Virology 426 (2012) 162–166

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Fig mosaic virus mRNAs show generation by cap-snatching

Jeewan Jyot Walia, Bryce W. Falk *

Department of Plant Pathology, University of California, Davis, USA

ARTICLE INFO

Article history:
Received 5 October 2011
Returned to author for revision
9 November 2011
Accepted 28 January 2012
Available online 20 February 2012

Keywords:
Negative strand viruses
Fig mosaic virus
Emaraviruses
Cap snatching
5′ Nucleotide extensions

ABSTRACT

Fig mosaic virus (FMV), a member of the newly described genus *Emaravirus*, has four negative-sense single-stranded genomic RNAs, and each codes for a single protein in the viral complementary RNA (vcRNA). In this study we show that FMV mRNAs for genome segments 2 and 3 contain short (12–18 nucleotides) heterogeneous nucleotide leader sequences at their 5′ termini. Furthermore, by using the high affinity cap binding protein eIF4E$_{K119A}$, we also determined that a 5′ cap is present on a population of the FMV positive-sense RNAs, presumably as a result of cap-snatching. Northern hybridization results showed that the 5′ capped RNA3 segments are slightly smaller than the homologous vcRNA3 and are not polyadenylated. These data suggest that FMV generates 5′ capped mRNAs via cap-snatching, similar to strategies used by other negative-sense multipartite ssRNA viruses.

Introduction

Fig mosaic disease (FMD) affects figs worldwide (Walia et al., 2009). Affected trees show mosaic symptoms on leaves and fruits, and can exhibit defoliation and premature fruit drop (Ferguson et al., 1990). FMD was first described to be of viral origin by Condit and Horne (1933), but the etiology was uncertain until quite recently when *Fig mosaic virus* (FMV) was identified as the causal agent (Elbeaino et al., 2009a ;Walia et al., 2009). Based on nucleotide sequence and deduced amino acid comparisons, the viruses most similar to FMV are *European mountain ash ringspot associated virus* (EMARAV) and Rose rosette virus (RRV), and the genus *Emaravirus* has been proposed, with EMARAV as the type species (King et al., 2011). These viruses are transmitted to plants by eriophyid mite vectors and induce similar but characteristic cytopathologies in their corresponding host plants, including the presence of double membrane bound bodies found in the cytoplasm of the virus-infected cells (not yet detected for EMARAV) (Anh et al., 1996; Appiano et al., 1995; Mielke-Ehret et al., 2010).

FMV has four negative-sense genomic ssRNAs ranging in size from 7.09 kb to 1.47 kb (Elbeaino et al., 2009a; Walia et al., 2009). The FMV genomic RNAs are not capped or polyadenylated (Elbeaino et al., 2009b), and all four FMV genomic RNAs contain complementary sequences (18–20 nt, depending on the RNA segment) at their 5′ and 3′ termini. These likely base pair to form a panhandle-like structure, which is one of the hallmarks of negative-sense multipartite ssRNA viruses, including tenuiviruses and viruses in the families *Bunyaviridae* and *Arenaviridae* (Falk and Tsai, 1998; Walter and Barr, 2011). Each FMV genome segment codes a single open reading frame (ORF) in the virus complementary polarity RNA (vcRNA). These ORFs have been annotated as follows. FMV vcRNA 1 (7093 nt) codes for the 264 kDa RNA dependent RNA polymerase protein (RdRp). The FMV RdRp has 47% amino acid identity with the EMARAV RdRp and 13% identity with the RdRps coded by some members of *Bunyaviridae* (Elbeaino et al., 2009b). The FMV RdRp also shows the presence of 6 motifs (pre motif A and A- E motifs) characteristic of negative strand segmented RNA virus RdRps and has the endonuclease signature at the N terminal region of the RdRp (Reguera et al., 2010) suggesting that the FMV RdRp might be involved in cap snatching. FMV vcRNA2 (2252 nt) codes for a 73 kDa putative glycoprotein (G) precursor. (Elbeaino et al., 2009b). FMV vcRNA3 (1490 nt) also has one ORF and codes for the putative 35 kDa nucleocapsid (N) protein. Despite other similarities, the FMV N protein shows no statistically significant amino acid similarity to N protein sequences for viruses in the family *Bunyaviridae.* FMV vcRNA4 (1472 nt) codes for a 40.5 kDa protein of unknown function. The FMV RNA4 and its coded protein do not show either nucleotide or amino acid identity or similarity to any plant viral sequences in GenBank (Elbeaino et al., 2009a; Walia et al., 2009) except for RRV (59% amino acid identity) (Laney et al., 2011).

Viruses of family *Bunyaviridae* and genus *Tenuivirus* have been shown to use “cap snatching” to initiate transcription and facilitate translation of their mRNAs (Bouloy et al., 1990; Duijsings et al., 1999; Estabrook et al., 1998; Reguera et al., 2010). For cap snatching, the virus coded RdRp binds to and cleaves host mRNAs to obtain the 5′ terminal methylated cap structure and the following ~15–20 nucleotides which serve as a primer for mRNA synthesis (Dhar et

* Corresponding author at: Department of Plant Pathology, One Shields Avenue, University of California, Davis, CA 95616, USA. Fax: +1 530 752 5634.
E-mail address: bwfalk@ucdavis.edu (B.W. Falk).

0042-6822/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2012.01.035

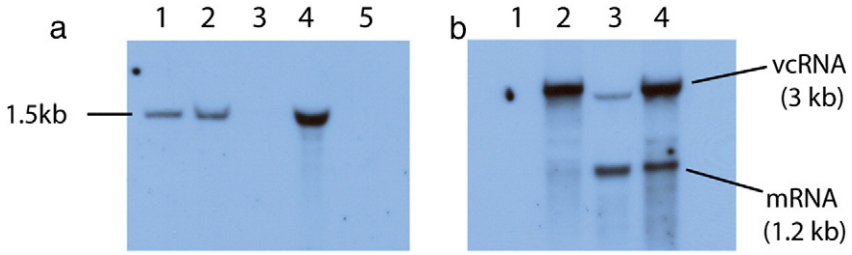

Fig. 1. Northern blot hybridization analysis of RNA from different fractions of polyribosomal and total RNAs extracted from FMV-infected figs and TSWV-infected *N. benthamiana* plants, respectively. RNAs were analyzed by agarose gel (1.6%) electrophoresis under denaturing conditions, transferred to nitrocellulose membrane and probed with biotinylated probes for FMV RNA3 (a) and TSWV S RNA (b).a: Lane 1: Total RNAs extracted from FMV infected figs, lane 2: polyribosomal RNAs, lane 3: Empty, lane 4: RNAs extracted from pre-polyribosomal membranous fraction and lane 5: total RNA extracted from healthy figs. Sizes of the corresponding RNAs are indicated.b: Lane 1: Total RNAs extracted from the healthy *N. benthamiana* plants, lane 2: RNA extracted from pre-polyribosomal membranous fraction, lane 3: polyribosomal RNAs; lane 4: total RNAs extracted from TSWV infected *N. benthamiana* plants. Sizes of the corresponding RNAs are indicated.

al., 1980; Duijsings et al., 2001; Garcin and Kolakofsky, 1990; Geerts-Dimitriadou et al., 2011; Patterson et al., 1984). The result is a 5′ capped viral mRNA which contains a short 5′ leader sequence derived from the 5′ end of any of many host mRNAs. This mechanism has been very well studied in the *Influenza A virus* (Braam et al., 1983; Fodor et al., 2002; Geerts-Dimitriadou et al., 2011; Krug et al., 1979; Li et al., 2001; Reguera et al., 2010; Ulmanen et al., 1981) but less so for the plant-infecting tospoviruses, family *Bunyaviridae* (Duijsings et al., 2001; Kormelink et al., 1992a; van Knippenberg et al., 2005) or for viruses in the genus *Tenuivirus* (Huiet et al., 1993; Ramirez et al., 1995; Shimizu et al., 1996; Yao et al., 2012).

The computer based analyses predict an endonuclease domain at the N terminus of the FMV RdRp (Reguera et al., 2010). This endonuclease signature has also been reported for other negative sense multipartite RNA viruses which show the presence of non-viral leader sequences on their mRNAs. As the FMV RdRp shows similarity to these negative sense multipartite viruses (members of family *Bunyaviridae* and genus *Tenuivirus*), this effort was executed to determine whether FMV mRNAs also are generated via cap snatching as they are for the related viruses.

Results and discussion

Evidence for presence of 5′ heterogenous non viral sequences on mRNA

A characteristic feature of the multipartite, negative sense ssRNA viruses is presence of non-viral nucleotides at the 5′ termini of their mRNAs which are acquired by cap-snatching, (Braam et al., 1983; Plotch et al., 1981). These are heterogeneous in length and sequence, and are typically of host mRNA origin. As FMV is a newly described multipartite, negative sense ssRNA virus, we attempted to identify and characterize FMV mRNAs to determine if FMV mRNAs exhibit hallmarks of cap snatching. Since fig leaves contain abundant amounts of latex and phenolic compounds (Ferguson et al., 1990) which can affect RNA quality and subsequent enzymatic reactions, we partially purified the polyribosomal RNAs from FMV-infected and healthy figs in order to concentrate mRNAs free of latex. To be sure that we were enriching for polyribosomal mRNAs we used *Tomato spotted wilt virus* (TSWV, family *Bunyaviridae*)-infected *N. benthamiana* plants as a positive control. Total and polyribosomal RNAs were analyzed by northern blot hybridization using probes complementary to both FMV RNA3 v and vcRNAs, and TSWV RNA3 (S) v and vc RNAs, the vcRNAs of each codes for the corresponding N protein. The resulting northern blots showed that the polyribosomal RNA fraction contained concentrated amounts of intact RNA (Figs. 1a, lane 2, and b, lane 3). Furthermore, for TSWV we observed enrichment of the N protein-coding mRNA (1.2 kb) in the polyribosomal RNA fraction compared to the total RNA extracted from TSWV-infected *N. benthamiana* plants (Fig. 1b, compare lanes 3 and 4). In contrast, we did not see clear size differences in the FMV polyribosomal RNAs vs. total RNAs, but we observed intact RNA from the polyribosomal RNA fraction (Fig. 1a, lane 2) compared to the RNA extracted from the pre-ribosomal membranous fraction (Fig. 1a, lane 4).

5′ RACE was next performed on the FMV polyribosomal RNAs in attempts to identify the 5′ end sequences of the FMV mRNAs (Fig. 2). Of 12 FMV RNA2 clones, 3 showed sequence complementarity to the 3′ end of vRNA 2, 3 showed the same complementary sequence but also had 5′ extensions of 12–14 nt, and 6 were apparently prematurely terminated transcripts. The corresponding results for 24 FMV RNA3 clones were 4 exact complements, 11 complements with extensions of 12–18 nt, and 9 prematurely terminated. The presence of 12–18 nt of non viral origin nucleotides at the 5′ termini of FMV mRNAs suggested that cap snatching may be involved in their synthesis. Furthermore, these extensions are similar in size to the host-derived leader sequences for tenuiviruses and members of the *Bunyaviridae* (Huiet et al., 1993; Kormelink et al., 1992b; Ramirez et al., 1995; Shimizu et al., 1996).

The endonucleolytic cleavage of host mRNAs has been shown to occur preferentially at the A residue for TSWV (Duijsings et al.,

```
1RNA3       ------AUCAGAUAAUUCAAGUAGUGAACUCC
2RNA3       ------AACCAAUCAGGAGAGUAGUGAACUCC
3RNA3       --ACACAAACAAACCUUAUAGUAGUGAACUCC
4RNA3       -------AAACCCUAGAAGAGUAGUGAACUCC
5RNA3       -----AGAACUGAUCGCACAGUAGUGAACUCC
6RNA3       -AGGAGUCUCUCUCUCUCUAGUAGUGAACUCC
7RNA3       ----ACUUCUGCUAUAGCUAGUAGUGAACUCC
8RNA3       -----AUUGCGCUCUCUCUAGUAGUGAACUCC
9RNA3       ------AUCUGAUCUCUUAAGUAGUGAACUCC
10RNA3      ------AUCUGAUCUCUUAAGUAGUGAACUCC
11RNA3      ----ACAACCGAAAUUACCAGUAGUGAACUCC
12RNA3      ------------------AGUAGUGAACUCC
13RNA3      ------------------AGUAGUGAACUCC
14RNA3      ------------------AGUAGUGAACUCC
15RNA3      ------------------AGUAGUGAACUCC
1RNA2       -----AAAACUCACAUCACAGUAGUGAACUCC
2RNA2       ------AGCGUCGGUUAAGAGUAGUGAACUCC
3RNA2       ------AGUCACCAAAAACAGUAGUGAACUCC
4RNA2       ------------------AGUAGUGAACUCC
5RNA2       ------------------AGUAGUGAACUCC
6RNA2       ------------------AGUAGUGAACUCC
                              **************
```

Fig. 2. Nucleotide sequences of the clones from 5′ RACE analysis. The blue colored nucleotides are extra nucleotides present at the 5′ termini of the viral mRNAs encoding for G (RNA2) and N (RNA3) proteins. The nucleotides shown in red are the 5′ terminal nucleotides present on the vcRNAs of FMV (RNA2 and RNA3). * indicates the conserved 5′ ends of the viral complementary RNAs. The clones from RNA 3 mRNA are labeled as 1RNA3 to 11RNA3 and from vcRNA 3 as 12RNA3–15RNA3. For RNA2 the mRNA clones are labeled as 1RNA2 to 3RNA2 and the corresponding vcRNA clones as 4RNA2 to 6RNA2.

2001), thereby resulting in a leader sequence having A complementarity with the ultimate or penultimate viral template nucleotide (U). The sequence preferences for the cleavage of the incorporated host mRNA fragments has also been reported for other negative strand multipartite viruses (Jin and Elliott, 1993a, 1993b), but we did not find any nucleotide residue preference among the limited sequences examined here for FMV (Fig. 2). Recent work with TSWV, *Rice stripe virus* (RSV) and *Influenza A virus* (Geerts-Dimitriadou et al., 2011; van Knippenberg et al., 2005; Yao et al., 2012) has shown that multiple base pair complementarity between the leader RNA and the vRNA increases the probability of it being used for transcription. In our study all of the analyzed clones (mRNAs 2 and 3) showed that the viral 5′ terminal sequence follows immediately after the non viral leader, and the non viral 3′ most nucleotide varied. As the sequence information of the host mRNAs used for the transcription initiation of FMV vRNA is not available, it is therefore difficult to comment on the sequence requisite at the 3′ end and cleavage specificity of the host mRNAs required for the transcription initiation of the FMV vRNAs.

Presence of 5′ 7-methylguanosine cap structure

If the 5′ leader sequences described above are derived from host mRNAs via cap-snatching, then the mRNAs should have a 5′ 7-methylguanosine cap. Although this has been inferred for tospoviruses, the 5′ cap structure has not been demonstrated, but immuno-selection using cap-specific antibodies indicated that for the *Tenuivirus*, *Rice hoja blanca virus*, the subgenomic RNAs may be capped (Ramirez et al., 1995). To determine if the FMV mRNAs contained a 5′ cap structure, we attempted to purify 5′-capped mRNAs from FMV-infected fig plants using GST-tagged recombinant eIF4E-$_{K119A}$. Compared to the wildtype eIF4E, the recombinant eIF4E has 10–15 fold greater affinity for the 5′-m7GpppN RNA cap structure (Choi and Hagedorn, 2003). FMV polyribosomal RNAs were incubated with immobilized eIF4E$_{K119A}$ and the bound RNAs were extracted from the GST 4E beads using phenol/chloroform extraction and ethanol precipitation (Gowda et al., 2010). Northern hybridization analysis for FMV positive-sense RNA3 (mRNA) indicated that FMV-specific RNAs were present in the eIF4E$_{K119A}$ extract, providing strong evidence for a 5′-m7GpppN RNA cap structure on these RNAs (Fig. 3, lane 2). As a positive control we analyzed TMV virion RNA (5′ capped), and hybridization results showed an abundance of TMV RNA in the eIF4E$_{K119A}$ extract but not in the wash fractions (data not shown).

It is also interesting to observe in our results the presence of two FMV RNA3-specific RNAs of slightly different electrophoretic mobilities (Fig. 3, lanes 1–5). The RNA of lesser migration (larger RNA) predominated for the total RNA (Fig. 3, lane 1) and the wash fractions (Fig. 3, lanes 3–5), presumably corresponding to the uncapped full-length vcRNA 3. The RNA of greater migration (smaller RNA) was more abundant for the fraction that had bound to, and was extracted from, the eIF4E$_{K119A}$ beads (Fig. 3, lane 2). This suggests that transcription generating 5′ capped FMV mRNA3 terminates internally on the vRNA3 template RNA.

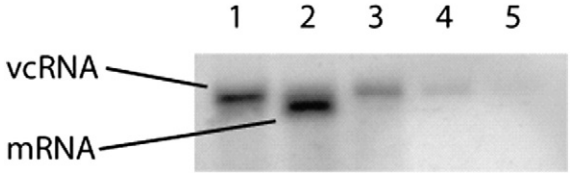

Fig. 3. Northern blot hybridization analysis of the FMV RNAs isolated by the eIF4E$_{K119A}$ cap-binding assay. RNAs were analyzed by denaturing agarose gel electrophoresis and transferred to nitrocellulose membranes. The RNAs were hybridized with P^{32} labeled probes complementary to the FMV RNA 3 vc and mRNAs. Lane 1: Total RNAs extracted from the FMV-infected fig plants. Lane 2: RNA recovered by affinity chromatography using immobilized cap binding protein eIF4E$_{K119A}$. Both lanes show two RNAs, with the slower migrating RNA (presumably vcRNA3) being the most intense for lane 1 and the faster migrating RNA (mRNA3) the most intense for lane 2. Lanes 3, 4 and 5 are from the RNA fractions unbound to the eIF4E$_{K119A}$, beads and extracted from the washes.

For the members of *Bunyaviridae*, mRNA transcription termination mechanisms are only partly known. Transcription termination for the entirely negative sense bunyaviruses (*Orthobunyavirus*, *Nairovirus* and *Hantavirus*) occurs internally on the vRNA template at a G/U rich sequence. However for bunyaviruses (*Tospovirus* and *Phlebovirus*) having ambisense RNAs the intergenic regions for both the vcRNA and vRNA are suggested to contain termination signals (Hutchinson et al., 1996; van Knippenberg et al., 2005). No universal, conserved transcription termination signals have been reported for viruses of the *Bunyaviridae*, however two sequences have been suggested. These are 3′-G/CUUUUU-5′ and 3′-GGUGGGGGGUGGGG-5′ (Acheson, 2007). We examined the 5′ regions of the FMV vRNAs (the negative sense templates used for mRNA synthesis) for the presence of possible transcription signals by comparison to those described for members of *Bunyaviridae* (Barr et al., 2006; Giorgi et al., 1991; Hutchinson et al., 1996; Walter and Barr, 2011) and found none of the previously identified termination sequences. Furthermore, we did not find a sequence near the template RNA 5′ termini that was common to all FMV vRNAs (excluding the conserved terminal nucleotides). We also used 3′ RACE in attempts to determine if in fact the FMV mRNA3 showed different 3′ termini than did the vcRNA, but we did not find consistent sequences that might be indicative of termination points (data not shown). We also analyzed the FMV RNAs with the PolyATtract mRNA isolation system and northern hybridization analysis on the RNA fraction bound to the PolyA binding beads showed the lack of FMV m/vcRNAs (RNA3, data not shown), suggesting FMV mRNAs (RNA3) are not polyadenylated.

Conclusions

Our data indicate that FMV mRNA2 and mRNA3, like those of other negative sense ssRNA multipartite viruses, have 5′ heterogeneous leader sequences. Affinity binding analysis with the eIF4E$_{K119A}$ mutant and northern blot hybridization analyses give strong support for the presence of 5′ cap structure at the 5′ termini of the leader sequences (mRNA3). This is the first demonstration of a 5′ cap and the presence of 5′ heterogeneous non-viral leader sequences on the mRNAs for Emaraviruses. It has recently been suggested that the mRNAs for another *Emaravirus*, RRV, may be capped (Laney et al., 2011), and this seems very likely.

Moreover, the 3′ RACE analysis and northern hybridization analysis have shown that FMV mRNA3 are not polyadenylated and appear to be produced by premature termination of transcription on the vRNA3 template.

Materials and Methods

Virus isolates

Eriophyid mites (*Aceria ficus*) from a FMV-infected fig (*Ficus carica*) tree were placed on two healthy fig seedlings. Approximately 10 days later leaves began to show typical mosaic symptoms, and were tested for FMV by RT-PCR using FMV specific primers (Walia et al., 2009). These FMV-infected fig seedlings were used as the FMV source for all the experiments performed in this study. *Tomato spotted wilt virus* (TSWV) was used for mechanical inoculation of *Nicotiana benthamiana* plants. These plants were used to isolate TSWV polyribosomal RNAs. *Tobacco mosaic virus* (TMV) virion RNA was extracted from purified virions for use as a 5′ 7-methyl guanosine cap-binding control.

Polyribosomal RNA extraction

Polyribosomes were extracted from leaf tissues of FMV-infected figs and TSWV-infected *Nicotiana benthamiana* plants, by following established procedures (Jackson and Larkins, 1976), except that polyribosomes were not purified using sucrose density gradients. The RNAs from the pre-polyribosome membranous fraction and the polyribosome fraction were extracted by slight modification of established protocols (Falk and Tsai, 1984). Both fractions were mixed 1:1 with RNA extraction buffer {50 mM Tris, pH 7.5 containing 2% sodium dodecyl sulfate (SDS) and 1% N-Lauroyl sarcosine (NLS)}, respectively. After 10 min incubation at room temperature, RNA was extracted using phenol: chloroform, followed by ethanol precipitation, and RNAs were resuspended in RNase free water.

5′ RACE and sequence analysis

5′ RACE was used for attempts to determine the 5′ termini of the FMV mRNAs (Invitrogen Corp., Carlsbad, California) for the G (vcRNA2) and N (vcRNA3) proteins by using 1–5 μg of polyribosomal RNA. The FMV-specific primers used for the reverse transcription and polymerase chain reactions are listed in Table 1. 5′ RACE products were analyzed by agarose gel electrophoresis and stained with SYBR Gold (Invitrogen, Molecular Probes Inc., Eugene, Oregon) as per manufacturer's instructions. Bands were excised from agarose gels, DNA was extracted by using the Minelute Gel extraction kit (Qiagen Sciences, Maryland, USA) and ligated into pGEM-T Easy (Promega), and then transformed into DH5α *Escherichia coli* electro competent-cells. Recombinant colonies were selected and grown on Luria Bertani broth containing 100 μg/ml of ampicillin (Sigma Chemical Co. St. Louis, MO.). Plasmids were extracted using QIAprep Spin Miniprep Kit (Qiagen Sciences, Maryland, USA) and sequenced using the ABI 3730 Capillary Electrophoresis Genetic Analyzer at the UC DNA Sequencing facility, University of California, Davis.

Affinity purification of 5′-methylguanosine-capped RNA

5′-Capped mRNAs were purified using the recombinant cap binding protein $eIF4E_{K119A}$ (Choi and Hagedorn, 2003). GST-tagged $eIF4E_{K119A}$ was bound to glutathione beads for 1 h in PBS and washed three times with binding buffer (Gowda et al., 2010). One hundred fifty μg of heat-denatured polyribosomal RNAs from FMV-infected fig and TMVvirion RNA was separately mixed with glutathione agarose GST4E beads for 1 h in binding buffer, followed by four washes with washing buffer (Choi and Hagedorn, 2003; Gowda et al., 2010). RNA bound to the GST 4E beads was extracted with phenol/chloroform, ethanol precipitated and dissolved in RNase free water.

Northern hybridization

Total RNAs (Qiagen RNeasy Plant mini kit), and polyribosomal RNAs (Jackson and Larkins, 1976) were extracted from FMV-infected as well as from healthy figs. Affinity purified 5′ capped FMV mRNAs and TMV virion RNAs were also analyzed by northern hybridization. The RNAs were denatured with dimethylsulfoxide and glyoxal, and analyzed by 1.6% agarose gel electrophoresis. The RNAs were then transferred to Hybond N^+ membranes (Amersham Biosciences, Piscataway, NJ). The [α-^{32}P]-UTP-labeled RNA probes were generated using the SP6/T7 MAXIscript *in vitro* transcription kit (Ambion, Texas) so as to generate probes of negative polarity. The prehybridization and hybridization steps for affinity purified mRNAs and RNAs were carried according to instructions provided by Perfect Hyb™ Plus buffer from Sigma (Sigma Chemical Co. St. Louis, MO.).

Biotinylated probes were generated for the detection of FMV RNA3 and TSWV RNA3 (S RNA) from the pre-polyribosomal membranous fraction and polyribosomal RNAs by using Bright Star psoralen–biotin nonisotopic labeling kit (Ambion Inc, Austin, Texas, USA). The prehybridization, hybridization and detection steps were carried out according to the manufacturer's instructions.

Table 1
Primer sequences for 5′ RACE experiments.

RACE primers	RNA segments	Primer sequences
5′ RACE	RNA2 GSP1	5′ CTATGGATGATTGATGTG 3′
	RNA2 GSP2	5′ CTTCATGGATGGTTGCAGTA 3′
	RNA3 GSP1	5′ TAGTATACCAGCTGACAT 3′
	RNA3 GSP2	5′ TTGGATGATACAATCAACCTCAA 3′

GSP: Gene specific primer.
GSP1 primer primes the first strand cDNA synthesis.
GSP2 is used for PCR amplification of cDNA along with anchor primer (provided with 5′ RACE system).

Acknowledgments

This research in part was supported by grants from the California Fig Advisory Board, University of California and Guru Gobind Singh fellowship (UC, Santa Cruz). The authors would like to thank Dr. Curt H. Hagedorn and Cassie Nelson (University of Utah School of Medicine and Huntsman Cancer Institute) for helpful suggestions and generously providing the recombinant $eIF4E_{K119A}$ recombinant protein. Authors are thankful to Dr. George Bruening for critical review of the manuscript.

References

Acheson, N.H., 2007. Fundamentals of Molecular Virology. John Wiley & Sons, Inc., New Jersey.
Anh, K.K., Kim, K.S., Gergerich, R.C., Jensen, S.G., Anderson, E.J., 1996. Comparative ultrastructure of double membrane-bound particles and inclusions associated with eriophyid mite-borne plant diseases of unknown etiology: a potentially new group of plant viruses. J. Submicrosc. Cytol. Pathol. 28, 345–355.
Appiano, A., Conti, M., Zini, N., 1995. Cytopathological study of double-membrane bodies occurring in fig plants affected by fig mosaic disease. Acta Hortic. 386, 585–592.
Barr, J.N., Rodgers, J.W., Wertz, G.W., 2006. Identification of the *Bunyamwera bunyavirus* transcription termination signal. J. Gen. Virol. 87, 189–198.
Bouloy, M., Pardigon, N., Vialat, P., Gerbaud, S., Girard, M., 1990. Characterization of the 5′ and 3′ ends of viral messenger RNAs isolated from BHK21 cells infected with Germiston virus (Bunyavirus). Virology 175, 50–58.
Braam, J., Ulmanen, I., Krug, R.M., 1983. Molecular model of a eucaryotic transcription complex: functions and movements of influenza P proteins during capped RNA-primed transcription. Cell 34, 611–618.
Choi, Y.H., Hagedorn, C.H., 2003. Purifying mRNAs with a high-affinity eIF4E mutant identifies the short 3′ poly(A) end phenotype. Proc. Natl. Acad. Sci. U. S. A. 100, 7033–7038.
Condit, I.J., Horne, W.T., 1933. A mosaic of the fig in California. Phytopathology 23, 887–896.
Dhar, R., Chanock, R.M., Lai, C.-J., 1980. Nonviral oligonucleotides at the 5′ terminus of cytoplasmic influenza viral mRNA deduced from cloned complete genomic sequences. Cell 21, 495–500.
Duijsings, D., Kormelink, R., Goldbach, R., 1999. Alfalfa mosaic virus RNAs serve as cap donors for tomato spotted wilt virus transcription during coinfection of *Nicotiana benthamiana*. J. Virol. 73, 5172–5175.
Duijsings, D., Kormelink, R., Goldbach, R., 2001. *In vivo* analysis of the TSWV cap snatching mechanism: single base complementarity and primer length requirements. EMBO J. 20, 2545–2552.
Elbeaino, T., Digiaro, M., Alabdullah, A., De Stradis, A., Minafra, A., Mielke, N., Castellano Maria, A., Martelli, G.P., 2009a. A multipartite single-stranded negative-sense RNA virus is the putative agent of fig mosaic disease. J. Gen. Virol. 90 (5), 1281–1288.
Elbeaino, T., Digiaro, M., Martelli, G., 2009b. Complete nucleotide sequence of four RNA segments of fig mosaic virus. Arch. Virol. 154, 1719–1727.
Estabrook, E.M., Tsai, J., Falk, B.W., 1998. *In vivo* transfer of barley stripe mosaic hordeivirus ribonucleotides to the 5′ terminus of maize stripe Tenuivirus RNAs. Proc. Natl. Acad. Sci. U. S. A. 95, 8304–8309.
Falk, B.W., Tsai, J.H., 1984. Identification of single- and double-stranded RNAs associated with maize stripe virus. Phytopathology 74, 909–915.
Falk, B.W., Tsai, J.H., 1998. Biology and molecular biology of viruses in the genus *Tenuivirus*. Annu. Rev. Phytopathol. 36, 139–163.
Ferguson, L., Michailidies, T.J., Shorey, H.H., 1990. The California fig industry. Hort. Rev. (Amer. Soc. Hort. Sci.) 12, 409–490.
Fodor, E., Crow, M., Mingay, L.J., Deng, T., Sharps, J., Fechter, P., Brownlee, G.G., 2002. A single amino acid mutation in the PA subunit of the influenza virus RNA polymerase inhibits endonucleolytic cleavage of capped RNAs. J. Virol. 76, 8989–9001.

Garcin, D., Kolakofsky, D., 1990. A novel mechanism for the initiation of *Tacaribe arenavirus* genome replication. J. Virol. 64, 6196–6203.
Geerts-Dimitriadou, C., Zwart, M.P., Goldbach, R., Kormelink, R., 2011. Base-pairing promotes leader selection to prime *in vitro* influenza genome transcription. Virology 409, 17–26.
Giorgi, C., Accardi, L., Nicoletti, L., Gro, M.C., Takehara, K., Hilditch, C., Morikawa, S., Bishop, D.H., 1991. Sequences and coding strategies of the S RNAs of *Toscana* and *Rift Valley fever* viruses compared to those of *Punta Toro*, *Sicilian Sandfly fever*, and *Uukuniemi* viruses. Virology 180, 738–753.
Gowda, M., Nunes, C.C., Sailsbery, J., Xue, M., Chen, F., Nelson, C.A., Brown, D.E., Oh, Y., Meng, S., Mitchell, T., Hagedorn, C.H., Dean, R.A., 2010. Genome-wide characterization of methylguanosine-capped and polyadenylated small RNAs in the rice blast fungus *Magnaporthe oryzae*. Nucleic Acids Res. 38, 7558–7569.
Huiet, L., Feldstein, P.A., Tsai, J.H., Falk, B.W., 1993. The maize stripe virus major non-capsid protein messenger RNA transcripts contain heterogenous leader sequences at their 5′ termini. Virology 197, 808–812.
Hutchinson, K.L., Peters, C.J., Nichol, S.T., 1996. Sin Nombre virus mRNA synthesis. Virology 224, 139–149.
Jackson, A.O., Larkins, B.A., 1976. Influence of ionic strength, pH and chelation of divalent metals on isolation of polyribosomes from tobacco leaves. Plant Physiol. 57, 5–10.
Jin, H., Elliott, R.M., 1993a. Non viral sequences at the 5′ ends of Dugbe nairovirus S mRNAs. J. Gen. Virol. 74, 2293–2297.
Jin, H., Elliott, R.M., 1993b. Characterization of *Bunyamwera* virus S RNA that is transcribed and replicated by the L protein expressed from recombinant vaccinia virus. J. Virol. 67, 1396–1404.
King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J., 2011. Virus Taxonomy, Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses (International Union of Microbiological Societies Virology Division). Elsevier Inc.
Kormelink, R., de Haan, P., Peters, D., Goldbach, R., 1992a. Viral RNA synthesis in tomato spotted wilt virus-infected Nicotiana rustica plants. J. Gen. Virol. 73, 687–693.
Kormelink, R., van Poelwijk, F., Peters, D., Goldbach, R., 1992b. Non-viral heterogeneous sequences at the 5′ ends of tomato spotted wilt virus mRNAs. J. Gen. Virol. 73, 2125–2128.
Krug, R.M., Broni, B.A., Bouloy, M., 1979. Are the 5′ ends of influenza viral mRNAs synthesized *in vivo* donated by host mRNAs? Cell 18, 329–334.
Laney, A.G., Keller, K.E., Martin, R.R., Tzanetakis, I.E., 2011. A discovery 70 years in the making: characterization of the Rose rosette virus. J. Gen. Virol. 92, 1727–1732.
Li, M.-L., Rao, P., Krug, R.M., 2001. The active sites of the influenza cap-dependent endonuclease are on different polymerase subunits. EMBO J. 20 (8), 2078–2086.
Mielke-Ehret, N., Thoma, J., Schlatermund, N., Mühlbach, H.P., 2010. Detection of European mountain ash ringspot-associated virus-specific RNA and protein P3 in the pear leaf blister mite *Phytoptus pyri* (*Eriophyidae*). Arch. Virol. 155, 987–991.
Patterson, J.L., Holloway, B., Kolakofsky, D., 1984. La Crosse virions contain a primer-stimulated RNA polymerase and a methylated cap-dependent endonuclease. J. Virol. 52, 215–222.
Plotch, S., Bouloy, M., Ulmanen, E., Krug, R.M., 1981. A unique cap (m7G pppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. Cell 23, 847–858.
Ramirez, B.C., Garcin, D., Calvert, L.A., Kolakofsky, D., Haenni, A.L., 1995. Capped non-viral sequences at the 5′ end of the mRNAs of rice hoja blanca virus RNA4. J. Virol. 69, 1951–1954.
Reguera, J., Weber, F., Cusack, S., 2010. *Bunyaviridae* RNA polymerases (L-protein) have an N-terminal, Influenza-like endonuclease domain, essential for viral cap-dependent transcription. PLoS Pathog. 6.
Shimizu, T., Toriyama, S., Takahashi, M., Akutsu, K., Yoneyama, K., 1996. Non-viral sequences at the 5′ termini of mRNAs derived from virus-sense and virus-complementary sequences of the ambisense RNA segments of rice stripe Tenuivirus. J. Gen. Virol. 77, 541–546.
Ulmanen, I., Broni, B.A., Krug, R.M., 1981. Role of two of the influenza virus core P proteins in recognizing cap 1 structures (m7G pppNm) on RNAs and in initiating viral RNA transcription. Proc. Natl. Acad. Sci. U. S. A. 78, 7355–7359.
van Knippenberg, I., Lamine, M., Goldbach, R., Kormelink, R., 2005. *Tomato spotted wilt virus* transcriptase *in vitro* displays a preference for cap donors with multiple base complementarity to the viral template. Virology 335, 122–130.
Walia, J.J., Salem, N.M., Falk, B.W., 2009. Partial Sequence and survey analysis identify a multipartite, negative-sense RNA virus associated with fig mosaic. Plant Dis. 93, 4–10.
Walter, T.C., Barr, J.N., 2011. Recent advances in the molecular and cellular biology of bunyaviruses. J. Gen. Virol. 92, 2467–2484.
Yao, M., Zhang, T., Zhou, T., Zhou, Y., Zhou, X., Tao, X., 2012. Repetitive prime-and-realignments convert short capped RNA leaders into longer ones that may be more suitable for elongation during *Rice stripe virus* transcription initiation. J. Gen. Virol. 93, 194–202.