in infected plants is also reviewed. Finally, areas of research that need further investigation are highlighted.

Keywords: integral membrane proteins, viral replication complexes, intracellular membranes, protein–membrane interactions, secoviridae, picornavirales, plant–virus interactions, membrane remodeling

CHARACTERIZATION OF COMOVIRUS AND NEPOVIRUS REPPLICATION COMPLEXES AND IDENTIFICATION OF PUTATIVE MEMBRANE ANCHORS

Positive-strand RNA viruses replicate in large complexes that are associated with host intracellular membranes (Salonen et al., 2005; Sanfacon, 2005; Miller and Krijnse-Locker, 2008; den Boon and Ablquist, 2010; Laliberte and Sanfacon, 2010; Nagy and Pogany, 2012). Some viruses require host membrane proteins to target their replication proteins to the membranes (Yamakana et al., 2000). However, many viruses encode proteins that interact with membranes directly and modify their intrinsic structure. These proteins have membrane association domains and contain protein–protein and/or protein–RNA interaction domains that allow them to recruit the viral RNA, other viral replication proteins, or host factors to the membranes. Well-characterized plant virus membrane proteins include the tombusvirus 33–36 kDa proteins, bromovirus 1a protein, potyvirus 6K protein, and tymovirus 140 kDa protein (Schaad et al., 1997; den Boon et al., 2002; Weber-Lotfi et al., 2002; Prod’Homme et al., 2003; Turner et al., 2004).

The family Secoviridae (order Picornavirales) includes the genera Comovirus, Fabavirus, Nepovirus, Squashvirus, Wavikavirus, Cher- avirus, Sadmovirus, and Torraviridae (Sanfacon et al., 2011). The best characterized members of the family are Cowpea mosaic virus (CPMV, comovirus), Grapevine fanleaf virus (GFLV, nepovirus), and Tomato ringspot virus (ToRSV, nepovirus; Pouwels et al., 2002a; Sanfacon et al., 2006). These viruses use a polyprotein strategy to express their proteins and have a replication block consisting of a nucleotide-binding protein (NTB), a genome-linked protein (VPg), a protease (Pro), and an RNA-dependent RNA polymerase (Pol, Figure 1C). Although they share these properties with picornaviruses (including the well-characterized poliovirus), nepo- and comoviruses differ in that they have bipartite genomes. The RNA1-encoded polyprotein contains all protein domains necessary for replication and RNA1 can replicate independently of RNA2 (Vis et al., 1988; Viry et al., 1993).

Plant cells infected with como- and nepoviruses are characterized by the presence of numerous membranous vesicles, which are derived from the endoplasmic reticulum (ER; Carette et al., 2000; Ritzenthaler et al., 2002; Han and Sanfacon, 2003). In CPMV-infected cells, vesicles first appear throughout the cytoplasm, but later coalesce in a large perinuclear structure (Carette et al., 2002a). Actin microfilaments are probably involved in this process (Carette et al., 2002a). Perinuclear membrane aggregates are also observed in ToRSV- and GFLV-infected cells (Ritzenthaler et al., 2002; Han and Sanfacon, 2003). Viral replication proteins, de novo RNA synthesis, and dsRNA intermediates co-localize with these structures, indicating that they are the site of viral replication (de Zetzen et al., 1974; Carette et al., 2000, 2002a; Ritzenthaler et al., 2002; Han and Sanfacon, 2003).

Figure 1A). These vesicles are similar to those observed in early
The bar indicates 200 nm. This model is based on electron tomography observations from Belov et al. (2012) and Limpens et al. (2011). (2) In cells infected with many plant and double-membrane vesicles are predominant in picornavirus-infected cells. Subsequent membrane fusion to allow its circularization. Late in infection, vesicles after the internal collapse of the single-membrane vesicle and single-membrane vesicles may bud out and form double-membrane membrane allowing the budding of tubular structures. Other viral integral membrane proteins (red ovals) induce positive curvature of the replication complexes. (1) In cells infected with poliovirus or coxsackie B3 virus, viral integral membrane proteins (red ovals) interact with the viral membrane proteins. Host factors and viral RNA (not shown) associate with membrane proteins allows budding of tubular structures from the outside of the single-membrane structures (fan);-immuno-precipitated by antibodies against viral replication proteins, is consistent with this model (Bienz et al., 1994; Carette et al., 2000). In contrast, membrane structures induced by many plant viruses (including bromo-, tombus-, and tymoviruses) are formed by membrane invagination and require negative membrane curvature. These replication complexes are sheltered inside spherules that are connected to the cytoplasm by a neck (Figure 1B, model 2). Of the replication proteins encoded by como- or nepovirus RNA1, two contain obvious hydrophobic regions: the comovirus Co-Pro and NTB proteins and the cognate nepovirus X2 and NTB proteins (Figure 1D). In infected cells, mature proteins are present either as a mature protein or within various intermediates (NTB–VPg, NTB–VPg–Pro, and NTB–VPg–Pro–Pol; Wellink et al., 1986). In contrast, processing at the nepovirus X2–NTB cleavage site is inefficient in vitro leading to the accumulation of X2–NTB and X2–NTB–VPg in addition to X2 and NTB (Wang and Sanfacon, 2000; Wetzl et al., 2010). In ToRSV-infected cells, NTB, NTB–VPg, and X2–NTB–VPg are tightly associated with ER membranes active in viral replication (Han and Sanfacon, 2003). In contrast, only a sub-population of a polyprotein containing the VPg, Pro, and Pol domains (VPg–Pro–Pol) is associated with replication-competent membranes and this association is peripheral, suggesting that it requires an interaction between stages of infection by poliovirus and consackie B3 virus (both picornaviruses).
VPg–Pro–Pol and a membrane protein (Chisholm et al., 2007). Similarly, only a fraction of VPg–Pro–Pol is membrane-bound in CPMV-infected cells (Dorssers et al., 1984). When expressed individually, the ToRSV X2, NTB and NTB–VPg and the CPMV Co-Pro and NTB–VPg associate with ER membranes, while proteins containing the ToRSV or CPMV VPg, Pro, and Pol domains remain in the soluble cytoplasmic fraction (Carette et al., 2002b; Zhang et al., 2005; Zhang and Sanfacon, 2006; Chisholm et al., 2007). Thus, the CPMV Co-Pro and NTB and ToRSV X2 and NTB and/or intermediate polyproteins containing these protein domains are likely to act as membrane anchors for the replication complex.

The nucleotide-binding motif of the nepo- and comovirus NTB is related to that of the poliovirus 2C protein (Figure 1C). The nepo- and comovirus NTB also contain a hydrophobic C-terminal domain, which is absent in 2C (Figure 1D). The poliovirus 3A protein (immediately downstream of 2C in the polyprotein) has a hydrophobic domain that corresponds to the C-terminal region of the nepo- and comovirus NTB, although polyproteins containing both 2C and 3A are not detected in infected cells (Figure 1C; Cameron et al., 2010). The ToRSV X2, and CPMV Co-Pro are highly hydrophobic and share a signature sequence (X-X-X-W-X-X-L-X-X-X-E; Rott et al., 1995), which is also found in the cognate proteins of nepo-, como-, faba-, and cheraviruses (Sanfacon et al., 2011). Co-Pro is a protease co-factor that slows the processing of the CPMV RNA1 polyprotein (Peters et al., 1992). However, there is no experimental evidence that X2 regulates the nepovirus protease activity (Wang and Sanfacon, 2000; Wetzel et al., 2008). Thus, the conserved motif may be important for another common activity of Co-Pro and X2. The poliovirus 2B protein is located immediately upstream of 2C (Figure 1D) but does not share sequence motifs with X2 and Co-Pro, other than a general hydrophobicity.

**MEMBRANE MODIFICATIONS AND SYMPTOMS INDUCED BY THE COMOVIRUS Co-Pro AND NTB–VPg**

When overexpressed from a viral vector, the CPMV NTB–VPg or Co-Pro induces the formation of small ER-derived perinuclear bodies (Carette et al., 2002b). Proliferation of the cortical ER is or Co-Pro induces the formation of small ER-derived perinuclear bodies (Carette et al., 2002b). Proliferation of the cortical ER is reminiscent of that observed in early stages of natural poliovirus infections (Suhy et al., 2000). Protein–protein interactions among these proteins are well-documented (Teterina et al., 2002). The C-terminal hydrophobic region of the ToRSV NTB contains a highly hydrophobic α-helix, which traverses the membrane. The VPg domain of NTB–VPg is translocated in the membrane lumen (topology 1, Figure 2C), allowing the recognition of a naturally occurring N-glycosylation site (Wang et al., 2004; Zhang et al., 2005). The luminal orientation of the C-terminal region of NTB–VPg was confirmed by proteinase K protection assays using these proteins in electron-dense bodies, which are probable sites of protein aggregation, may help reduce their toxicity (Carette et al., 2002b). Comparison of the symptomatology induced by chimeric constructs of two isolates of bean pod mosaic virus (another comovirus) also points to Co-Pro and NTB as symptom severity determinants (Guz and Ghahrial, 2005). Chimeric constructs containing Co-Pro or NTB from the severe isolate induce increased symptomatology and accumulate to higher level than the mild isolate. Co-Pro and NTB may regulate the rate of virus replication, in agreement with their proposed role in replication complex assembly. Although the severe symptoms may be correlated with symptom induction, viral products, possibly triggering plant defense responses, it may be a direct consequence of the membrane alterations induced by NTB and Co-Pro. Poliovirus 2B and 3A induce apoptosis when overexpressed (Madan et al., 2008). At least for 2B, the induction of apoptosis was correlated with its viroporin activity, which affects the integrity of various membranes, including mitochondrial membranes (Madan et al., 2008, 2010). Although a sub-population of the CPMV NTB–VPg targets plastosomal membranes (Carette et al., 2002b), there is no experimental evidence that mitochondria are targeted. Further studies will be necessary to investigate possible correlations between membrane alterations and symptomatology induced by the comovirus NTB and Co-Pro proteins and to determine whether the nepovirus X2 and NTB proteins can alter membrane structures and induce symptoms.

**EVIDENCE FOR OLIGOMERIZATION AND VIROPORIN ACTIVITY**

Membrane association of integral membrane proteins can be directed by transmembrane α-helices, which are highly hydrophobic, or by amphipathic α-helices. Amphipathic helices initially insert parallel to the membranes with their hydrophobic face inserted in the lipid bilayer (Figures 2A,B). Oligomerization of amphipathic helices can lead to the formation of aqueous pores whereby the hydrophobic faces of the helices orient toward the pore and the hydrophobic faces interact within the membrane environment (Gonzalez and Carrasco, 2005; Figure 2B). Hydrophobic intra- and intermolecular interactions among amphipathic and adjacent hydrophobic helices can stabilize the oligomers (Figure 2B), as suggested for the poliovirus 2B protein (Agirre et al., 2002; Martinez-Gil et al., 2011).

The hydrophobic C-terminal domain and a predicted N-terminal amphipathic helix of the ToRSV NTB protein (Figure 1D) are each sufficient to target GFP fusion proteins to ER membranes in plant cells or to direct the insertion of NTB or NTB–VPg into canine microsomal membranes in vitro (Wang et al., 2004; Zhang et al., 2005). These domains are conserved in the sequence of NTB from other nepo- and comoviruses (Figure 1D). The C-terminal hydrophobic region of the ToRSV NTB contains a highly hydrophobic α-helix, which traverses the membrane. The VPg domain of NTB–VPg is translocated in the membrane lumen (topology 1, Figure 2C), allowing the recognition of a naturally occurring N-glycosylation site (Wang et al., 2004; Zhang et al., 2005). The luminal orientation of the C-terminal region of NTB–VPg was confirmed by proteinase K protection assays using...
membrane-fractions of ToRSV-infected cells (Han and Sanfacon, 2003). However, these results do not exclude the possibility that a sub-population of the protein adopt an alternate topology. In vitro, a second weakly predicted transmembrane α-helix traverses the membranes when the first transmembrane helix is deleted (Wang et al., 2004). In an alternate topology (topology 2, Figure 2C), the NTB C-terminal hydrophilic region traverses the membrane twice allowing a cytosolic orientation of the VPg. Experiments are required to determine whether this alternate topology exists in infected cells. Alternative topologies for NTB–VPg could regulate the presentation of the VPg to the cytoplasmic face of the membrane where protein–protein interactions and viral replication take place.

The N-terminus of NTB is translocated in the membrane lumen, suggesting oligomerization of the amphipathic helix and pore formation (Zhang et al., 2005; Figure 2B). The open circle represents the VPg domain and the red oval indicates the conserved NTB motif. (B) Model for the induction of positive membrane curvature by hydrophobic interactions of membrane protein oligomers, shown for NTB–VPg. On the left, blue arrows represent possible hydrophobic interactions. These interactions (shown by broken blue lines on the right) would induce positive membrane curvature. Similar hydrophobic interactions are predicted to occur in X2 or X2-NTB–VPg oligomers (not shown).

FIGURE 2 | Topology model for ToRSV membrane replication proteins. (A) Model for the parallel insertion of an amphipathic helix. The hydrophobic side of the helix (blue) inserts in one leaflet of the lipid bilayer while the polar charged hydrophilic side of the helix (yellow) is exposed to the cytosolic face of the membrane. This insertion displaces the lipid headgroups causing the acyl chain to orient and inducing positive membrane curvature. (B) Model for the oligomerization of amphipathic helices and formation of an aqueous pore. In the top panel, an amphipathic helix is inserted parallel to the lipid bilayer (horizontal gray lines) of the membrane (left). Formation of an aqueous pore (double-ended red arrow) requires oligomerization of four or six amphipathic helices (middle). In the aqueous pore, the hydrophobic side of the helix (yellow) is exposed toward the pore while its hydrophilic side (blue) is orientated toward the membrane lipid bilayer. A simplified representation of the pore shows only two molecules to better visualize each side of the amphipathic helixes relative to the pore (right). In the bottom panel, a membrane protein consisting of an amphipathic helix and a hydrophobic helix (blue) is shown. After initial membrane insertion of the monomer with the amphipathic helix parallel to the membrane (left), an aqueous pore is formed by oligomerization of the amphipathic helixes (middle). The hydrophobic helix of each molecule is located on the outside of the pore alongside the amphipathic helixes (model shown for a hexamer). Hydrophobic interactions between the hydrophobic side of the amphipathic helixes and the hydrophobic helixes stabilize pore formation. A simplified representation of the pore shows only two molecules (right). (Continued)
protein oligomerization occurs through hydrophobic interactions (Zhang and Sanfacon, 2006). A topology model of X2 oligomers implies the formation of an aqueous pore by oligomerization of the amphipathic helix (Figure 2C). However, in vivo evidence in support of this model is still lacking. Due to its highly hydrophobic nature, it has not been possible to produce antibodies against X2. Thus, although the presence of mature X2 in ToRSV-infected cells is likely, it could not be confirmed. However, polyproteins corresponding to the expected molecular mass for X2–NTB–VPg were detected with anti-NTB and anti-VPg antibodies (Han and Sanfacon, 2003). Efforts are underway to develop ToRSV infectious clones, which may allow the insertion of epitope tags in X2 to confirm its presence in ToRSV-infected cells and examine its topology in vivo (Chisholm and Sanfacon, unpublished). Although insertion of hydrophilic epitope tags into hydrophobic membrane proteins can hinder their function, a recent study described tolled insertion sites in poliovirus membrane proteins (Teterina et al., 2011a).

The topology models for X2 and NTB–VPg pose some problems when applied to the X2–NTB–VPg polyprotein. The cytosolic orientation of the N-terminus of NTB in apparent conflict with the luminal orientation of the N-terminus of NTB. However, the presence of two strong transmembrane domains in the X2 domain of X2–NTB–VPg may prevent the membrane translocation of the NTB amphipathic helix, forcing it to insert parallel to the membranes (Figure 2C). Thus, processing at the X2–NTB cleavage site may influence the orientation of the NTB amphipathic helix and alter the ability of NTB and/or X2 to modify intracellular membranes. The impact of proteolytic cleavage on membrane topology was demonstrated for the poliovirus 3A and 3AB (Fujita et al., 2007). Using a fluorescence quenching method, 3AB was shown to adopt a single topology, in which the hydrophobic domain is parallel to the membrane. In contrast, 3A adopts two possible orientations, one of which traverses the membrane. It was suggested that the hydrophilic VPg domain prevents the membrane translocation of the 3A hydrophobic domain in 3AB. Regulated cleavage of the poliovirus 2BC also impacts its membrane-modification activities. Although 2B, 2C, and 2BC can target to membranes, only 2BC induces a proliferation of membraneous vesicles (Suhy et al., 2000). On the other hand, poliovirus mutants with decreased processing efficiency at the 2BC cleavage site have reduced membrane permeabilization activity, suggesting that the release of mature 2B from 2BC is essential for its viroporin function (van Kuppevelt et al., 1996).

INTERACTION OF VIRAL MEMBRANE PROTEINS WITH HOST FACTORS: TOWARD A MECHANISM FOR MEMBRANE MODIFICATION

The experimental evidence points to a role for como- and nepovirus membrane replication proteins in altering host membranes and assembling the replication complexes. Positive membrane curvature can be induced by parallel insertion of amphipathic helices (Figure 2A) or by intra- and inter-molecular hydrophobic interactions among membrane protein oligomers (as shown for NTB–VPg, Figure 2D, McMahon and Gallop, 2005).

Host factors are also likely to play an important role. The secretory pathway is hijacked by poliovirus to help the formation of membranous vesicles, resulting in an inhibition of host protein transport (Hsu et al., 2010). The 2B and 3A proteins inhibit the secretory pathway (Doedens and Kirkegaard, 1995). 3A interacts with several components of the secretory pathway, including ABCL, a Golgi adaptor protein (Greninger et al., 2012; Sasaki et al., 2012) and GDF1, a guanine nucleotide exchange factor that activates Arf1, a cellular GTPase and regulator of the secretory pathway (Wessels et al., 2006; Belov et al., 2008; Teterina et al., 2011b). Arf1 is also the known target of brefeldin A, an inhibitor of the secretory pathway that blocks poliovirus infection (Furuzan et al., 1992; Maynell et al., 1992). The 3A–GDF1 and 3A–ABCL interactions may assist in the recruitment of P1KIII, an enzyme involved in phospholipid synthesis, to the replication complex (Hsu et al., 2010; Greninger et al., 2012; Sasaki et al., 2012). P1KIII would alter the membrane lipid composition, possibly affecting the membrane curvature and facilitating the formation of virus factories. However, the sensitivity of picornaviruses to brefeldin A varies greatly and the GDF1–3A interaction is not conserved for all picornaviruses, suggesting that the interaction between viruses and the host secretory pathway varies.

How do these findings apply to como- and nepoviruses? Repli- cation of CPMV and GFLV is hindered by cerulin (Carette et al., 2000; Ritzenthaler et al., 2002), an inhibitor of type II fatty acid synthase, suggesting that de novo phospholipid synthesis is required for membrane proliferation, possibly involving changes in membrane lipid composition. GFLV and CPMV replication is inhibited by brefeldin A (Pouwels et al., 2002b; Ritzenthaler et al., 2002). However, the interaction of nepo- and comoviruses with the secretory pathway is not well understood and their ability to block protein secretion has not been investigated. Two SNARE-like proteins from Arabidopsis thaliana were shown to interact with the CPMV NTB–VPg (Carette et al., 2002c). Although their function is not known, they may regulate membrane fusion and vesicle formation. Identification of additional interaction partners of the nepo- and comovirus membrane proteins will be essential to better understand membrane remodeling directed by these proteins.

ACKNOWLEDGMENTS

This work was supported in part by an NSERC discovery grant. I wish to thank Joan Chisholm for critical reading of the manuscript and Andrew Wieczorek for taking the EM picture (shown in Figure 2A), while he was in my lab.

REFERENCES

Agirre, A., Barco, A., Carrasco, L., and Naya, J. L. (2002). Viroporin-mediated membrane permeabilization. Pure formation by

nonstructural poliovirus 2B protein. J. Biol. Chem. 277, 40434–40441.

Belov, G. A., Feng, Q., Nikovics, K., Jack- son, C. L., and Ehrenfeld, E. (2008). A critical role of a cellular membrane traffic protein in poliovirus RNA replication. PLoS Pathog. 4, e1000216. doi:10.1371/journal.ppat.1000216

Belov, G. A., Nair, V., Hansen, B. T., Højt, F. H., Fischer, E. R., and Ehrenfeld, E. (2012). Complex dynamic development of poliovirus membrane replication complexes. J. Virol. 86, 302–312.
Biemesderfer, C., Egger, D., and Pfister, T. (1994). Characteristics of the poliovirus replication complex. Arch. Virol. Suppl. 6, 147–157.

Cameron, C. E., Oh, H. S., and Mountsi, I. M. (2010). Expandability of 3′-protease 2B in the poliovirus polyprotein. Future Microbiol. 5, 487–498.

Carette, J. E., Stuiver, M., Van Lent, J., Wellink, J., and Van Kammen, A. (2000a). Coalescence of the sites of cowpea mosaic virus RNA replication into a cytoplasmic structure. J. Virol. 74, 6235–6243.

Carette, J. E., Van Lent, J., MacFarlane, S. A., Wellink, J., and Van Kammen, A. (2000b). Coalescence of plant proteins that interact with cowpea mosaic virus 3′R-protein targeting to the cytosol and vesicular system. J. Gen. Virol. 81, 885–893.

Chahal, N., Jiang, G., Wang, A., and Sanfaçon, H. (2007). Periphedral association of a polypeptide precursor form of the RNA-dependent RNA polymerase of tomato ringpot virus with the membrane-bound viral replication complex. Virolgy 368, 135–144.

Della Sanave, G., Gruizki, H., and Leut, J. O. (2005). How do helix–helix interactions help to define the limits of membrane proteins? Perspectives from the study of homo-oligomeric lipid bilayers. Proteins Sci. 12, 647–655.

den Boon, J. A., Chen, J., and AlQUIp, P. (2001). Identification of sequences in Brome mosaic virus replicase protein 1a that modulate association with endoplasmic reticulum membranes. J. Virol. 75, 12070–12083.

den Boon, J. A., AlQUIp, P. (2010). Organelle-like membrane compartmentalization of positive-strand RNA virus replication factories. Annu. Rev. Microbiol. 64, 241–260.

de Zwaan, G. A., Assink, A. M., and Van Kammen, A. (1974). Association of cowpea mosaic virus-induced double-stranded RNA with a cytopathological structure in infected cells. Virolgy 59, 342–355.

Doudna, J. A., and Kirkegaard, K. (1995). Inhibition of cellular protein secretion by poliovirus proteins 2B and 3A. EMBO J. 14, 848–857.

Dumont, L., Van der Kolk, S., Van der Meij, J., Van Kammen, A., and Teldner, P. (1998). Purification of cowpea mosaic virus RNA replication complex: identification of a virus-encoded 110,000-dalton polyprotein responsible for RNA chain elongation. Proc. Natl. Acad. Sci. U.S.A. 95, 1951–1955.

Fujita, K., Kishioka, S., Franco, D. P., Aul, V., London, E., and Wimmer, E. (2007). Membrane topography of the hydrophobic anchor sequence of poliovirus 3A and 3B proteins and the functional effect of 3A/3AB membrane association upon RNA replication. Biochemistry 46, 5185–5199.

Gnäule, M. E., and Carrasco, L. (2005). Virusproteins. FEBS Lett. 572, 28–34.

Gorbalenya, A. E., Komarova, G. M., Bardin, M., Bushunova, A. L., and Derijts, T. J. L. (2012). The 3A protein from multiple picornaviruses utilizes the golgi adaptor protein ACBD3 to recruit HflB130. J. Virol. 86, 3015–3016.

Gu, H., and Schnaitman, C. A. (1998). The Raf-related membrane protein endoplasmic reticulum-targeting cofactor and putative fusogenic activity of an endoplasmic reticulum snare inhibit the synthesis of poliovirus-like particles. Virolgy 333, 273–281.

Han, S., and Sanfaçon, H. (2003). Tomato ringpot virus 3A polyprotein targeting the endoplasmic reticulum and characterization of its targeting domain. J. Virol. 77, 523–534.

Huang, H., and Sanfaçon, H. (2005). Tomato ringpot virus 3A polyprotein targeting the endoplasmic reticulum and three membrane proteins that associate with the endoplasmic reticulum and cofractionate with the endoplasmic reticulum-derived membranes. J. Virol. 80, 5327–5337.

Irurzun, A., Perez, L., and Carrasco, L. (2003). How do helix–helix interactions help to define the limits of membrane proteins? Perspectives from the study of homo-oligomeric lipid bilayers. Proteins Sci. 12, 647–655.

Iwami, A., Kishikawa, K., Tsurumaki, Y., Hasegawa, T., and Kato, N. (1995). Inhibition of cellular protein secretion by poliovirus proteins 2B and 3A. EMBO J. 14, 848–857.

Joshi, A., Van der Vliet, R., Wellink, J., Willekens, T., et al. (2011). Family Secoviridae. In Virus Taxonomy: Classification and Nomenclature of Viruses, ed. T. E. Maizel, Jr. (San Diego: Elsevier), 540–546.

Kirkegaard, K., and Tschopp, M. W. (1992). Inhibition of poliovirus RNA synthesis by hsf1/C. J. Virol. 66, 1995–1994.

Kleiman, H. T., and Godolphin, J. L. (2005). Membrane curvature and mechanisms of dynamic cell membrane remodeling. Nat Rev. 438, 380–396.

Klopper, J. M., and Krivine-Louchon, J. (2008). Modulation of intracellular membrane structures for virus replication. Nat. Rev. Microbial. 6, 363–374.

Kato, N., and Tschopp, M. W. (2000). The 3A protein of picornaviruses is responsible for RNA chain elongation. J. Virol. 74, 8953–8965.

Kita, M., Ishikawa, K., Anta, M., and Tanimura, K. (2012). ACBD3-mediated recruitment of Poliovirus to the plasma membrane RNA replication sites. EMBO J. 31, 756–766.

Kojo, S. C., Jemasi, P. E., and Carrington, C. J. (1995). Formation of plant RNA virus replication complex on membranes: role of an endoplasmic reticulum-targeted viral protein. EMBO J. 14, 4089–4099.

Kyle, D. A., Guldberg, T. H. Jr., and Krijnse-Locker, J. (2008). Remodeling the endoplasmic reticulum by poliovirus infection: Implications for the general role of individual viral proteins: an aptophagy-like origin for virus-induced vesicles. J. Virol. 84, 8955–8960.

Kuwahara, N., Levenson, E., Rinne, M., A., Egger, D., Beute, K., Gorbalenya, A. E., et al. (2004). Evidence for functional protein interactions required for poliovirus RNA replication. J. Virol. 80, 3327–3337.

Lamb, L. E., Johnson, K. S., Levenson, E. A., Gorbalenya, A. E., and Helenius, E. (2012). Identification of tolerated insertion sites in poliovirus non-structural proteins. Virology 428, 1–11.

Levenson, N. L., Pinto, Y., Zweier, J. L., Johnson, K. S., and Helenius, E. (2011a). Analysis of poliovirus protein 3A interactions with viral and
cellular proteins in infected cells. J. Virol. 85, 4284–4296.

Turner, K. A., Su, Y. L., Callaway, A. S., Allen, N. S., and Lommel, S. A. (2004). Red clover mosaic virus replication proteins accumulate at the endoplasmic reticulum. Virology 320, 276–280.

van Kuppeveld, F. J., van den Hurk, F. J., Zoll, J., Galama, J. M., and Melchers, W. J. (1996). Mutagenesis of the coxackie B3 virus 2B/2C cleavage site: determinants of processing efficiency and effects on viral replication. J. Virol. 70, 7632–7640.

Viry, M., Serghini, M. A., Hans, F., Ritzenthaler, C., Pinck, M., and Pinck, L. (1993). Biologically active transcripts from cloned cDNA of genomic grapevine fanleaf nepovirus RNAs. J. Gen. Virol. 74, 169–174.

Vo, P., Jaegle, M., Wellink, J., Verker, J., Eggien, R., Van Kammen, A., et al. (1992). Infectious RNA transcripts derived from full-length DNA copies of the genomic RNAs of cowpea mosaic virus. Virology 165, 35–42.

Wang, A., and Sanfacon, H. (2000). Proteolytic processing at a novel cleavage site in the N-terminal region of the tomato ringspot nepovirus RNA-1-encoded polyprotein in vitro. J. Gen. Virol. 81, 2771–2781.

Wang, A., Han, S., and Sanfacon, H. (2004). Topogenesis in membranes of the NTR-VPg protein of tomato ringspot nepovirus: definition of the C-terminal transmembrane domain. J. Gen. Virol. 85, 557–565.

Wang, A., Han, S., and Sanfacon, H. (2004). Topogenesis in membranes of the NTR-VPg protein of tomato ringspot nepovirus: definition of the C-terminal transmembrane domain. J. Gen. Virol. 85, 557–565.

Wang, A., Han, S., and Sanfacon, H. (2004). Topogenesis in membranes of the NTR-VPg protein of tomato ringspot nepovirus: definition of the C-terminal transmembrane domain. J. Gen. Virol. 85, 557–565.