A Sequence Located 4.5 to 5 Kilobases from the 5′ End of the Barley Yellow Dwarf Virus (PAV) Genome Strongly Stimulates Translation of Uncapped mRNA*

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An infectious, in vitro transcript from a full-length cDNA clone of the barley yellow dwarf virus (PAV serotype) genome translated efficiently in a wheat germ translation extract. Deletions in a region that we call the 3′ translational enhancer, located between bases 4,513 and 5,009 in the 5,677-base genome, reduced translation of the 5′-proximal open reading frames from un capped RNA by at least 30-fold. Deletions elsewhere in all but the 5′ end of the genome had no effect on translation. Presence of a m^G(5′)ppp(5′)G cap on the 5′ end fully restored translational efficiency of transcripts lacking the 3′ translational enhancer. The translation enhancer reduced inhibition of translation by free cap analog, did not affect RNA stability, and did not function in reticulocyte lysates. When placed in the 3′-untranslated region of uncapped mRNA encoding the β-glucuronidase gene, the translation enhancer stimulated translation more than 80-fold, in the presence of the viral, but not a plasmid-derived, 5′ leader. A polyadenylate tail could not substitute for the 3′ translational enhancer. These observations provide an extreme example, in terms of distance from the 5′ end and level of stimulation, of an mRNA in which a sequence near the 3′ end stimulates translation.

Luteoviruses are particularly rich in unusual translational control mechanisms (1, 2), e.g. ribosomal framingshifting (3, 4) and stop codon suppression (5). The genomic RNA of luteoviruses is about 5.7 kilobases long and is not polyadenylated (6, 7). The genes in the 5′-half of the genome are translated directly from genomic RNA, whereas the 3′-half is expressed via subgenomic mRNA(s) (5, 8, 9). The luteoviruses, including the barley yellow dwarf viruses (BYDVs), have been classified into subgroups I and II, according to genome organization and other biological properties (1, 2, 10, 11). The 5′-halves of the genomes of the two subgroups are essentially unrelated, whereas the 3′-halves of all luteoviruses have significant homology, with the exception of the approximately 800 3′-terminal bases of subgroup I genomes which are absent in subgroup II luteoviruses. The 5′-half of the genome of BYDV-PAV, a member of subgroup I, encodes a 39-kDa protein (39K ORF) and an overlapping open reading frame (60K ORF) which has the conserved amino acid motifs shared by RNA-dependent RNA polymerases (6). This 60K ORF is expressed via a 1 ribosomal frameshift at the end of the 39K ORF to produce a 99-kDa protein (Fig. IA) (3, 4).

The 5′ termini of eukaryotic cellular mRNAs have a cap structure (m^G(5′)ppp(5′)N) (12, 13), while the 5′ termini of viral RNAs can be either capped, uncapped (14), or covalently linked to a viral protein (VPg) (15). Genomes of viruses with any of these structures can have sequences near the 5′ end that enhance translation. For example, the 5′-untranslated regions (UTRs) of naturally capped RNAs of tobacco mosaic virus (16), alfalfa mosaic virus (17), and potato virus X (18) and of VPg-linked viral RNAs such as tobacco etch virus (19) and the picornaviruses (20) all stimulate translation by a variety of mechanisms. Although a VPg is attached to the 5′ termini of subgroup II luteoviral genomes (6, 21), the chemical nature of the 5′ terminus of BYDV-PAV and other subgroup I luteoviruses has not been reported.

The 3′-UTRs of eukaryotic mRNAs can also regulate initiation of translation (22, 23). A ubiquitous 3′-UTR sequence that enhances translation initiation is the polyadenylate sequence (poly(A) tail) of 30 to 200 adenosine residues at the 3′ termini of most eukaryotic mRNAs (24–27). Other sequences can functionally substitute for a poly(A) tail. For example, the pseudoknot-rich portion of the 3′-UTR of tobacco mosaic virus RNA, which is not polyadenylated, enhances the translation efficiency in vivo (28). The 5′ cap and this pseudoknot-rich domain or a poly(A) tail stimulate translation synergistically (27). In the case of satellite tobacco necrosis virus (STNV), which has an uncapped, nonpolyadenylated, 1239-nucleotide (nt) RNA genome (14, 29), the 3′-UTR facilitates cap-independent translation in vitro of its coat protein gene or heterologous genes in the presence of the viral 5′-UTR (30, 31). Here we report that BYDV-PAV genes are translated cap-independently in wheat germ extracts. A sequence of at most 500 bases, located between 4.5 and 5 kilobases from the 5′ end of the viral genome, stimulates translation of viral or heterologous reporter genes from uncapped RNA by 30- to over 100-fold. This cap-independent translation may also depend on the presence of a portion of the 5′ end of the viral genome. This report should further our understanding of interactions between mRNAs and eukaryotic translation machinery.

MATERIALS AND METHODS

Construction of Internal Deletion Mutants—DNA manipulations were performed essentially as described by Sambrook et al. (32). All plasmids were cloned in Escherichia coli (strain DH100) except when BclI was used to digest the DNA, in which case plasmids were prepared
in dam strain GM39. Restriction sites discussed below are numbered by the base position in the BYDV-PAV genome (7), pAGUS1 (33) was a gift from J. Skuzecki, now at Oregon State University.

Construction of a full-length clone (pPAV6), from which infectious BYDV-PAV genomic RNA can be transcribed in vitro, was described previously (4). To make pPAVM1, pPAV6 was digested with EcoRI to release the EcoRI-EcoRI fragment and religated. The strategy was used to also make pPAVM2, in which the BamHI, BamHI fragment was deleted from pPAV6. To construct pSPM, pPAV6 was first digested with Scal which cuts at base 4515 in the BYDV-PAV cDNA genome and in the amplicin resistance gene in the vector. The resulting 5.5-kilobase fragment was purified by 1% low melting point agarose gel electrophoresis and digested with PstII, and this Scal site in the vector PmlI Scal fragment was ligated with Scal site in the vector Scal site was digested with Scal site was deleted from the PMLI Scal region of the genome, pPAV6 was digested with these enzymes and religated. After gel purification, the Balnl Scal Scal fragment was discarded, and the remaining two fragments were ligated to give rise to pBS6.

### Table 1

<table>
<thead>
<tr>
<th>Restriction endonuclease</th>
<th>5’ Terminus of transcript</th>
<th>Relative translation efficiency</th>
<th>-Fold increase due to capping</th>
<th>-Fold decrease due to 3’ truncation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>SmaI</em></td>
<td>m’GpppG</td>
<td>100</td>
<td>2.3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>ppG</td>
<td>43.4 ± 5.4</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td><em>PstI</em></td>
<td>m’GpppG</td>
<td>78.3 ± 9.7</td>
<td>2.7</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>ppG</td>
<td>29.4 ± 2.3</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td><em>SacI</em></td>
<td>m’GpppG</td>
<td>78.4 ± 17.2</td>
<td>49</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>ppG</td>
<td>1.6 ± 0.1</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td><em>KpnI</em></td>
<td>m’GpppG</td>
<td>37.0 ± 0.9</td>
<td>27</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>ppG</td>
<td>1.0 ± 0.2</td>
<td>43</td>
<td>43</td>
</tr>
</tbody>
</table>

*a* Translation efficiency was based on linearized pPAV6 prior to *in vitro* transcription (see Fig. 1).

*b* Translation efficiency was based on the amount of 39-kDa protein produced from 5 μg of transcript (e.g., 0.2 μg of transcript from *SacI*-linearized pPAV6) in 1 h in wheat germ extract (as in Fig. 1). The relative amount of 39-kDa product from capped mRNA transcribed from *SmaI*-linearized pPAV6 was defined as 100% translation efficiency. Percentages, ± S.D., are from three experiments.

*c* Translation efficiency of capped transcript/(translation efficiency of uncapped transcript from pPAV6 linearized with the same restriction endonuclease).


50-fold less 39-kDa product (Table I, Fig. 1B, lanes 10 and 12) than either their capped forms or the larger uncapped transcripts. Thus, the translation of viral RNA was nearly cap-independent when transcribed from viral cDNA digested with *SmaI*, *BclI*, or *PstI*. In contrast, translation of transcripts from pPAV6 linearized at the *SacI* or *PstI* sites was highly cap-dependent. Thus, a sequence between the *SacI* and the *PstI* site is at least in part responsible for efficient translation in the absence of a 5’ cap.

These experiments also revealed that a more distal portion of the genome appears to be required for efficient 1’ ribosomal frameshifting. As reported previously (4), the 99-kDa product of ribosomal frameshifting can be seen clearly among the translation products of full-length (*SmaI*-linearized) transcript (Fig. 1B, lanes 3 and 4). However, transcripts truncated at the *BclI* site gave less frameshift product, and those with larger deletions yielded no frameshift products at all (Fig. 1B, lanes 7–12). Thus, sequences near the 3’ end of the genome are required for efficient frameshifting at bases 1152–1155. This remarkable phenomenon was unaffected by capping and it will be discussed in a separate publication.

To further define the sequence(s) necessary for cap-independent translation, transcripts synthesized from internal deletion mutants and linearized with various restriction enzymes were translated in wheat germ extracts (Fig. 2). Mutants containing any deletions between the *BalI* and *SacI* sites were still translated cap-independently, if the transcripts were synthesized from *PstI* or *SacI*-linearized templates (Fig. 2). Cap-independent translation was abolished in transcripts linearized at *BamHI* (Fig. 2). Transcripts synthesized from *SacI* and *PstI*-linearized pPAV2, in which the sequence immediately 5’ of *BamHI* was deleted, also failed to permit cap-independent translation. We conclude that (i) no sequences from the *BalI* to *SacI* sites and from *PstI* to 5090 are required for cap-independent translation, (ii) the regions from *SacI* to *BamHI* are required for cap-independent translation, and (iii) reducing the spacing between the *SacI* and *PstI* regions and the 5’ end of the genome did not reduce cap-independent translation. Thus, a region of the RNA between bases 4513 and 5090 is required for BYDVPV RNA to be translated independently of the presence of a 5’-m’G(5’)-ppp(5’)G cap. We define the required *SacI* to *PstI* region as a 3’ translation enhancer (3’-TE).

In the following experiments, the transcripts having intact 3’-TE are called 3’-TE(+), whereas transcripts having no, or an incomplete 3’-TE, are 3’-TE(−).

#### Analysis of Conditions for Cap-independent Translation

The reduced amount of 39-kDa product from uncapped, 3’-TE(−) transcripts could result from (i) a sequence-specific difference in optimal ionic conditions for *in vitro* translation, (ii) sharply decreased stability of the transcript, (iii) inactivation of critical factor(s) involved in translation in the wheat germ extract due to unknown reasons, or (iv) a decreased level of translatability of the transcript. These possibilities were tested. Because different mRNAs can have different optimal potassium ion concentrations (38, 41), these concentrations were varied. At all potassium ion concentrations, uncapped, 3’-TE(−) transcripts gave extremely low amounts of 39-kDa product which was about 25- to 50-fold less than that from uncapped 3’-TE(+) transcripts (data not shown). Thus, the extreme cap dependence of the 3’-TE(−) transcripts could not be compensated for by changes in ionic conditions.

To test the second possibility that 3’-TE(−) transcripts were much less stable than 3’-TE(+) transcripts, their stabilities in the translation reaction were assessed by Northern blot hybridization. Approximately 50% of the transcripts from pPAV6 linearized with either *SacI* (3’-TE(−)) or *SmaI* (3’-TE(+)) remained intact after 1 h of incubation in the wheat germ extract under our standard translation conditions (Fig. 3). Thus, the stabilities of 3’-TE(+) and 3’-TE(−) transcripts were indistinguishable.

To test the third possibility, that the absence of cap-independent translation in the uncapped, 3’-TE(−) transcripts was due to some kind of selective inactivation of the wheat germ translation system, translation of naturally capped brome mosaic virus (BMV) RNAs was observed in the presence of various BYDVPV transcripts. BMV RNA was translated with the same efficiency in the presence of capped or uncapped, 3’-TE(−) or 3’-TE(+) BYDVPV transcripts (Fig. 4, lanes 1 and 6–9). Conversely, the presence of BMV RNA did not affect the translation of capped or uncapped, 3’-TE(−) or 3’-TE(+) transcripts (Fig. 4, compare lanes 2 and 6, 3 and 7, 4 and 8, and 5 and 9). Thus, there was no inhibition of the wheat germ translation machinery by the uncapped, 3’-TE(−) BYDVPV transcript.

The lack of competition between BMV and BYDVPV RNAs verified that the mRNA levels were saturating and thus rate-limiting. All the above results taken together support the hypothesis that the 3’-TE acts to increase efficiency with which ribosomes and translation factors initiate translation of the uncapped viral transcript.

The 3’-TE appears to functionally substitute for a cap structure. Thus, we tested whether addition of free cap analog
Fig. 2. Effect of deletions in BYDV-PAV transcripts on cap-independent translation. Schematic diagram of BYDV-PAV cDNA clones with internal deletions and corresponding transcripts from these clones after linearization with various restriction enzymes. Bold lines below genome organization represent transcribed RNAs containing deletions indicated by the dotted lines. Deletion and 3′ truncation sites correspond to the restriction endonuclease sites indicated below genome organization. The dependence of each transcript on a 3′ cap for efficient translation is indicated at the right. “+” indicates that the translation efficiency (yield of product from 39K ORF or its deleted forms) of uncapped transcripts is no more than 3-fold lower than that of their capped counterparts, and 30–50-fold higher than that of uncapped transcripts indicated by “−”. “+” also indicates that the translation efficiency of uncapped transcripts is 30–50-fold lower than that of their capped counterparts. The 3′-TE is shaded.

Fig. 3. The 3′-TE does not affect transcript stability in wheat germ extracts. pPAV6 was linearized with SmaI or Scal (Fig. 1A) prior to transcription. Transcripts were added to a standard wheat germ translation system as in Fig. 1, with aliquots being removed at indicated time points. Total RNA was extracted from the translation mixture and analyzed by Northern blot hybridization (see “Materials and Methods”). A, uncapped transcripts from SmaI-cut pPAV6. B, uncapped transcripts from Scal-cut pPAV6. C, quantitation of transcripts as in A and B. Data represent averages of two and three experiments for SmaI-linearized (filled circles) and Scal-linearized (open circles) transcripts, respectively.

Inhibited translation of 3′-TE(+) transcripts differently from 3′-TE(−) transcripts. As shown in Fig. 5, twice as much m7G(5′)ppp5′G (27 μM) was required to achieve 50% inhibition of translation of uncapped, 3′-TE(+) transcript as was required for 50% inhibition of capped, 3′-TE(−) transcript (13 μM). Consistent with this, inhibition of translation of the transcript containing both a cap and 3′-TE falls in between. Although translation of capped transcripts was higher than uncapped 3′-TE(+) transcripts in these conditions (Table 1), their sensitivity to inhibition by free cap analog was greater.

The 3′-TE did not stimulate translation of uncapped transcripts in reticulocyte lysates. Uncapped 3′-TE(+) or 3′-TE(−) RNAs both gave about one-eighth as much 39-kDa product as capped 3′-TE(+) and 3′-TE(−) transcripts (Fig. 6). Because reticulocyte lysates were less discriminatory against the un-capped transcripts than were wheat germ extracts, any enhancement by the 3′-TE would have been less extreme than in wheat germ. This relatively less cap dependence in reticulocyte lysates has been observed previously (42).

Fig. 4. Effect of capped and uncapped, 3′-TE(+) and 3′-TE(−) pPAV6 transcripts on translation of BMV RNA. The reactions are the same as in Fig. 1B, except that, in lanes 6–9, 0.1 μg of BMV RNA was added to the translation mixture. Even-numbered lanes contain products of capped transcripts (C); lanes 3, 5, 7, and 9 contain products of uncapped transcripts (U). Lane 1, BMV RNA only; lanes 2, 3, 6, and 7, transcripts from Scal-linearized pPAV6; lanes 4, 5, 8, and 9, transcripts from Scal-linearized pPAV6. Mobilities (in kilodaltons) of BMV translation products and expected major translation product from pPAV6 transcripts (arrow) are at left.

BYDV-PAV Sequences Confer Cap-independent Translation on a Heterologous Gene—To determine the role of the portion of the 39K ORF that was in all the deletion mutants, this ORF was replaced with a nonviral gene. Construct pCIGUS has the 5′-terminal 169 nt of BYDV-PAV, including all 141 bases of the BYDV-PAV 5′-UTR and the first nine codons of the 39K ORF fused in-frame with the E. coli uidA (GUS) gene lacking its own start codon (Fig. 7). In this plasmid, the 3′-UTR of the GUS gene contains the 3′ end of the BYDV-PAV genome from the Scal to the SmaI sites, including the 3′-TE. Because the Scal site was destroyed in construction of pCIGUS, linearization with EcoRI in the GUS gene-derived portion of the 3′-UTR was used to create transcripts lacking all viral sequence in the 3′-UTR. The presence of both the viral 5′ sequence and 3′-TE flanking the GUS gene enhanced translation of uncapped transcript by more than 80-fold (Fig. 7, compare lanes 7 and 9 to
Fig. 5. Inhibition of translation by free cap analog. Capped and uncapped transcripts (4 nM of pPAV6 linearized with Smal (3'-TE(+)) or Scal (3'-TE(-))) were translated in wheat germ extracts containing the indicated amounts of cap analog (mG5'ppp5'G). The 100% relative amount of 39-kDa product is defined for each transcript as the relative radioactivity incorporated in the 39-kDa product (measured using Imagequant 3.22) in the absence of added cap analog. Data represent the averages of two separate experiments.

Fig. 6. Comparison of 3'-TE function in wheat germ and reticulocyte lysates. Yield of 39-kDa product produced in wheat germ (A) or reticulocyte lysates (B) containing 4 nM transcripts from Smal (3'-TE(+)) or Scal (3'-TE(-))–linearized pPAV6 is plotted with the amount of product from capped, 3'-TE(+) transcript defined as 100%. Experiments were repeated three times. Error bars represent 1 S.D. Conditions for translation in rabbit reticulocyte lysates (Promega) were as described previously (5).

lane 5). Unlike in the viral genomic context, capping of these transcripts did not increase their ability to be translated. These capped transcripts gave about 4 times as much product as the capped transcript lacking the 3'-TE (Fig. 7, compare lanes 6 and 8 to lane 4). To test the role of the 5' leader sequence, pT7GUS3'-d2 was constructed. This plasmid contains a 5' leader derived from pGEM5Z(f+) in place of the BYDV-PAV 5' sequence. All transcripts of this plasmid translated poorly in the absence of a 5' cap, whether or not they contained the 3'-TE (Fig. 7). The translation efficiency of transcripts from EcoI CR I–linearized pT7GUS1, which lacks all viral sequences, was similar to those corresponding transcripts synthesized from pT7GUS3'-d2 (Fig. 7, compare lanes 2 and 3 with lanes 10–15). Thus, the 3'-TE does not function in the presence of the vector-derived 5' leader sequence. The 5' viral leader sequence itself can enhance the translation efficiency by about 4-fold relative to the leader derived from the multiple cloning site of pGEM5Z(f+) (Fig. 7, compare lane 4 with 2 and lane 5 with 3). This stimulation is reminiscent of other efficient viral leader sequences such as the tobacco mosaic virus 5' sequence (16).

A poly(A) Tail Does Not Substitute for the BYDV-PAV 3' Translational Enhancer—Because a poly(A) tail confers stability on eukaryotic mRNAs and also enhances translation initiation in vivo (24–27), we compared the effect of a poly(A) tail on translation of GUS from capped and uncapped transcripts. The GUS gene was subcloned from pT7GUS1 into vector pSP64poly(A) which contains a run of 30 adenosine residues in the multiple cloning site, to create plasmids pT7GUS(A+) and pGUSEA1 (Fig. 8). Transcripts from these plasmids differ only in their 5'UTRs. The 5'UTR of the pT7GUS(A+) transcript is derived from the vector, whereas that in pGUSEA1 contains the same 169-base 5'UTR and first 9 codons as pCIGUS. Transcription of either of these plasmids when linearized with EcoRI gives a transcript ending in ApoCCAGAUU. Linearization with EcoI CR I gives an otherwise identical transcript lacking this 3'-terminal sequence. All uncapped transcripts translated very poorly whether or not they contained a poly(A) tail or a viral 5' leader (Fig. 8). In all cases, capped transcripts yielded 20 to 50 times as much GUS as uncapped transcripts, although the amount of GUS protein made from uncapped transcripts was so low that these -fold increases are very approximate. Any increase in translation owing to polyadenylation was 2-fold or less, regardless of whether the transcripts were capped. Thus, the poly(A) tail does not substitute for the 3'-TE, nor does it act synergistically (43) with a 5' cap to stimulate translation in the wheat germ extract translation system.

**DISCUSSION**

The results presented here reveal that sequence(s) within a region near the 3'-end of the genome (bases 4513–5009), of BYDV-PAV RNA confer the ability of a BYDV-PAV gene or a
heterologous gene to be translated very efficiently from uncapped mRNAs in wheat germ extracts. The viral 5' leader (bases 1–169) may also be required, but only one alternative leader was tested. It is possible that other efficient (e.g. viral) leaders may substitute for that of BYDV-PAV. Other known stimulatory sequences include the 5' leaders of tobacco mosaic virus (16), alfalfa mosaic virus RNA4 (17), and potato virus X (18), but these do not replace the need for a cap. Indeed, the 5'-UTR of BYDV-PAV alone stimulates such cap-dependent translation relative to a vector-derived 5'-UTR (Fig. 7). The 5'-UTRs of tobacco etch virus (19), potato virus S (44), and picorna viruses (45) confer cap-independent translation, but do not require the presence of a sequence 3' of the coding region. The poly(A) tail in the 3'-UTRs of most mRNAs stimulates translation, but has only modest effects in vitro as observed by us (Fig. 8) and others (25, 46). Thus, the observation reported here in which the 3'-UTR functionally substitutes for a 5' cap structure requires a revision of the traditional concepts regarding the mechanisms of translation initiation in which mRNA recognition requires only 5'-terminal structures and sequences (47).

The stimulation of translation of uncapped mRNA by the 3'-UTR resembles the behavior of the 3'-UTR of STNV RNA. On STNV RNA, the 5' 150 bases of the 3'-UTR and a portion of the 5'-UTR act together to facilitate cap-independent translation (30, 31) in wheat germ extracts. However, this naturally uncapped RNA (14) differs in several ways from BYDV-PAV RNA. (i) It is only 1239 nt long (29), with a 29-base 5'-UTR, 600-base ORF that encodes coat protein, and a 600-base 3'-UTR. (ii) The 5' stimulatory region in the 3'-UTR is adjacent to the ORF under stimulus in contrast to the 3'-UTR in BYDV-PAV RNA which is separated from the stimulatory (39K) ORF by several ORFs and kilobases. (iii) The 5'- and 3'-UTRs of STNV stimulate translation of heterologous genes (α-globin or GUS) less than the native coat protein gene (30, 31), whereas the BYDV-PAV sequences provided greater stimulation and more complete cap-independent translation of GUS than of viral genes.

The discovery of this cap-independent translation enhancer has led us to wonder whether the 5' end of the BYDV-PAV genome is capped. Previously (7), we proposed that the BYDV-PAV genome contains a VPG because subgroup II luteoviruses have VPGs (6, 21). However, being a subgroup I luteovirus, much of the genome of BYDV-PAV, including the essential replication genes, is more closely related to dianthoviruses which have capped mRNAs (48). Preliminary evidence indicates that neither of these structures is present and that BYDV-PAV RNA is uncapped: the 5' terminus of BYDV-PAV RNA from virions is accessible to alkaline phosphatase and polynucleotide kinase, with or without pretreatment with the cap-removing enzyme, tobacco acid pyrophosphatase. In support of this, uncapped, full-length in vitro transcripts of BYDV-PAV RNA (49) are far more infectious than capped transcripts (34). Furthermore, another group has found that BYDV-PAV RNA seems to lack a VPG.

Absence of a 5' cap would suggest that the 3'-TE is necessary for virus viability. Consistent with this, all deletions in the 3'-TE render the RNA noninfectious in protoplasts (34), even though the proteins encoded by the 50K and 6.7K ORFs in which much of the 3'-TE resides are unnecessary for replication in protoplasts. Even if BYDV-PAV RNA were naturally capped, a role for a cap-independent translation element would not be unprecedented. Naturally capped mRNAs of the immunoglobulin heavy chain binding protein gene and a few other eukaryotic cellular genes contain sequences that allow efficient cap-independent translation in vivo (45).

How can a 3' sequence behave like a cap? Gallie and Tanguay (46) showed that a poly(A) tail is bound by the same factors (elF-4F and elF-4B) that bind the m7G cap (50), but with lower affinity. If these or other factors bound the 3'-TE with higher affinity, it could perhaps function like a cap. The fact that more free cap analog is required to inhibit translation of 3'-TE-containing RNA than capped RNA suggests that the 3'-TE would have a higher binding affinity to initiation factors than cap analog. Furthermore, translation of uncapped RNAs containing a piconaviral internal ribosome entry site requires the presence of cap-binding protein elF-4F (51). Thus, the concept that the same factors can recognize both a sequence and a modified nucleotide (m7G) provides an explanation for how a sequence like the 3'-TE can substitute for a cap structure.

Mechanisms by which the 3'-TE may act by enhancing translation initiation at the 5' end can be envisioned by comparison with STNV RNA. Danthine et al. (30) and Timmer et al. (31) proposed that direct base pairing occurs between small stretches of the 3' translation enhancing domain of STNV RNA and the 5'-UTR to facilitate return of ribosomes that have completed translation to the 5' end of the genome. In BYDV-PAV RNA, there were no obvious, phylogenetically conserved regions in the 5'-UTR to which portions of the 3'-TE might base pair, nor were any striking similarities between STNV and BYDV-PAV primary or secondary structures detected. Danthine et al. (30) identified a potential 18 S ribosomal RNA binding sequence in the 3'-UTR of STNV RNA. Upon sequence comparison of three complete subgroup I luteoviral genomes (52, 53) and partial sequences of nine additional BYDV-PAV isolates (54), we found an intriguing 17-base sequence, beginning at the BamH I site, GGAUCCUGGAAACACGG, that is absolutely conserved. Consistent with the model of Danthine et al. (30), the underlined hexanucleotide has the potential to base pair near the 3' end of 18 S rRNA (55). No conserved, potential 18 S ribosomal RNA binding sequence was identified in the 5'-UTRs. Obviously, further structural comparisons await narrowing down the 3'-TE to its minimal functional size and determining the specific role(s) of sequence(s) in the 5'-UTR.

A possibility remains that the 3'-TE acts by preventing degradation of uncapped mRNA. Because stability of total added mRNA in wheat germ was unaffected by the presence of the

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2 W. A. Miller, unpublished observation.
3 L. Domier, personal communication.
3'-TE (Fig. 3), differential stability could be explained only if just a few percent of the mRNA molecules were actually associated with polysomes and being translated. If this polysomal fraction alone were subject to instability in the absence of 3'-TE, the degradation might not be detected in our Northern blots. In this case, either the 3'-TE or a 5' cap must prevent instability.

Although the mechanism by which a 3' sequence can substitute for a 5'-terminal cap structure is still unclear, the occurrence of 3' sequence-mediated cap-independent translation in BYDV-PAV RNA and the unrelated STNV RNA which have little sequence similarity suggests that these two viruses arrived by convergent evolution at a similar strategy. This provides yet another example of novel mechanisms by which viruses interact with host translational machinery to control their gene expression.

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