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Bromovirus-induced remodeling of host membranes during viral RNA replication

Arturo Diaz[1] and Xiaofeng Wang[2]

With its high yield, small genome, and ability to replicate in the yeast *Saccharomyces cerevisiae*, *Brome mosaic virus* (BMV) has served as a productive model to study the general features of positive-strand RNA virus infection. BMV RNA is replicated in spherules, vesicle-like invaginations of the outer perinuclear endoplasmic reticulum membrane that remain connected to the cytoplasm via a neck-like opening. Each spherule contains the viral replicase proteins as well as genomic RNAs. Recent advances indicate that multiple interactions between the viral proteins with themselves, cellular membranes, and host factors play crucial roles in BMV-mediated spherule formation. These findings are probably applicable to other positive-strand RNA viruses and might potentially provide new targets for antiviral treatments.

Addresses
[1] Department of Biology, La Sierra University, Riverside, CA 92505, United States
[2] Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech University, Blacksburg, VA 24061, United States

Corresponding authors: Diaz, Arturo (adiaz@lasierra.edu) and Wang, Xiaofeng (reachxw@vt.edu)

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Introduction

The RNA replication compartments of positive-strand RNA viruses are mini-organelles that feature the close association of both viral proteins and host cellular components [1]. Viral RNA replication takes place in close association with cellular membranes and although the architecture of these compartments differs among viral families, they usually involve membrane vesicle formation or other membrane rearrangements. Cellular membranes provide a scaffold for the enrichment of the viral replication factors as well as exploited cellular proteins, provide an environment to protect the viral RNA from cellular defense mechanisms and help to separate and coordinate the various stages of the viral life cycle. Because of the limited coding capacity of positive-strand RNA viruses, the formation and function of the viral replication compartments require complex interactions between the viral proteins, viral genome, and co-opted host factors. Recent work has shown that cellular host proteins that either structurally induce and/or support membrane curvature are required to form the replication compartments [2,3,4••]. Moreover, cellular lipid synthesis and appropriate lipid composition are essential for viral replication [5], implying that the membrane is an essential, functional component of the RNA replication compartments. Host membrane remodeling and the role of host genes in viral replication of other positive-strand RNA viruses have been summarized in the following reviews [1,3,6,7•]. This review will focus on recent advances on the formation of the BMV-induced RNA replication complexes.

Brome mosaic virus (BMV) is the type member of the *Bromoviridae* family and a representative member of the alphavirus-like superfamily of human, animal, and plant positive-strand RNA viruses. With its high yield, tripartite genome and ability to replicate in the yeast *Saccharomyces cerevisiae*, BMV has served as a productive model to study some of the general features of positive-strand RNA virus infection, including viral genomic RNA replication, gene expression, recombination, and virus–host interactions [8]. In the following sections we will summarize recent work that has enhanced our understanding of BMV replication complex assembly and structure, both of which are probably applicable to other positive-strand RNA viruses as seemingly diverse membrane rearrangements may represent topologically and functionally related structures.

General features of spherule formation

BMV has three genomic RNAs and one subgenomic mRNA. RNA1 encodes the multifunctional replication factor 1a, which contains an N-proximal RNA capping domain that is separated from a C-terminal NTPase/RNA helicase-like domain by a short proline-rich sequence that may be a flexible spacer [9–12]. $2a^{pol}$ is encoded from RNA2 and contains a central polymerase domain and an N-terminal domain that interacts with the helicase-like domain of 1a [13,14]. Genomic RNA3 encodes the 3a movement protein, which is required for cell-to-cell spread in plants, while subgenomic RNA4 encodes for the coat protein [15].

In both yeast and plant cells, BMV RNA replication depends on the viral 1a and $2a^{pol}$ proteins and specific

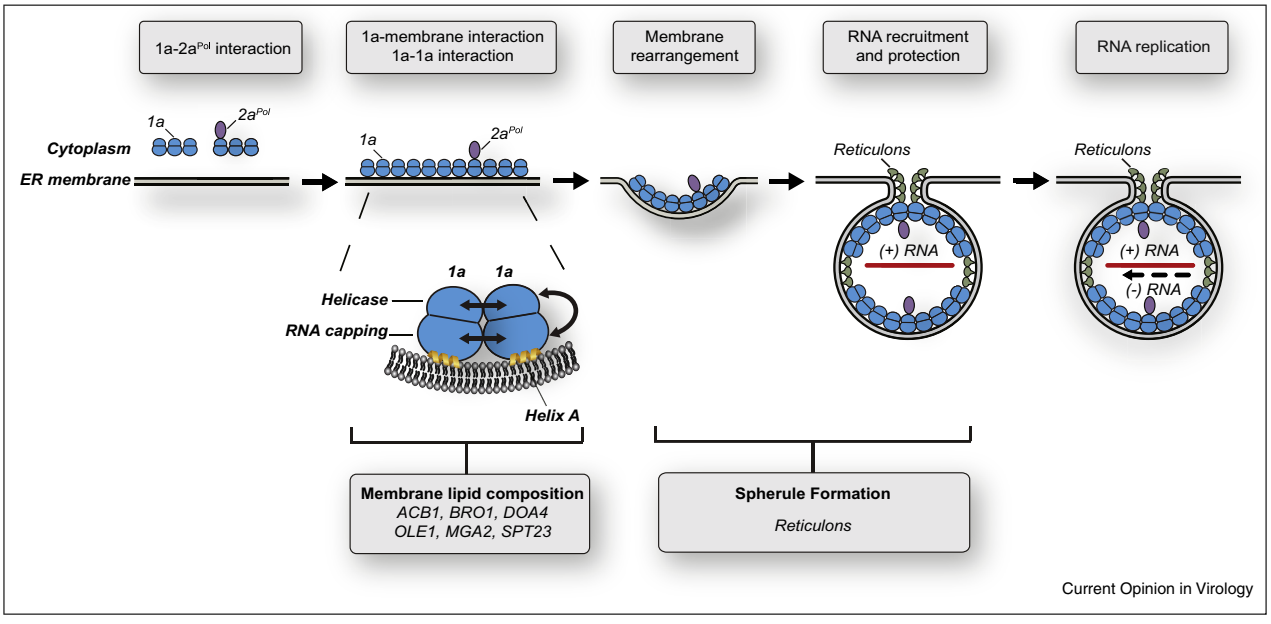
cis-acting RNA signals [16], generates a considerable excess of positive-strand to negative-strand RNA [17••], and efficiently directs subgenomic RNA4 synthesis [17••]. In yeast, in the absence of other viral factors, 1a induces 60–80 nm vesicular invaginations in the outer membrane of the perinuclear endoplasmic reticulum (ER), which are referred to as spherules [18••] (Figure 1). Spherules are bounded by a single lipid bilayer and the interior of these vesicles is connected to the cytoplasm through a neck-like opening, probably providing a channel for ribonucleotide import and product RNA export [18••]. Confocal microscopy shows that 1a accumulates in discrete ER patches that expand during the course of infection [19•] while biochemical and immunogold labeling analyses suggest that there are hundreds of 1a's per spherule and only a few copies of $2a^{pol}$ [18••]. Consistent with this, 1a interacts with and recruits $2a^{pol}$ to the perinuclear ER [18••,19,20]. The 1a-mediated recruitment and stabilization of genomic RNA appear to include two steps, a 1a-induced recruitment of RNA to the ER membrane followed by the translocation of the RNA into preformed spherules [12]. Moreover, incorporation of BrUTP shows nascent RNA synthesis occurs within the spherules [18••] (Figure 1). However, modulating the relative levels and interactions between replication factors 1a and $2a^{pol}$ shifts the membrane rearrangements from spherular compartments to large and multilayer stacks of appressed double-membrane layers that support RNA replication as efficiently as spherules [21].

The following two sections will summarize recent work that sheds light into the manner by which 1a interacts with itself, lipid membranes, and other viral components and the details by which it invaginates the ER membranes to induce spherule formation.

Roles of Helix A

1a has no trans-membrane domain and fully resides on the cytoplasmic side of the ER membrane [22]. A small amphipathic α-helix, termed Helix A, is crucial for both 1a's membrane association and 1a-induced membrane rearrangement as well as 1a-mediated recruitment of viral RNA templates and $2a^{pol}$ [22,23••] (Figure 1). Genetic, biochemical, and NMR analyses showed that mutations within Helix A give rise to two classes of mutants with distinct properties. Class I mutants, which disrupt helix formation, significantly inhibit 1a membrane affinity and fail to induce spherule formation but are over twice as effective as wild-type (wt) 1a at stabilizing and recruiting $2a^{pol}$ to nonperinuclear ER membranes [23••]. By contrast, Class II mutants interact with $2a^{pol}$ inefficiently but increase the frequency of 1a-induced spherules by 5-fold, although these compartments are ~30% smaller in diameter than those induced by wt 1a [23••] (Figure 2b). Correspondingly, Class I mutants fail to recruit the viral

Figure 1



Interactions and host factors required for the formation of functional BMV RNA replication complexes. 1a and $2a^{pol}$ interact in the cytoplasm before membrane association. 1a associates with the outer ER membrane, where 1a–1a and 1a–membrane interactions, in conjunction with host factors, lead to the invagination of the ER membrane to form spherules. 1a-mediated recruitment of viral RNA templates occurs after the formation of spherules, followed by the synthesis and retention of negative-strand RNA (dashed black lines), and asymmetric synthesis and export of positive-strand progeny RNA (red lines). Host factors involved in maintaining proper membrane lipid composition around the spherule membranes and in generating and/or maintaining the virus-induced membrane rearrangements are shown.

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