Nucleotide sequence of raspberry bushy dwarf virus RNA-3

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A nucleotide sequence is reported for RNA-3, the smallest of the three major RNA species found in particles of raspberry bushy dwarf virus (RBDV). The sequence of 946 nucleotides contains a single large open reading frame which encodes an Mr 30509 polypeptide. In vitro translation of RNA-3 yielded an Mr 30000 product that reacted specifically with antiserum to RBDV particles and we conclude that the amino acid sequence deduced from the sequence of RNA-3 is that of the RBDV coat protein, or an immediate precursor of it. No affinities were detected by comparing the nucleotide sequence of RNA-3 with the sequences of other plant viruses.

Raspberry bushy dwarf virus (RBDV; Murant, 1976) infects and causes disease throughout the world in wild and cultivated Rubus plants, especially red raspberry (R. idaeus) (Murant, 1987). RBDV is transmitted in association with pollen from infected plants both to the seed and to the pollinated plant (Murant et al., 1974); and this is the only known method of natural spread.

RBDV particles are about 33 nm in diameter and appear slightly flattened in electron micrographs of negatively stained preparations (Barnett & Murant, 1970). The particles sediment as a broad band (115S) in sucrose gradients (Murant, 1975) and are readily disrupted, for example by as little as 0-01% SDS (Murant, 1976). The particle coat protein has an Mr of about 30000. These particle properties resemble those of ilarviruses which also are transmitted in association with pollen from infected plants. However, RBDV particles differ from those of ilarviruses in that they contain three, not four, RNA species with $M_r$ ($\times 10^{-6}$) of about 2 (RNA-1), 0-8 (RNA-2) and 0-3 (RNA-3) (Murant, 1975). Murant et al. (1986) found that RBDV-infected Chenopodium quinoa leaves contain dsRNA species equivalent in size to replicative forms of RNA-1 and RNA-2 but not RNA-3; they speculated that RNA-3 may be derived during virus multiplication from one of the other RNA species. In this paper we report the nucleotide sequence of RNA-3 and present evidence to confirm our preliminary report (Murant et al., 1986) that RNA-3 is an mRNA for the coat protein of RBDV.

RBDV isolate RI5 (Murant et al., 1986) was propagated in C. quinoa and the virus particles were purified from systemically infected leaves, essentially as described by Murant (1976) and Murant et al. (1986), by adjusting leaf extracts to pH 4-8, precipitating the virus by adding polyethylene glycol, and differential centrifugation. RNA was prepared as described by Murant et al. (1986) by heating a mixture of virus particles and an equal volume of 0-02 m-Tris–HCl, 1 mM-EDTA and 4% SDS pH 9 for 20 min at 60 °C. RNA-3 was separated from the other RNA species by centrifugation in gradients of 10% to 40% sucrose containing 0-1 m-Tris–HCl, 0-1 m-NaCl, 1 mM-EDTA pH 7-5 and 0-1 mg/ml bentonite for 16 h at 30000 r.p.m. at 20 °C in a Beckman SW50.1 rotor (Murant et al., 1986). RNA-3 was collected as the slowest sedimenting RNA species and was precipitated from 70% ethanol.

For cDNA cloning, RNA-3 collected from a sucrose gradient was polyadenylated, essentially as described by Mayo et al. (1989), annealed to oligo(dT) and reverse transcribed as described by Gubler & Hoffman (1983). cDNA was ligated with Smal-cut pUC19 and used to transform Escherichia coli DH5α. Recombinant plasmids containing RNA-3-specific cDNA were selected by colony hybridization (Maniatis et al., 1982) with randomly primed cDNA. Suitable recombinant plasmids were selected by restriction enzyme mapping and overlapping fragments of cDNA were sequenced by dideoxynucleotide chain termination (Sanger et al., 1977). The 14 5'–terminal nucleotides of RNA-3 were not represented in the cDNA library and were therefore sequenced by reverse transcription (Zimmern & Kaesberg, 1978), for which the primer was synthetic oligonucleotide 5' TACGATTGGTGGAACAGC 3', which is complementary to the sequence between nucleotides 52 and 71 of the complete sequence (Fig. 1). The 5'-terminal sequence was determined after the addition of homopolymer tails by adding terminal transferase to the sequencing reaction (DeBorde et al., 1986). Sequences were assembled by the programs DBUTIL and DBAUTO (Staden, 1982) and analysed.
The sequence of RNA-3 contains only one open reading frame (ORF) that encodes a polypeptide of more than 24 amino acids. Fig. 1 shows the amino acid sequence of this polypeptide. Its size ($M$, 30509, 30K) corresponds to that of the coat protein of RBDV particles.

RNA was translated at 100 µg/ml (unfractionated RNA) or 50 µg/ml (RNA-3) in wheatgerm extracts or reticulocyte lysate (P&S Biochemicals; Amersham), as described by Mayo & Reddy (1985). Alternatively, particles of RBDV were suspended in 1 mM-Tris–HCl, 1-5 mM-NaCl pH 7-6, and added to reticulocyte lysate to a final concentration of 0-4 mg/ml in place of RNA. Reaction mixtures contained $[^35]$methionine and were incubated for 90 min at 30°C. Samples were then mixed with an equal volume of 2% SDS, 0-15 M-dithiothreitol and 20% glycerol in 0-15 M-Tris–HCl pH 6-8, heated for 3 min at 100°C and analysed by electrophoresis in polyacrylamide gels as described by Laemmli (1970), followed by autoradiography. Translation products were allowed to react with antisera and the immunoreactive products were isolated and analysed as described by Mayo & Reddy (1985).

Translation of RBDV RNA in either wheatgerm extracts or reticulocyte lysates yielded many polypeptides of different sizes (Murant et al., 1986). However, when purified virus particles were added to reticulocyte lysate in place of RNA, a less complex mixture of products was made (Fig. 2, lane 2). The main specific products had estimated Mr of 200 000, 44 000 and 31000.

When translation products of unfractionated RNA were isolated and analysed as described by Mayo & Reddy (1985).Translation of RBDV RNA in either wheatgerm extracts or reticulocyte lysates yielded many polypeptides of different sizes (Murant et al., 1986). However, when purified virus particles were added to reticulocyte lysate in place of RNA, a less complex mixture of products was made (Fig. 2, lane 2). The main specific products had estimated Mr of 200 000, 44 000 and 31000. RBDV RNA particles are readily disrupted (Murant, 1976), possibly permitting co-translational disassembly (Wilson, 1985) of well preserved RNA.

When purified RNA-3 was translated, the predominant product had an $M$, of about 30000 (Fig. 2, lane 3) and comigrated with protein extracted from purified virus particles. Trace amounts of other translation products were probably the result of premature termination of translation, or contamination with other RNA species or with degradation products from RNA-3.

When translation products of unfractionated RNA (Fig. 3, lane 1) were allowed to react with antisera, the 30K product was precipitated by antiserum raised against RBDV particles (Fig. 3, lane 1) but not antiserum to potato leafroll virus particles (Fig. 3, lane 3). Similar results were obtained with purified RNA-3 (data not shown); in these tests, about 10-fold more radioactivity was recovered after reaction with RBDV antiserum than after reaction with PLRV antiserum. The $M$, 30000 polypeptide encoded by the ORF in RNA-3 presumably corresponds to the translation product of RNA-3 and the polypeptide is thus RBDV coat protein. However, the possibility cannot be excluded that a few amino acids are removed from either or both termini of the $M$, 30509
Fig. 2. Products of \textit{in vitro} translation of RBDV RNA-3 in reticulocyte lysate after electrophoresis in a 10\% polyacrylamide gel. CP indicates the position of RBDV particle protein. Expt. 1: lane 1, endogenous activity with no added messenger; lane 2, products made during 90 min after the addition of RBDV particles. The positions of \textit{M}, marker proteins phosphorylase (94K), ovalbumin (45K) and carbonic anhydrase (29K) are shown on the left. Expt. 2: lane 3, products made during 90 min after the addition of purified RNA-3; lane 4, control with no added RNA. The positions of internal \textit{M}, markers (translation products of brome mosaic virus RNA, B1, 94K; B2, 32-5K; B3, 21K; Symons 1985) are shown on the right.

![Fig. 2](image)

The putative coat protein sequence (Fig. 1) has an overall positive charge and the N-terminal half of the molecule is relatively hydrophilic. Comparison of the amino acid composition of RBDV putative coat protein with those of other viruses in a multidimensional classification (Fauquet \textit{et al.}, 1986a, b) suggested that RBDV has greatest similarity to viruses in the ilarivirus, luteovirus, tombusvirus and cucumovirus groups (D. Desbois, personal communication). However, no marked sequence similarities were detected with proteins in the EMBL database, which included the coat proteins of tobacco streak ilarivirus, potato leafroll luteovirus, cucumber mosaic virus and alfalfa mosaic virus. Also no obvious similarities were detected between the non-coding sequences of RBDV RNA-3 and those of other viruses, including those with tripartite genomes (Symons, 1985).

Northern blot analysis has shown that RNA-3-specific cloned cDNA reacted with RNA-2 but not RNA-1 (data not shown), as would happen for example with ilarivirus RNA. However there is only weak support from the sequence of RBDV RNA-3 for the suggestion, based on particle and transmission properties, that RBDV has affinities with ilarviruses. A further test of this possible relationship may come from knowledge of the sequences of the other RNA species.

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\textbf{References}


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