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Viral and host determinants of RNA virus vector replication and expression

Paul Ahlquist[a,b,*], Michael Schwartz[a,1], Jianbo Chen[a,2], David Kushner[a,3], Linhui Hao[a], Billy T. Dye[a,b]

[a] *Institute for Molecular Virology, University of Wisconsin – Madison, 1525 Linden Drive, Madison, WI 53706, USA*
[b] *Howard Hughes Medical Institute, University of Wisconsin – Madison, 1525 Linden Drive, Madison, WI 53706, USA*

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Abstract

Positive-strand RNA viruses have proven to be valuable vectors for delivery and expression of antigens for direct vaccination of animals and vaccine production in plants. However, optimal use of these viruses as vectors for vaccine and other purposes is limited by incomplete understanding of their replication pathways and associated constraints on inserted foreign genes. Further insights into RNA virus vector design and optimization are emerging from recent advances on the function of viral RNA replication factors, the nature of the viral RNA replication complex as a membrane-bounded compartment sequestering replication components from competing processes and host defenses, and identification of surprisingly diverse host genes contributing to many virus replication steps.

Keywords: RNA virus expression vectors; Vaccine expression; Virus–host interactions

1. Introduction

As the premier natural agents for gene transfer and expression, viruses and their derivatives are valuable tools for engineered gene expression in medicine, biotechnology and research. This includes not only DNA viruses and the reverse transcribing retroviruses, but also RNA viruses, which replicate and express their genes solely through RNA intermediates. The largest class of RNA viruses are the positive-strand RNA viruses, which package messenger-sense, single strand RNA in their virion particles. These viruses include many important pathogens such as hepatitis C virus, the severe acute respiratory syndrome (SARS) coronavirus, potential bioterrorism agents, and the vast majority of known plant viruses. Nevertheless, such positive-strand RNA viruses also have beneficial uses, serving as useful expression vectors in both animals and plants [1,2]. Among other advantages, such viruses generally have small genomes, high wild type replication and gene expression levels, and lack DNA forms to genetically transform host DNA. Vector derivatives of such viruses can provide high level expression of recombinant proteins and RNAs for many purposes including direct immunization of humans and animals, and vaccine production in plants.

Efficient use of such RNA virus vectors presently is limited in part by incomplete understanding of their replication cycle and its constraints. For example, insertion of foreign genes in RNA viruses as a payload for vaccine production or other directed expression often decreases the efficiency of viral genomic RNA replication and subgenomic mRNA expression for reasons that are not well understood. The degree to which viral genomic RNA replication is reduced varies with the gene inserted, is often substantial, and is not simply a function of the length of the inserted foreign sequence.

* Corresponding author. Tel.: +1 608 263 5916; fax: +1 608 265 9214.
E-mail address: ahlquist@wisc.edu (P. Ahlquist).
[1] Present address: Department of Biological Sciences, Vanderbilt University, Nashville TN 37235, USA.
[2] Present address: HIV Drug Resistance Program, National Cancer Institute, Frederick, MD 21702, USA.
[3] Present address: Department of Biology, Dickinson College, Carlisle, PA 17013, USA.

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Such reductions in replicative fitness not only reduce the level of gene expression but usually make the expression vector genetically unstable, since the high levels of RNA recombination in such viruses lead to the appearance of deletion variants that have lost part or all of the payload gene, and thereby gained increased replicative fitness that allows them to overgrow the starting vector ([3] and references therein).

Since the mechanism(s) by which foreign genes inhibit viral genome replication are not understood, such results imply the existence of presently unrecognized requirements for viral RNA replication. Better understanding of such constraints and the overall mechanisms by which positive RNA viruses replicate their genomes and synthesize mRNAs could greatly increase the utility of these viruses as vectors for protein expression, RNA expression, RNA silencing and other applications. Advances in understanding these viral replication mechanisms also offer targets for improved virus control, better understanding of virus pathology, and other benefits.

One virus that is being used as a model to study positive-strand RNA virus replication is brome mosaic virus (BMV). BMV is a representative member of the alphavirus superfamily of human, animal and plant viruses. All members of this superfamily share multiple conserved domains in their RNA replication proteins and conserved features in their RNA replication pathways. BMV was the first RNA virus engineered to express foreign genes [4], related human and animal alphaviruses have been found to be valuable vectors for gene expression in animal cells [5,6], and related plant viruses such as tobacco mosaic virus are useful vectors for gene expression in plants [2].

BMV encodes two large, multifunctional RNA replication proteins, designated 1a and 2a. 1a has a C-terminal helicase domain and an N-terminal domain with m^7GTP methyltransferase and covalent GTP binding (putative guanylyltransferase) activities required for capping viral RNA in vivo. 2a has a central polymerase-like domain and an N-terminal extension that interacts with the helicase-like domain of 1a. Below we discuss selected recent findings on viral RNA replication mechanisms from studies of BMV, including the nature of the viral RNA replication complex and the role of surprisingly diverse, host-encoded functions in viral RNA replication and gene expression.

2. Structure, assembly and function of the viral RNA replication complex

Some advances with potentially important mechanistic implications for RNA virus vectors have come from the realization that BMV RNA replication does not occur in the open cytoplasm but rather in a virus-induced, membrane-bounded compartment [7]. These findings appear to have relevance for additional positive-strand RNA viruses since all such viruses replicate their RNA on intracellular membranes, usually in association with vesicles or other membrane rearrangements. The structure of such replication complexes, their interaction with the host, and the processes by which RNA templates are recruited and progeny RNA products exported appear likely to have significant practical effects on the optimal design and performance of RNA virus vectors.

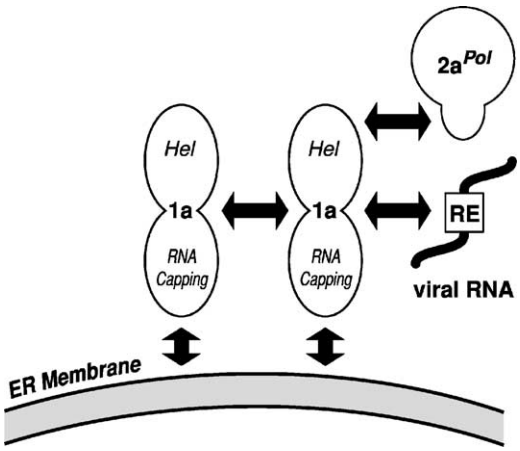

Fig. 1. Diagram of basic interactions by which the multifunctional BMV 1a protein directs assembly of the membrane-bound viral RNA replication complex, including: 1a interaction with the cytoplasmic face of the ER membrane; 1a–1a interaction; interaction of the 1a helicase-like domain with the N-terminal extension of the 2a polymerase; and 1a interaction with the cis-acting recognition element (RE) on viral genomic RNAs, leading to recruitment of viral RNA to the RNA replication compartment. See main text for further details.

The recent BMV results emerged from a combination of genetic, biochemical and cell biology approaches [7–9]. These findings show that the BMV 1a RNA replication protein plays key roles in directing the form and assembly of the RNA replication complex (Fig. 1). 1a localizes to the cytoplasmic face of the perinuclear endoplasmic reticulum (ER) membrane, and induces the membrane to invaginate into the ER lumen to form 50–70 nm vesicles or spherules [7]. The interiors of these ER luminal, membrane-bound spherules, which remain connected to the cytoplasm by a narrow, membranous neck, become compartments or mini-organelles for viral RNA synthesis. 1a is the sole viral factor needed to induce spherule formation. By other interactions (Fig. 1), 1a also independently recruits viral RNA templates and 2a polymerase to these compartments, which become the sites of negative-strand RNA synthesis. Negative strand RNAs then are retained in these compartments and used as templates to synthesize new positive-strand RNA for further viral translation and assembly of new infectious virions. Thus, these replication compartments concentrate the viral replication factors and RNA templates and link successive RNA replication steps. Viral positive- and negative-strand RNA templates in these structures also are protected from nucleases [7], suggesting that these compartments also protect potentially double stranded (ds) viral RNA replication intermediates from dsRNA-induced host defense responses including RNA interference and interferon responses [10].

Immunogold electron microscopy and biochemical approaches show that each spherule contains one to a few hun-

dred copies of 1a [7]. Since 1a self-interacts [11], these large numbers of 1a proteins may form a capsid-like protein shell to direct the formation and membrane envelopment of the spherular replication compartment. Structure and assembly of the spherular replication compartment thus appear potentially very similar to those of a budding, membrane-enveloped virion particle. In particular, BMV RNA replication complex assembly closely parallels the steps by which the reverse-transcribing, replicative cores of retrovirus virions assemble and become membrane enveloped. Specifically, the functions discussed above for BMV 1a, 2a polymerase, and certain 1a-recognized cis-acting signals on BMV genomic RNAs recapitulate the functions of Gag (the major capsid protein), Pol (polymerase or reverse transcriptase) and RNA packaging signals in virion assembly by retroviruses like HIV [7]. The similarities revealed bridge retroviruses, positive strand RNA viruses and dsRNA viruses, which also package RNA templates and RNA polymerase in a protein shell for replication. These and other similarities suggest that all three virus classes use related mechanisms for nucleic acid replication and may have evolved from common ancestors.

3. Host factors in viral RNA replication: a functional genomics approach

In addition to virus-encoded factors, most if not all steps in virus infections involve host factors [12]. Such virus–host interactions are crucial determinants of virus host range, replication, and pathology, offer insights to viral and cellular function, and provide antiviral targets. Identifying such interactions and the associated host factors thus is a major frontier in virology.

An unusual feature of BMV for identifying and characterizing host functions in viral replication is that BMV directs RNA replication, gene expression and virion formation in the genetic model yeast, *Saccharomyces cerevisiae* [13]. The potent approaches of yeast genetics and the large and growing understanding of yeast molecular biology thus can be applied to studying BMV replication and virus–host interactions. Interestingly, such yeast genetic studies of BMV replication depend heavily on using engineered virus derivatives as vectors to express selectable, counter-selectable, or screenable marker genes, making colony-level yeast phenotypes dependent on viral RNA replication. In recent years, classical yeast genetics have been used to identify host genes that function in controlling BMV translation [14,15], selecting BMV RNAs as replication templates [16], activating the viral RNA replication complex [17], maintaining a lipid composition required for membrane-associated RNA replication [18,19], and other steps.

To more globally and systematically identify host factors affecting virus replication, we also have used engineered BMV derivatives and high-throughput approaches to individually assay viral RNA replication in each strain of an ordered, genome-wide set of ∼4500 yeast single-gene-deletion strains, covering ∼80% of all yeast genes [20]. Specifically, we transformed each of the yeast deletion strains with plasmids expressing BMV 1a, 2a and a BMV RNA replication template with the capsid gene replaced by a luciferase reporter gene. Luciferase expression then depended on viral RNA replication and RNA-dependent mRNA synthesis. BMV-directed luciferase expression levels in this entire collection of deletion strains were independently assayed twice, and selected strains were analyzed further with detailed assays for various viral RNAs, proteins and activities.

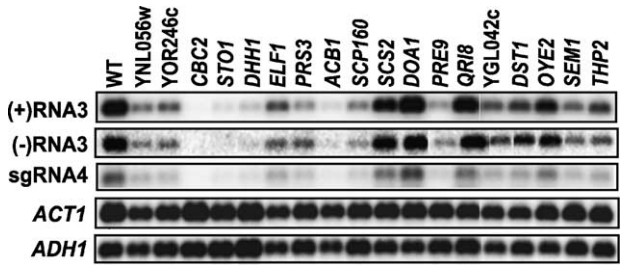

Fig. 2. Northern blots showing the varying accumulation of BMV RNA replication products in wt yeast and in selected isogenic yeast strains with deletions of the indicated genes, all transformed with plasmids expressing BMV RNA replication factors 1a and 2a and viral genomic RNA3 as a replication template. For comparison, northern blots of cellular *ACT*1 and *ADH*1 mRNAs are shown from the same cells. Modified from [20].

This systematic approach identified nearly 100 genes whose absence either inhibited or, in a smaller number of cases, stimulated BMV RNA replication and gene expression by 3- to >25-fold [20]. Examples of some of the host genes identified and the effects of their deletion on the accumulation of viral RNA replication products are shown in Fig. 2. Of the pool of yeast genes identified, several had previously been shown to function in BMV replication, confirming that this approach could identify relevant host genes. Yeast genes that were newly implicated in BMV RNA replication by this screen included genes in RNA, protein or membrane modification pathways, and many genes of presently unknown function. Thus, these screens identified many new host factors that affect BMV replication and implicated previously unconsidered pathways in the virus lifecycle. Further studies should determine more directly the diverse roles by which these host factors contribute to virus replication and identify additional host genes, such as essential genes not covered by this screen, that contribute to BMV replication. Moreover, since even the cellular function of many of these genes is unknown, these virus-motivated studies also should help to illuminate basic cell biology.

Like the growing understanding of virus-encoded replication functions discussed earlier, developing a basic understanding of the essential ways in which RNA viruses interact with their hosts to replicate and express their own genes should provide new insights for using such viruses to optimally deliver, maintain, replicate, and express foreign genes, such as those for vaccine antigens. In these and other ways, basic studies should provide the foundation for

translating the full potential of these viruses, most frequently considered as harmful pathogens, into useful tools.

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