Characterization of a potato leafroll luteovirus subgenomic RNA: differential expression by internal translation initiation and UAG suppression

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Northern blot analysis of Solanum tuberosum infected with potato leafroll luteovirus revealed the 6 kb genomic RNA and a major 2.3 kb subgenomic RNA. The 5' end of the subgenomic RNA was located at nucleotide 3653 in an intergenic region located at the centre of the viral genome upstream of three open reading frames (ORFs). Transient expression in tobacco and potato protoplasts of the β-glucuronidase reporter gene fused to various putative regulatory sequences present in the subgenomic RNA was used to study their influence on expression levels. We observed a suppression of the amber stop codon separating the coat protein (CP) gene from a downstream ORF (56K protein), to a level of 0.9% to 1.3%. Translation initiation at the AUG of an ORF (17K protein) which is nested within the CP gene, exceeds translation of the CP gene itself by a factor of 7.

Introduction

Luteoviruses are transmitted by aphids in a persistent manner and replicate tissue-specifically in the phloem of infected plants (for a review see Francki et al., 1985). The icosahedral virus particles contain a single-stranded 6 kb RNA of positive polarity, which is not polyadenylated and possesses a genome-linked small protein (VPg) (Mayo et al., 1982; Harrison, 1984). Recently, cloning and nucleotide sequence analysis have been described for the RNAs of three luteoviruses, barley yellow dwarf (BYDV) (Miller et al., 1988), beet western yellows (BWYV) (Veidt et al., 1988) and potato leafroll (PLRV) (Mayo et al., 1989; van der Wilk et al., 1989; Keese et al., 1990).

The 3'-located gene cluster includes the coat protein (CP) gene, which is separated from a 56K ORF by an amber stop codon. On the basis of homologies of the sequences adjacent to this UAG to those found next to leaky UAG codons in other viruses (Miller et al., 1988; Mayo et al., 1989), as well as from in vitro translation studies (Veidt et al., 1988), it is assumed that the 56K protein is translated by suppression of the CP gene amber stop codon.

Here we show that the expression of genes located on the 3' proximal half of the PLRV genome is from a subgenomic 2.3 kb RNA which contains the 3' proximal gene cluster ORF 3 (M, 23000, 23K, CP), ORF 4 (17K protein) and ORF 5 (56K protein). Translational efficiencies of the various ORFs were tested.

Methods

Preparation of total RNA. Material was harvested from stems and leaves of Solanum tuberosum plants infected with a PLRV field isolate (Sarkar, Hohenheim, F.R.G.). Plant tissue (10 g) was homogenized in 60 ml of a two-phase system consisting of equal volumes of phenol and lysis buffer (1% Triton X-100, 50 mM-MOPS pH 7.0, 50 mM-EDTA, 2 M-urea, 1% 2-mercaptoethanol). After extraction, the aqueous phase was adjusted to 350 mM-NaCl and passed through an ion-exchange column (QIAGEN-pack 100; DIAGEN) equilibrated in buffer A (400 mM-NaCl, 50 mM-MOPS pH 7.0, 15% ethanol). The column was washed with buffer A to remove proteins and polysaccharides. RNA was eluted with a buffer containing 1050 mM-NaCl, 50 mM-MOPS pH 7.0, 15% ethanol, 2 M-urea and precipitated by adding 0.8 volumes of isopropanol.
Northern blot analysis. Samples of 10 μg of total RNA were glyoxalated, separated by electrophoresis in a 1:2:2 agarose gel, transferred to nitrocellulose filters and baked for 2 h at 80 °C (Thomas, 1980). Virus-specific hybridization probes were prepared by isolating DNA fragments from the PLRV cDNA clones pCPL1 and pCPL2 (Tacke et al., 1989) after restriction with appropriate endonucleases (EcoRI, 2173 bp fragment 1, co-ordinates 640 to 2812 according to the published PLRV sequence of Mayo et al., 1989; AatI/SacI, 788 bp fragment 2, co-ordinates 2711 to 3498; AatI/SacI, 987 bp fragment 3, co-ordinates 2711 to 3679; BssHII/EcoRI, 1514 bp fragment 4, co-ordinates 3773 to 5286; see Fig. 1) (Maniatis et al., 1982). These fragments were isolated by electrophoretic separation and electrophoration and labelled to high specificity as described by Feinberg & Vogelstein (1983, 1984). Additionally, an rRNA-specific probe was prepared as follows. A 3417 bp fragment (co-ordinates 3498 to 3777) was isolated from the cDNA clone pCPL1 (Tacke et al., 1989). The 270 bp fragment, forming part of the intergenic region and the initial coding sequences for both CP and 17K, was treated with mung bean nuclease or the Klenow fragment of DNA polymerase, and translationally fused to the GUS gene by insertion into the Neo-restricted and mung bean nuclease-treated vector pRT103GUS (Töpfer et al., 1988).

For chimeric constructs of GUS with PLRV sequences surrounding the UAG stop codon between the genes for the CP and the 56K protein, an AatI–BsrNI fragment, containing the CP gene and additional 218 bp upstream and 21 bp downstream sequences, was isolated from the cDNA clone pCPL1 (Tacke et al., 1989; co-ordinates 3500 to 4433). This fragment was cloned into the vector pRT103GUS between the cauliflower mosaic virus (CaMV) 35S promoter and the GUS gene. The polymerase chain reaction (PCR) was used for primer-dependent in vitro mutagenesis. The upstream primer (UP; antisense strand orientation; Fig. 5) of the primer extension experiment (see above) and the downstream primer (DP; sense strand orientation) 3'-GCCATGTTACGAAACCCGGAUAGGUAGACUCCGGAUCA-5' were employed with circular plasmid DNA as a template, in a PCR in order to delete the central part of the CP gene. A second downstream primer, in which the UAG stop codon was replaced by the GCC codon for alanine, served in a subsequent PCR mutation experiment to replace the amber stop codon. The integrity of all constructs obtained was verified by sequence analysis. From this analysis, a construct was identified which contained an extra nucleotide (T) at the junction of UP- and DP-initiated cDNA which resulted in a frameshift mutation of the CP gene and 17K ORF, respectively, was annealed with 100 μg of total RNA as described above. The nucleic acids were precipitated, resuspended in 300 μl of 50 mM-Tris-HCl pH 8.3, 50 mM-KCl, 6 mM-MgCl2, 100 μM of each dNTP, 5 mM-EDTA, 30 μg/ml ampicillin and 50 μg/ml BSA and incubated for 1 h at 42 °C with 200 units of murine leukemia virus reverse transcriptase. After alkaline hydrolysis (see above), the elongation products were analysed on a 16% sequencing gel (Garoff & Ansorge, 1981).

Transcription initiation mapping. Total RNA (100 μg) was annealed at 47 °C for 20 h to 50 ng of a 361 bp PstI–BssHI fragment (co-ordinates 3417 and 3777) which was 5'-labelled at the BssHI site. Annealing conditions were as described by Weaver & Weissmann (1979). Nucleic acids were precipitated with ethanol and dissolved in 500 μl of 30 mM-sodium acetate pH 4.6, 50 mM-NaCl, 1 mM-ZnCl2, 0.1 mg/ml bovine serum albumin (BSA). Unhybridized single-stranded nucleic acids were degraded by digestion with 400 units of mung bean nuclease for 2 h at 30 °C. After ethanol precipitation, the RNA was hydrolysed in 20 mM-NaOH at room temperature for 4 h. The solution was neutralized with acetic acid and precipitated with ethanol. The reaction products were analysed on 8% sequencing gels (Garoff & Ansorge, 1981) and detected by autoradiography.

For determination of the transcriptional start point by a primer extension experiment, 50 ng of the 5'-32P-labelled oligodeoxynucleotide 5'-TACACCCACCTTTGACATTTCTCCCTAACCAGAC-3' was annealed with 100 μg of total RNA as described above. The nucleic acids were precipitated, resuspended in 300 μl of 50 mM-Tris-HCl pH 8.3, 50 mM-KCl, 6 mM-MgCl2, 100 μM of each dNTP, 5 mM-EDTA, 30 μg/ml ampicillin and 50 μg/ml BSA and incubated for 1 h at 42 °C with 200 units of murine leukemia virus reverse transcriptase. After alkaline hydrolysis (see above), the elongation products were analysed on a 16% sequencing gel (Garoff & Ansorge, 1981).

Chimeric PLRV/GUS constructs. Constructs of the β-glucuronidase (GUS) gene from E. coli (Jefferson et al., 1986) translationally fused to the AUG start codons of the CP gene and 17K ORF, respectively, were prepared as follows. A SacI-BssHI fragment (co-ordinates 3503 and 3777) was isolated from the cDNA clone pCPL1 (Tacke et al., 1989). The 270 bp fragment, forming part of the intergenic region and the initial coding sequences for both CP and 17K, was treated with mung bean nuclease or the Klenow fragment of DNA polymerase, and translationally fused to the GUS gene by insertion into the Neo-restricted and mung bean nuclease-treated vector pRT103GUS (Töpfer et al., 1988).
Results

Characterization of viral RNAs

Total nucleic acids were isolated from PLRV-infected potato plants and purified by ion-exchange chromatography. Northern blot analysis was performed after electrophoretic separation of glyoxalated RNAs by hybridization with $^{32}$P-labelled cDNA probes specific for different regions of the viral genome (Fig. 2). All probes detected the genomic 6 kb RNA (Fig. 2a, upper arrowhead). Probe 3, covering the non-coding intergenic region, revealed a virus-specific RNA with an apparent size of about 2-6 kb, whereas hybridization with a probe to the more 5' located ORF 2b (probe 2) did not react with RNA of that size. With probe 4, carrying sequences 3' distal to the internal region, the 2-6 kb signal was stronger than that obtained with probe 3. This RNA was also detected when a labelled oligodeoxynucleotide, specific for the 3' end of genomic RNA, was used for hybridization analysis (data not shown). The subgenomic RNA was apparently not polyadenylated as it did not bind to oligo(dT)-cellulose. This large subgenomic RNA, the existence of which has already been mentioned by Mayo et al. (1989), will be referred to in the following as subgenomic RNA 1 (sgRNA 1).

sgRNA 1 was further characterized by hybridization with single-stranded riboprobes (probes 5+ and 5- in Fig. 2b). Only $^{32}$P-labelled transcripts of antisense orientation (probe 5-) showed hybridization to RNA from PLRV-infected plants, (lanes 1 and 2) indicating that sgRNA 1 is single-stranded and of positive-sense orientation. The specificity of this probe for PLRV sequences was shown in a Northern blot experiment in which the antisense riboprobe (5-) did not react with total RNA from healthy plants (lanes 3 and 4). The integrity of the plant RNA was proven by rehybridizing the filter with a probe for ribosomal sequences (lane 4, -PLRV).

In some Northern blot experiments additional hybridization signals smaller than 2 kb were detected e.g. by probe 5- (Fig. 2). As the genomic 6 kb RNA was clearly visible in this experiment, we can rule out degradation of the RNA sample prior to electrophoretic separation. In order to test whether these signals were artefacts due to comigration of PLRV RNA with excess cellular RNA, a $^{32}$P-labelled single-stranded sense RNA (sRNA), representing 2-5 kb of the 3' half of the viral genome, was synthesized in vitro. After addition of total RNA from healthy plants or E. coli tRNA, these mixtures, as well as pure sRNA, were glyoxalated and electrophoresed in an agarose gel. In all three lanes, a band corresponding to sRNA is visible (Fig. 2c, arrow), with additional smaller RNA of undefined size at the bottom, presumably due to incomplete RNA synthesis or partial degradation. In lane 1, additional bands were detected that are comparable in size to ribosomal RNAs (visualized by a toluidine-stained gel of total plant RNA; Fig. 2c, lane T). This indicates that some of the PLRV sRNA comigrates with
ribosomal RNAs under the conditions employed. Such comigration of viral RNAs with excess ribosomal RNA was also observed during Northern blot analysis of total RNA extracts from tobacco mosaic virus (TMV)-infected plants (Zaitlin et al., 1983).

Transcriptional start analysis for sgRNA 1

To determine the position of the transcriptional start of sgRNA 1, total RNA from PLRV-infected potato plants was hybridized to a 32P-labelled 365 bp *BssHII/PstI* fragment representing the intergenic region of the viral genome. The resulting RNA/DNA hybrids were treated with mung bean nuclease and the protected labelled DNA was analysed on sequencing gels with appropriate size markers (Fig. 3). Fragments of 125, 126 and 130 nucleotides in size were apparent after autoradiography. Similar heterogeneity in protection experiments has been reported for other systems (see e.g. Sommer & Saedler, 1986).

To characterize further the start of transcription, a primer extension experiment was carried out using a 32P-labelled primer complementary to sequences in the 5' terminus of ORF 3. This was hybridized to total RNA and the template/primer complex was incubated with reverse transcriptase and deoxynucleoside triphosphates. Gel electrophoresis revealed an elongation product of 82 nucleotides in length. This corresponds to the 125 nucleotide fragment detected in the mung bean nuclease protection experiment. This would place the start of transcription at a guanosine residue 40 nucleotides upstream of the AUG translational start codon of ORF 3 (capsid protein), corresponding to residue 3653 in the PLRV sequence (Mayo et al., 1989). The additional two bands visible in the nuclease experiment may represent incomplete digestions of the DNA/RNA hybrids in the presence of the vast excess of cellular RNA, as the primer extension experiment does not show this ambiguity. From this analysis, the size of sgRNA 1 would be only 2334 bases, thus being overestimated in the Northern blot experiment (total length of PLRV genomic RNA 5987 nucleotides, according to Mayo et al., 1989).

The PLRV sequence encompassing the putative transcriptional start site and the untranslated leader of the sgRNA 1 was compared with the corresponding regions upstream of the CP gene AUG start codon of the luteoviruses BWYV and BYDV. Two conserved sequence blocks were evident (Fig. 4). One is located immediately upstream of the AUG start codon whereas the second is composed of a sequence stretch of some 27 nucleotides, rich in uridine residues as noted before by Mayo et al. (1989). This conserved sequence is centred...
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PLRV coat protein gene (ORF 3)

\[
\begin{array}{c}
\text{ATG} \\
\text{UAG}
\end{array}
\]

\begin{align*}
\text{Alul} & \quad \text{BstNI} \\
\text{Pr} & \quad \text{GUS} \\
\text{PCR}
\end{align*}

Fig. 5. Translational fusion of the amber termination codon to a GUS reporter gene. Synthesis of chimeric PLRV/GUS constructs starting from an Alul/BstNI fragment of cDNA clone pCPL1 (Tacke et al., 1989) and the wild-type GUS gene in pRT103GUS (Töpfer et al., 1988). Pr and T are CaMV promoter (35S) and terminator sequences, respectively. UP and DP are oligodeoxynucleotide primers used for PCR-mediated mutagenesis.

around the transcriptional start site of the PLRV sgRNA 1. The sequence UUAUAUU, located 5’ to the transcription start site, is very similar to the sequence UUAUUAUU, which is part of the subgenomic promoter of brome mosaic virus (BMV) and is present in the intergenic region of other RNA plant viruses (March et al., 1988). Additionally, the 5’ untranslated end of sgRNA 1 is rich in adenosine and uracil residues, as observed for the 5’ ends of subgenomic RNAs of other RNA viruses (TMV, Goelet et al., 1982; BMV, Ahlquist et al., 1981; alfalfa mosaic virus, Koper-Zwarthoff et al., 1980).

### Transient expression of chimeric PLRV/GUS constructs

To determine the frequency of suppression of the UAG stop codon separating ORF 3 (CP) and ORF 5 (56K protein), the CP gene and the first 21 bases of ORF 5 were translationally fused to the GUS gene (Jefferson, 1987), with the CaMV 35S promoter and terminator as control sequences (Fig. 5). As GUS activity may decrease in fusion proteins with increasing length of the foreign protein, most of the CP gene (encoding a 208 amino acid coat protein) was internally deleted in a PCR using 5’ UP and 3’ DP terminal primers (Fig. 5). Readthrough of this chimeric construct would result in a GUS fusion protein containing 28 foreign N-terminal amino acids (20 amino acids of CP, one corresponding to the suppressed UAG codon and seven amino acids of the 56K protein). In the final construct, the amber stop codon remained located within the original sequence of 18 bp to the 5’ side and 21 bp downstream (Tacke et al., 1989), which is considered to be sufficient for suppressor tRNA interaction (Bossi, 1983). By an additional PCR mutation, the UAG codon of the construct was changed into the GCC codon for the uncharged amino acid alanine. This GCC construct served as a positive control for GUS expression. A third GUS construct, carrying an out-of-frame mutation in the PLRV sequence preceding the GUS coding sequence, was used as a negative control in order to account for any putative GUS activity resulting from internal translation initiation.

These constructs were tested for transient expression of GUS activity in potato and tobacco protoplasts. The data summarized in Table 1 were obtained with different protoplast preparations and each value represents the mean of four experiments. Both solanaceous plant species allowed in vivo suppression of the amber stop codon leading to the synthesis of GUS enzyme at a frequency of 0-9 to 1-3%, normalized with respect to the two control constructs. Although tobacco is not a natural host for PLRV (Rich, 1983), isolated tobacco mesophyll protoplasts support PLRV multiplication (Takanami & Kubo, 1979; Mayo et al., 1982). Our results also showed no significant difference between host species.

The second approach to the study of translational signals present on the sgRNA 1 was the determination of the relative expression of the CP gene and ORF4 (17K protein).

Table 1. Results of transient GUS expression experiments in potato and tobacco protoplasts using the constructs described in Fig. 4.

<table>
<thead>
<tr>
<th>Protoplasts</th>
<th>Construct</th>
<th>pmol MU/mg protein/min*</th>
<th>Readthrough (%)</th>
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<tbody>
<tr>
<td>Potato</td>
<td>GCC</td>
<td>19 278</td>
<td>1-15</td>
</tr>
<tr>
<td></td>
<td>UAG</td>
<td>221</td>
<td>0-93</td>
</tr>
<tr>
<td></td>
<td>GCC</td>
<td>26 177</td>
<td>1-06</td>
</tr>
<tr>
<td></td>
<td>UAG</td>
<td>243</td>
<td>1-11</td>
</tr>
<tr>
<td></td>
<td>GCC</td>
<td>27 215</td>
<td>1-24</td>
</tr>
<tr>
<td></td>
<td>UAG</td>
<td>289</td>
<td>0-92</td>
</tr>
<tr>
<td></td>
<td>GCC</td>
<td>29 300</td>
<td>1-24</td>
</tr>
<tr>
<td></td>
<td>UAG</td>
<td>294</td>
<td>1-24</td>
</tr>
<tr>
<td>Tobacco</td>
<td>GCC</td>
<td>38 744</td>
<td>1-24</td>
</tr>
<tr>
<td></td>
<td>UAG</td>
<td>38 480</td>
<td>0-92</td>
</tr>
<tr>
<td></td>
<td>GCC</td>
<td>37 626</td>
<td>0-92</td>
</tr>
<tr>
<td></td>
<td>UAG</td>
<td>37 776</td>
<td>0-92</td>
</tr>
<tr>
<td></td>
<td>GCC</td>
<td>38 871</td>
<td>1-24</td>
</tr>
<tr>
<td></td>
<td>UAG</td>
<td>485</td>
<td>1-25</td>
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* GUS activity of the GCC construct (GCC replacing the UAG stop codon) was set equal to 100% and percentage of readthrough determined for the UAG construct after subtraction of the background value measured in control experiments with an out-of-frame mutant (see Results). Background activities had a mean value of 55 pmol MU/mg protein/min for potato protoplasts and 32 pmol MU/mg protein/min for tobacco protoplasts.
protein). The latter gene is nested within the CP gene coding sequence (Fig. 1) and has its first AUG translational start codon only 22 nucleotides downstream of the CP gene AUG start and a second AUG six nucleotides further downstream (Tacke et al., 1989). A 270 bp SacI/BssHII cDNA fragment was treated with mung bean nuclease for translational fusion of ORF 3 with the GUS reporter gene, or with the Klenow fragment of DNA polymerase for translational fusion of ORF 4 with the GUS gene (Fig. 6). The resulting constructs therefore expressed a GUS protein with amino-terminal fusion of either 29 amino acids (translational initiation at the CP gene AUG; CP construct) or either 20 or 18 amino acids (translational initiation at one of the two 17K AUGs; 17K construct). GUS activity was tested by transient expression in potato and tobacco protoplasts, as described above, with the vector pRT103GUS (encoding GUS wild-type protein) as the positive control. The results demonstrate (Table 2) that the 17K-GUS construct induced GUS activity approximately sevenfold greater than the CP-GUS construct, even through the 17K AUGs are located downstream of the CP gene AUG codon. Even more surprisingly, the 17K-GUS construct induced more GUS activity than did the wild-type GUS protein expressed from the control construct pRT103GUS (110% of the wild-type GUS protein construct activity).

### Table 2. GUS activity of 17K/CP constructs in protoplasts

<table>
<thead>
<tr>
<th>Protoplasts</th>
<th>Construct</th>
<th>pmol MU/mg protein/min*</th>
<th>Ratio CP:17K</th>
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<tr>
<td>Potato</td>
<td>17K-GUS</td>
<td>388630</td>
<td>1:6-8</td>
</tr>
<tr>
<td></td>
<td>CP-GUS</td>
<td>57127</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17K-GUS</td>
<td>237023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP-GUS</td>
<td>33653</td>
<td>1:7</td>
</tr>
<tr>
<td>Tobacco</td>
<td>17K-GUS</td>
<td>51466</td>
<td>1:6-7</td>
</tr>
<tr>
<td></td>
<td>CP-GUS</td>
<td>7734</td>
<td></td>
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<tr>
<td></td>
<td>17K-GUS</td>
<td>108254</td>
<td>1:6-9</td>
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<tr>
<td></td>
<td>CP-GUS</td>
<td>15653</td>
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</table>

* Results obtained as in Table 1.

Negative and/or positive effects of the N-terminal GUS modification present in both chimeric constructs could have contributed to the 7:1 ratio in favour of 17K protein synthesis. However, another possible factor is that the second putative AUG start codon of the 17K protein (Fig. 6) is in a sequence that more closely resembles the consensus sequence for optimal translation initiation in plants (Lütke et al., 1987) than that of the CP gene AUG.

### Discussion

Sequence analysis of the RNA of two luteoviruses, BWYV (Veidt et al., 1988) and PLRV (Mayo et al., 1989; van der Wilk et al., 1989), has identified a conserved cluster of three genes, ORF 3 (CP gene), ORF 4 (17K protein) and ORF 5 (56K protein), in the 3' half of the genomic RNA (Fig. 1). Earlier attempts to translate the CP gene from genomic PLRV RNA in vitro have failed (Mayo et al., 1982), suggesting that the CP gene is translated from a subgenomic RNA, similarly to those of several other RNA plant viruses (Davies, 1985). Luteoviral RNAs smaller than unit length have been reported for BYDV (3-0 kb; Gerlach et al., 1987) and BWYV (2.9 kb; Falk et al., 1989). This communication characterizes a subgenomic RNA detected in PLRV-infected potato plants of 2.3 kb in length (sgRNA 1). The subgenomic PLRV RNA is 3' coterminal with PLRV genomic RNA and is transcribed from the intercistronic region. Its transcriptional start maps at the guanosine residue within the uridine-rich sequence UAUUUGGUUUC, a region which is highly conserved with respect to nucleotide sequence (Fig. 4) as well as distance (34 nucleotides) from the CP gene initiation codon in the RNAs of PLRV, BWYV and BYDV. By analogy to PLRV, this region may therefore represent part of the subgenomic promoter for all luteoviruses, with A presumably being the start of transcription for the subgenomic RNAs of BWYV and BYDV. As pointed...
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out by Mayo et al. (1989), an additional nucleotide sequence is conserved within the intergenic region of the three luteoviruses PLRV, BWYV and BYDV. For PLRV, this sequence is located some 50 nucleotides upstream of the transcription start site described in this paper. If the size of the PLRV subgenomic promoter is similar to that of BMV (extending at least 74 nucleotides upstream of the transcriptional start site; French & Ahlquist, 1988), then these conserved sequences might also be part of the PLRV subgenomic promoter.

Using constructs containing parts of the PLRV genome fused to the GUS gene, we observed suppression of the amber stop codon separating the CP gene and ORF 5 (Fig. 1) in both tobacco and potato protoplasts. GUS activity was from 0.9 to 1.3% of the level measured for a construct in which the UAG stop codon had been replaced by the alanine codon GCC. These and other data (see below) provide molecular evidence that protoplasts of the non-host tobacco can support PLRV replication by expression of a suppressor tRNA capable of recognizing the PLRV UAG codon, and possibly interacting with flanking sequences. Tobacco tRNAs promoting suppression of UAG stop codons of TMV RNA have been identified and characterized by sequence analysis (Beier et al., 1984a, b). It will be interesting to see whether these tobacco suppressor tRNAs will promote readthrough of PLRV subgenomic RNA in vitro.

The low amount of CP/ORF 5 readthrough product measured in protoplasts (Fig. 5) may reflect a possible regulatory role of the fusion protein, or the released ORF 5 product, during virus replication, either in the infected host plant or in the aphid vector that transmits the virus. Such regulatory effects of readthrough products are known for E. coli phage Qβ replication. The Qβ CP gene terminates in a leaky stop codon and readthrough occurs at a frequency of about 2% (Weiner et al., 1973). In the absence of readthrough, infectious phage particles are not formed (Hofstetter et al., 1974). In this context it should be noted that the PLRV 56K protein is detected on purified virus particles by monospecific antisera directed against this protein (E. Tacke, unpublished results). This protein could possibly play an important role in transmission by the insect vector Myzus persicae.

Our data suggest that, in contrast to low level ORF 5 expression by UAG readthrough, translation initiation at an internal AUG of the sgRNA 1 to produce the ORF 4 17K protein is very efficient and exceeds by sevenfold the synthesis of CP in both potato and tobacco protoplasts. These values, however, may not necessarily reflect the actual accumulation in vivo, as the CP is sequestered for morphogenesis of progeny virus particles and the 17K protein may be subject to high turnover rates. Experiments are under way to study the synthesis and distribution of this non-structural protein in transgenic as well as virus-infected plants by the use of monospecific antisera.

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References


