In vitro Transcription and Translation of Potato Mop-Top Pomovirus RNA 3: Coat Protein and Read-through Cistron

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Abstract: RNA transcripts from Spe-1 linearised PMTV-T RNA 3 cDNA clone were translated in wheat germ extract system. Two major translation products of 19.7K and 65K correspond to the predicted translation products of RNA 3: 19.7K produced by coat protein and 65K by read-through of translation termination codon. The mean density of the coat protein band was 3.253 as determined by Whole Band analysis of different exposures of autoradiographs. Similarly, the mean density of the coat protein plus read-through protein band was 0.209. This gives a ratio of 0.064 for the density of the coat protein plus read-through protein: coat protein. However, there are three methionine residues in the coat protein and 8 in the coat protein plus read-through protein. Normalising for this gives a ratio of 0.024 for amount of coat protein plus read-through protein: coat protein. In other words -2.4 percent of the ribosomes which reach the coat protein gene termination codon suppress the termination and go on to produce coat protein plus read-through protein.

Key word: Potato mop-top furovirus, transcription translation, coat protein, red-through protein

Introduction
Potato mop-top furovirus (PMTV) has fragile tubular rod-shaped particles of two predominant lengths (100-150 nm and 250-300 nm), a tripartite, single-stranded plus RNA genome (Harrison and Jones, 1970; Scott et al., 1994) and is a species of the genus Pomovirus (Torrance and Mayo, 1997). PMTV occurs in potato growing regions of Northern and Central Europe, the Andean region of South America, China, Japan, and in potato growing regions of Canada. PMTV is transmitted by the plasmodiophorid fungus genus (Harrison and Jones, 1970; Scott et al., 1994) and is a species of the genus (Torrance and Mayo, 1997). PMTV occurs in potato growing regions of Northern and Central Europe, the Andean region of South America, China, Japan, and in potato growing regions of Canada. PMTV is transmitted by the plasmodiophorid fungus (Harrison and Jones, 1970; Scott et al., 1994).

Materials and Methods

In vitro transcription: PMTV RNA 3 cDNA plasmid clone, pPMTV-21 (Kashiwazaki et al., 1995) was linearised by digestion with Spe-1. RNA transcripts were produced with a T3 RNA polymerase using an in vitro transcription kit (Stratagene). The transcription reaction contained 5 µl of 5 x transcription buffer (200 mM Tris-ph 8.0; 40 mM MgCl2; 10 mM spermidine; 250 mM NaCl), 1 µg restricted plasmid (pPMTV-21) DNA template, 1 µl 10 mM rATP, 1 µl 10 mM rCTP, 1 µl 10 mM rGTP, 1 µl 1 mM rUTP, 1 µl ‘CAP’ (m7GpppG), 1 µl 0.75 M dithiothreitol (DTT), 1 µl RNase inhibitor (RNAguard TM, Pharmacia) and 10 units of 13 DNA polymerase (1 µl of 1/5 dilution). The final volume was adjusted to 25 µl with DEPC-treated water. The transcription reaction was incubated at 37°C for 30 min in a water bath. After incubation, the RNA transcript was extracted with phenol:chloroform (1:1), precipitated with ethanol and resuspended in DEPC-treated water.

In vitro Translation: Two microlitres of transcription reaction (above) were used for translation in a wheat germ extract in vitro translation kit (Promega). The translation reaction contained 25 µl wheat germ extract; 2 µl reaction buffer; 1 µl 1 mM amino acid mixture (minus methionine), 4 µl 35S-methionine (1,000 Ci/mmol at 10m Ci/µl); 1 µl RNAsin ribonuclease inhibitor and 2 µl transcription reaction. The final volume was maintained at 50 µl with nuclelease-free water and incubated at 30°C for 120 min.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis: The translated protein was denatured with 2 percent SOD, 1 percent 2-mercaptoethanol, 50 mM Tris-HCl, pH 8.8, 15 percent sucrose at 100°C for 5 min and separated by 12.5 percent PAGE as described by Fransen et al. (1982). Gels were stained in 0.2 percent Coomassie Brilliant Blue R in 50 percent methanol, 7 percent acetic acid and destained in 50 percent methanol, 3.8 percent acetic acid.

Autoradiography and gel analysis: After staining, the gels were soaked twice in water for 15 min and dried on filter paper (Whatman 3 MM) using a gel dryer (Hedodrier, Heto Lab Equip., Denmark). Multiple exposures were made to X-ray film (Fuji RX) at room temperature. Analysis of autoradiographs was performed using a Kodak Megaplus CCD high-resolution Video Camera linked
Results

In vitro transcription of coat protein and coat protein read-through gene: A cDNA plasmid clone of PMTV RNA 3 linearised with Spe was transcribed with T3 RNA polymerase. Figure 1 shows the production of RNA transcripts at time zero (T0) and after 1 h (T60) at 37°C.

Analysis of coat protein and coat protein/read-through gene in vitro translation product: RNA 3 transcripts were translated in wheat germ extract system for 90 min and analysed by SDS-PAGE followed by autoradiography. Two major translation products were observed. Fig. 2 shows the two major proteins, 19.7K and 65K correspond to the predicted translation products of RNA 3, 19.7K product produced by coat protein and 65K by read-through of translation termination codon.

The mean density of the coat protein band was 3.253 as determined by whole band analysis of different exposures of autoradiographs. Similarly, the mean density of the coat protein plus read-through protein band was 0.209. This gives a ratio of 0.064 for the density of the coat protein plus read-through protein: coat protein. However, there are three methionine residues in the coat protein and 8 in the coat protein plus read-through protein. Normalising for this gives a ratio of 0.024 for amount of coat protein plus read-through protein: coat protein. In other words, 2.4 percent of the ribosomes which reach the coat protein gene termination codon suppress the termination and go on to produce coat protein plus read-through protein.

Discussion

In this paper, we present results of the in vitro translation of PMTV RNA 3 coat protein and coat protein/read-through protein genes. It is assumed that a common initiation codon is used for the viral coat protein gene and for the coat protein/read-through gene. Two major translation products of 19.7K and 65K correspond to the predicted translation products of RNA 3: 19.7K produced by the coat protein and 65K by read-through of the coat protein termination codon. Measurement of the relative intensities of these two products on autoradiographs indicated that 2.4 percent of the ribosomes which reach the coat protein gene termination codon suppress the termination and produce coat protein plus read-through protein. Similar strategy of genome expression involving a leaky termination codon has been reported for number of plant viruses (Joshi and Haenni, 1984). RNA 2 of SBWMV, like BNYVV RNA 2 (Ziegler et al., 1985) and PMTV RNA 3, can undergo translational suppression of the termination codon of its 5'-terminally situated coat protein cistron to produce longer read-through polypeptides in addition to the viral coat protein (Hsu and Brakke, 1985; Shirako and Ehara, 1986).

Cell-free translation of peanut clump virus (PCV; Indian peanut clump virus, IPCV), another furovirus showed that RNA 2 encodes the viral coat protein but does not undergo read-through to produce a longer polypeptide (Mayo and Reddy, 1985) indicating that it may be different from BNYVV, SBWMV and PMTV. It is believed that 39K protein may be involved in vector transmission (Herzog et al., 1995) and that of BNYVV (Bouzoubaa et al., 1986; Tamada and Kusume, 1991). We detected 543nt deletion in read-through domain of PMTV RNA 3 and this mutated isolate (PMTV-T, Harrison and Jones, 1970) could not be acquired and transmitted by the single cystosoral culture of S. subterranea f. sp. subterranea (Reavy et al., 1998; Arif et al., 1993).
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1999), while a wild-type isolate (PMTV-S, Arif et al., 1994) was successfully acquired and transmitted by the same fungal culture (Arif et al., 1995; 1999).

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References


