Evidence for the role of subgenomic RNAs in the production of potato virus S coat protein during in vitro translation

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Purified virus particles of potato virus S (PVS) when disrupted yielded only one readily resolved band of 7.5 kb in ethidium bromide-stained agarose gels. However Northern hybridization of viral RNA, probed with a clone specific to the viral coat protein gene, revealed a region of subgenomic RNAs of approximately 1.3 kb. Sucrose gradient fractionation of subgenomic RNA revealed that it coded for viral coat protein when translated in vitro in rabbit reticulocyte lysate. Virus particle lengths suggest that this subgenomic RNA may be encapsidated in 100 to 220 nm particles.

Recently the 3' nucleotide sequences of two carlaviruses, potato virus M (PVM) (2630 nucleotides) and an Andean strain of potato virus S (PVS) (3553 nucleotides) (Rupasov et al., 1989; MacKenzie et al., 1989) have been published. In both papers the role of subgenomic RNAs in the translation of the 3' open reading frames has been suggested, but to date no evidence has been reported to support the existence of these putative subgenomic RNAs. In our analyses of the in vitro translation of PVS RNA presented here, we found evidence for the role of an encapsidated subgenomic RNA species coding for the coat protein gene.

An isolate of PVS was propagated in the potato cultivar Orion and purified from infected leaves as described by Foster & Mills (1990). Particle lengths of purified virus were determined from electron micrographs of particles negatively stained with 2% phosphotungstic acid. A distinct population was apparent at 660 nm, with secondary peaks at approximately 220 nm and 100 nm (Fig. 1a). This correlates well with previously published particle length measurements of purified virus (Koenig, 1982) and virus particles found in leaf dips (Rosc. 1983).

PVS RNA isolated from purified virus particles by the method of Shields & Wilson (1987) was analysed on 0.8% agarose gels under non-denaturing conditions (Maniatis et al., 1982) and on denaturing formaldehyde–agarose gels as described by Meinkoth & Wahl (1984). A clone of 1350 nucleotides covering the region from 100 nucleotides upstream from the initiation codon of the coat protein gene through to the poly(A) tail was 32P-labelled (Pharmacia oligolabelling kit) and used to hybridize with viral RNA transferred to nitrocellulose as described by Perbal (1984). The nature of the clone was confirmed by sequence analysis (data not shown) and comparison with sequence data reported by MacKenzie et al. (1989).

Results showed two distinct hybridization bands of approximately 7.5 kb (genomic RNA) and 1.3 kb in addition to a faint RNA species of 2.5 kb (Fig. 1c). BMV RNAs transferred to nitrocellulose from the same gel and hybridized with randomly primed single-stranded cDNA to BMV were used as molecular size markers.

To investigate the translation products of total viral RNA, RNA from purified virus preparations was translated in vitro in rabbit reticulocyte lysate. Translation mixtures contained 66% (v/v) reticulocyte lysate (P & S Biochemicals), 82 mM-KCl, 0.4 mM-MgCl2, 8 mM-creatine phosphate, 40 μM each of essential amino acids except methionine, 1 mCi/ml L-[35S]methionine (800 μCi/mmol; Amersham), 1 unit/μl human placental ribonuclease inhibitor (Amersham) and 40 μg/ml RNA. All incubations were at 30 °C in a standard reaction volume of 50 μl. Translation products were separated on 12-5% polyacrylamide gels (Laemmli, 1970) and visualized by autoradiography. Antiserum to PVS (Bioreba
Fig. 1. (a) Particle length distribution of purified PVS particles. The major population is indicated at 660 nm with two smaller secondary peaks at 220 nm and 100 nm. (b) Analysis of viral RNA by agarose gel electrophoresis. RNA extracted from purified virus particles was electrophoresed in 0.8% non-denaturing agarose gels. Lane 1, size markers from TMV (1 µg) and BMV (1 µg); lane 2, PVS RNA (1 µg). (c) Northern hybridization of RNA (sizes in kb) extracted from purified virus particles hybridized with a 1.3 kb gel-purified clone, covering the coat protein gene, oligolabelled with $[^{32}\text{P}]\text{dCTP}$.

AG) was used for immunoprecipitation essentially as described by Mayo & Reddy (1985).

Time course experiments showed the early appearance of significant quantities of 34K protein after only 10 min incubation (Fig. 2). The 34K product was confirmed as viral coat protein by immunoprecipitation with PVS antiserum. Small quantities of a larger 44K peptide also immunoprecipitated, confirming the results of MacKenzie et al. (1989). Production of large quantities of coat protein during in vitro translation of PVS RNA does not, however, support the results reported for another carlaviruse, potato virus M (Szybiak & Legocki, 1981), where little or no coat protein was synthesized. After 60 min incubation a broad size range of peptides was produced with $M_t$ up to 190K.

To investigate the translation products of genomic RNA and the 1.3 kb subgenomic RNA, total viral RNA was fractionated on 10 to 50% sucrose gradients as described by Harbison et al. (1984). Small molecular size RNA fractions, containing the 1.3 kb subgenomic RNA, directed the synthesis of the 34K coat protein. Fractions containing slightly larger RNA directed the synthesis of the faint immunoprecipitable 44K peptide and small quantities of 26K and 50K proteins in addition to the
Fig. 3. Translation products of the 1-3 kb and 7-5 kb RNAs. Lanes 1 and 2, translation products of fractions from sucrose gradients containing low molecular size RNA equivalent to the 1.3 kb subgenomic RNA. Lane 3, translation products of the 7-5 kb genomic RNA. Lane 4, translation of unfractionated viral RNA. The position and size of the coat protein are indicated (closed arrow).

34K coat protein (Fig. 3, lanes 1 and 2). Genomic RNA directed the synthesis of a 190K peptide with smaller quantities of 90K and 150K products (Fig. 3, lane 3).

The evidence presented here reinforces the overall similarity in genome organization between PVS and those members of the potexviruses group which have encapsidated subgenomic RNA encoding the coat protein (Forster et al., 1987; Short & Davies, 1983).

Further work is required to investigate the presence of subgenomic RNA species in infected tissue and possible expression strategy of genomic RNA.

References


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