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Nucleotide Sequence of Rice Dwarf Virus Genome Segment 9

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SUMMARY

The complete nucleotide sequence of the phytoreovirus rice dwarf virus (RDV) genome segment 9 is presented. It consisted of 1305 nucleotides and had an open reading frame that codes for a putative polypeptide of 351 amino acids. $M_r$ of the protein was calculated to be 38 598. The terminal nucleotides 5' GGUAAA --GAU 3' were the same as those of RDV genome segment 10. The fourth nucleotide from the 3' end of an expected conserved sequence was a C rather than the U found in the previously sequenced genome segment 10. A structure similar to the segment-specific inverted repeat of wound tumour virus was also found in the terminal region of segment 9.

Rice dwarf virus (RDV) belongs to phytoreovirus subgroup 1 and has a genome comprising 12 segmented dsRNAs (Boccardo & Milne, 1984). The virus multiplies both in plant hosts and its insect vectors and has a virion-associated RNA polymerase (Kodama & Suzuki, 1973; Uyeda & Shikata, 1984). In order to elucidate the mechanisms operating in replication and transcription, it is essential to determine the structure of the genome. The nucleotide sequence of genome segment 10 has already been determined (Uyeda et al., 1987; Omura et al., 1988). In this paper, we present the complete nucleotide sequence of genome segment 9.

Rice dwarf virus was maintained in rice plants in a greenhouse by periodic transfers through an insect vector, the leafhopper Nephrotettix cineticeps. The virus was purified as previously described (Uyeda & Shikata, 1982).

The viral RNA was extracted from the purified virus and cDNA cloning of genome segment 9 was done as previously described (Uyeda et al., 1987). Two clones, pRD538 and pRD546, were made from the denatured genome dsRNA. They reacted positively with a 32P-labelled genome segment 9 probe (Jordan & Dodds, 1983) by Southern blot hybridization (Southern, 1975). The sizes of the inserts of pRD538 and pRD546 were about 1350 and 1200 nucleotides respectively. A restriction endonuclease cleavage map of the overlapping inserts showed that these clones covered about 1400 nucleotides of genome segment 9.

In order to examine whether the cDNA clones cover the entire nucleotide sequence of genome segment 9, the 5'- and 3'-terminal regions were directly sequenced by a dideoxynucleotide chain termination method using reverse transcriptase (Seikagaku Kogyo) as described by Meshi et al. (1983). For 5'-terminal sequencing, a HindIII - AccII restriction endonuclease cleavage fragment of the cDNA at nucleotides 96 to 71 was used as a primer and the transcript as a template. The transcript was prepared as described previously (Uyeda & Shikata, 1984). Analyses showed that the clone pRD545 contained the 5'-terminal region with a sequence identical to that derived from direct RNA sequencing. For sequencing the 3'-terminal region of the genome segment, a synthetic oligonucleotide, 5' GAAGTTTGTGACCGA 3' at nucleotides 1228 to 1244, was used as a primer and the genome dsRNA as a template (Fig. 1). The genome dsRNA was denatured in 90% DMSO for 30 min at 50°C, quickly chilled in ice and precipitated by ethanol. The template was annealed with the primer at 40°C for 6 h in a buffer described by Meshi et al.
Fig. 1. Analyses of the 3'-terminal region of RDV genome segment 9. (a) Direct RNA sequencing of the 3'-terminal region of the genome segment 9 by a dideoxynucleotide chain termination method. (b) PEI-cellulose thin-layer chromatography of genome segment 9 terminally labelled by polynucleotide kinase and then completely digested with nuclease P1. Positions of the mononucleotides are shown on the right-hand side of the chromatograph.

(1983). It was found that the cDNA clone pRD538 lacked 11 nucleotides of the 3'-terminal region.

The nucleotide at the 3' terminus of the (+) strand was determined by identifying that of the 5' terminus of the (-) strand. The 5' termini of the genome dsRNAs were labelled with $[^{32}\text{P}]\text{ATP}$ by T4 polynucleotide kinase (Takara Shuzo). Genome segment 9 was separated on a 10% polyacrylamide gel according to Laemmli (1970), eluted from the gel as described by Smith (1980), and the labelled genome segment was completely digested with nuclease P1. The digested RNA was separated on PEI-cellulose in 0.4 M-LiCl using the four mononucleotides as standards. Autoradiography after chromatography showed that the radioactive phosphate was predominantly incorporated into the adenosine mononucleotide. Incorporation into GMP was about 50% of that in AMP (Fig. 1). Direct RNA sequencing by two-dimensional electrophoresis (De Wachter & Fiers, 1972; Rensing & Schoenmakers, 1973) indicated that the radioactivity was predominantly incorporated into the 5' terminus of the (-) strand (unpublished). Thus we concluded that the 3'-terminal nucleotide residue was U, complementary to the 5' terminus of the (-) strand.

Restriction endonuclease cleavage fragments of the cDNAs were subcloned in M13 phage mp18 or 19 (Messing, 1983) and sequenced by the dideoxynucleotide chain termination method (Sanger et al., 1977) using a sequencing kit purchased from Takara Shuzo. When subcloned cDNAs were too long for the sequence to be read, they were deleted sequentially for sequencing using exonuclease III (Takara Shuzo) and mung bean nuclease (Takara Shuzo) as described by Henikoff (1984). When it was compared with the sequence of RDV genome segment 10 (Uyeda et al., 1987; Omura et al., 1988), hexanucleotide 5'-GGUAAA--- at the 5' terminus were found to be conserved, and the first four, GGUA, were also as found in wound tumour virus (Asamizu et al., 1985). Tetranucleotide ---UGAU 3' of wound tumour virus was conserved in all 12 segments (Asamizu et al., 1985) and the sequence is the same as that of RDV genome segment 10. However, segment 9 was found to have ---CGAU 3'. That is, the fourth base from the 3' terminus is C instead of U. Whether this change is peculiar to our isolate, which has been maintained for at least 20 years in our greenhouse, is not known at the moment.
Fig. 2. Complete nucleotide sequence and predicted amino acid sequence of RDV genome segment 9.

The complete nucleotide sequence of genome segment 9 is presented in Fig. 2. It consisted of 1305 nucleotides and the longest predicted open reading frame started with AUG at nucleotides 25 to 27 and terminated with UGA at nucleotides 1078 to 1080. The initiation codon had the strong and consensus eukaryotic initiator context GXXAUGG (Kozak, 1987). The M, of the
Fig. 3. Inverted repeat and a possible secondary structure found in the terminal region of RDV genome segment 9.

protein was calculated to be 38598. The predicted gene product of segment 9 is probably a non-structural protein, because its $M_r$ is lower than the smallest structural protein of about 45000 (Nakata et al., 1978; Matsuoka et al., 1985).

A structure similar to the segment-specific inverted repeat of wound tumour virus (Anzola et al., 1987) is also found in genome segment 9 (Fig. 3).

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