Evidence for translation of apple stem grooving capillovirus genomic RNA

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Apple stem grooving virus (ASGV) RNA was translated in a rabbit reticulocyte lysate system and shown to direct the synthesis of several polypeptides of Mr ranging from 200K to 43K. A polypeptide of 200K was a major product, but no polypeptide with electrophoretic mobility the same as that of the ASGV coat protein was synthesized. Immunoprecipitation experiments showed that a polypeptide of 200K was selectively precipitated by antiserum against purified ASGV. These results indicate that ASGV coat protein is translated as part of a 200K polyprotein.

Apple stem grooving virus (ASGV) is distributed worldwide in apple trees (Lister, 1970; Németh, 1986). The virus is one of the causative agents of apple topworking disease in Japan (Yanase, 1974, 1981) and induces decline syndrome in Mitsuba Kaido (Malus sieboldii) plants, which are used as rootstocks of apple trees in Japan (Yanase, 1981). ASGV is also reported to infect pear and apricot trees (Németh, 1986; Motoshima et al., 1983; Sawamura et al., 1988; Takahashi et al., 1990).

ASGV is the type member of the Capillovirus group, which also includes potato virus T and possibly citrus tatter leaf virus (Francki et al., 1991; Salazar & Harrison, 1978; Nishio et al., 1989). It has very flexuous thread-like particles of approximately 600 to 700 nm in length and 12 nm in diameter (De Sequeira & Lister, 1969; Lister, 1970), and contains a polyadenylated plus-sense ssRNA of Mr 2.30 x 10^6 and a single coat protein of Mr 27000 (Yoshikawa & Takahashi, 1988). In ultrathin sections, the virus particles are present either singly or as aggregates in the cytoplasm of mesophyll as well as phloem parenchyma cells (Ohki et al., 1989). No data on the genome structure and gene expression strategy of any capillovirus have been reported. In this paper we report the results of in vitro translation of ASGV RNA.

ASGV (isolate P-209) was propagated in Chenopodium quinoa and purified as described previously (Yoshikawa & Takahashi, 1988). Viral RNA was extracted from purified virus by dissociation in 2% SDS for 15 min at room temperature, followed by extraction with phenol/chloroform. RNA was precipitated with 70% ethanol, dried in vacuo and suspended in water.

Rabbit reticulocyte lysate (BRL) was used for in vitro translation. The reaction conditions were similar to those reported previously (Yoshikawa & Takahashi, 1989). Reaction mixtures (usually 30 µl) contained 10 µl lysate, 8 µM-haemin, 17 µg/ml creatine phosphate, 25 mM-HEPES pH 7.2, 0.3 mM-CaCl₂, 0.7 mM-EGTA, 0.17 mM-DTT, 0.3 to 0.5 µg ASGV RNA, 5 µCi [³⁵S]methionine (approximately 1500 Ci/mmol; Amersham) and all other essential amino acids at 50 µM. Magnesium acetate (1 mM) and potassium acetate (87 mM) were also added. The mixture was incubated at 30 °C for 1 h. After translation, samples were mixed with an equal volume of 2 x loading buffer (4% SDS, 2% 2-mercaptoethanol, 100 mM-Tris–HCl pH 8.8, 30% sucrose) and heated at 100 °C for 5 min. The translation products were analysed by electrophoresis in a 10 to 20% linear gradient SDS–polyacrylamide gel using the buffer system of Laemmli (1970). For fluorography, gels were soaked in 1 M-sodium salicylate for 60 min, dried onto Whatman 3MM paper and exposed to Fuji RX X-ray film at -80 °C for 1 to 2 days (Shirako & Ehara, 1986).

Immunoprecipitation of translation products was conducted according to Sambrook et al. (1989) using Protein A–Sepharose (Amersham). The reaction mixture (20 µl) was adjusted to 200 µl with NET gel buffer (50 mM-Tris–HCl pH 7.5, 150 mM-NaCl, 0.1% NP40, 1 mM-EDTA 0.25% gelatin, 0.02% sodium azide) and incubated with 10 µl of antiserum against purified ASGV overnight at 4 °C. After the addition of 50 µl of Protein A–Sepharose (10% in NET gel buffer), the mixture was incubated for 1 h at 4 °C. The Sepharose, collected by centrifugation, was suspended in 1 ml of NET gel buffer and incubated for 20 min at 4 °C. This step was repeated three times. Finally, the Sepharose was resuspended in 1 x loading buffer and heated at 100 °C for 5 min. Electrophoresis and fluorography were done as described above.

In the rabbit reticulocyte lysate, ASGV RNA directed the synthesis of several polypeptides of Mr ranging from 200K to 43K (Fig. 1, lane 2). These polypeptides were not synthesized in a reaction mixture without ASGV RNA.
Fig. 1. Fluorogram of \textit{in vitro} translation products encoded by ASGV RNA in a rabbit reticulocyte lysate. Lane 1, no RNA control sample; lane 2, translation products of ASGV RNA; lane 3, ASGV coat protein; lane 4, Mr standards consisting of a mixture of myosin (200K), phosphorylase b (97.4K), bovine serum albumin (68K), ovalbumin (43K), carbonic anhydrase (29K), $\alpha$-lactoglobulin (18.4K) and lysozyme (14.3K). Lanes 3 and 4 were stained with Coomassie blue. (Fig. 1, lane 1). A polypeptide of 200K was a major product, accounting for about 80\% of the total coding capacity of ASGV RNA. No polypeptide with electrophoretic mobility identical to that of ASGV coat protein was synthesized in several repeat experiments. In time course experiments, significant changes in the amount of 200K polypeptide and in its ratio to other products of lower Mr were not found in samples taken between 60 min and 120 min of incubation. Addition of DTT, reported to promote the proteolytic cleavage of comoviruses (Pelham, 1979; Xiong \textit{et al.}, 1988), to the reaction mixture at a final concentration of 5 mM produced an electrophoretic pattern of synthesized polypeptides identical to that of a reaction mixture containing 0.17 mM-DTT (data not shown).

Immunoprecipitation experiments using antiserum against ASGV revealed that a polypeptide of 200K was selectively precipitated by the antiserum (Fig. 2, lane 2). No translation product was precipitated by a preimmune antiserum (Fig. 2, lane 3). The addition of unlabelled coat protein (5, 10 and 20 $\mu$g) as a competitor prior to immunoprecipitation significantly reduced the precipitation of labelled 200K polypeptide (data not shown). These results indicate that ASGV coat protein is translated as part of a 200K polyprotein. We have recently found that a coat protein gene is located in the 3'-terminal region of the ASGV genome by using an expression vector in \textit{Escherichia coli} (unpublished results). Therefore, a polyprotein of 200K may be the primary translation product, the products of lower Mr arising by premature termination of translation.

We then investigated whether the 200K protein could be detected \textit{in vivo} by immunoblot analysis of ASGV-infected and healthy \textit{C. quinoa} leaves using antiserum against purified ASGV. Infected and healthy leaves (0.5 g) were homogenized with 0.5 ml of 50 mM-phosphate buffer pH 8.0. After centrifugation, the supernatants were mixed with 2 $\times$ loading buffer and heated at 100 $^\circ$C for 5 min. Samples (20 $\mu$l) were electrophoresed in a 10\% SDS-polyacrylamide gel and blotted onto a nitrocellulose membrane. Anti-ASGV antiserum (diluted 1:2000) and an alkaline phosphatase-conjugated goat anti-rabbit IgG (Tago) diluted 1:2000 were used as the first and second antibodies, respectively. Colorimetric detection steps were carried out as described by Yoshikawa \textit{et al.} (1986).


(Received 8 December 1991; Accepted 28 January 1992)