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Short communication

The double-stranded RNA genome of maize rough dwarf *Fijivirus* contains both mono and dicistronic segments

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Abstract

An existing cDNA library of the segmented double-stranded RNA genome of maize rough dwarf virus, a plant-infecting reovirus of the genus *Fijivirus* was used to further characterise three genome segments. Segments S7, S8 and S10 were completely sequenced and found to consist of 1936, 1900 and 1802 base pairs, respectively. They all contained the conserved structural motifs present in all genome segments of plant-infecting reoviruses. Computer analysis indicated that the coding strands of segments S7 and S10 each contained a single large open reading frame [ORF], consisting of 591 and 558 codons, respectively, as is the norm for reovirus genome segments. Segment S8 contained two large non overlapping ORFs consisting of 347 and 209 codons, located in the 5′ and 3′ terminal domains, respectively; an arrangement already described for segment S6. *In vitro* translation experiments confirmed that the gene products of gel-purified dsRNA genome segments S7 and S10 were single polypeptides of 68 and 63 kDa, respectively. Transcription/translation of a plasmid carrying a full-length cDNA copy of segment S8 yielded two (40 and 25 kDa) polypeptides. The nucleotide sequences of segments S7 and S10 were aligned with those of the corresponding segments of rice black-streaked dwarf virus, another member of the genus *Fijivirus*, and found to be almost identical.

Plant-infecting members of the family Reoviridae have been divided into three genera [*Phytoreovirus, Fijivirus* and *Oryzavirus*] on the basis of particle morphology, number and size of genome segments and vector specificity (Holmes et al., 1995). Genome sequences obtained so far confirm this classification. For reviews, see Nuss and Dall (1990), Kudo et al. (1991), Nakashima et al. (1990), Noda et al. (1991), Suzuki (1995), Uyeda et al. (1995) for the *Phytoreovirus* genus and Uyeda et al. (1990), Marzachì et al. (1991, 1995) and Azuhata et al. (1993) for the *Fijivirus* genus. The available information on the *Oryzavirus* genus has been published by Yan et al. (1992, 1995) and Upadhaya et al. (1995).

Terminal structural motifs of the same nature, although of different sequence, are present in at least three segments [S6, S7 and S8] of the maize rough dwarf Fijivirus [MRDV] genome (Marzachì et al., 1991) as in all other reovirus genome segments. The conserved, genus-specific, terminal sequences [+] 5′ AAGUUUUU[UU] ...and ...[U]GUC 3′ as contained in all ten segments of rice black-streaked dwarf Fijivirus [RBSDV] genome (Azuhata et al., 1992) are also shared by all MRDV segments sequenced so far (Marzachì et al., 1991).

Viruses with segmented double-stranded [ds] RNA genomes, except the *Cystoviridae*, generally have monocistronic genome segments (see Pereira, 1991). Within the genus *Fijivirus*, this seems to be the case for the smallest segment [S10] of the genome of RBSDV (Uyeda et al., 1990); whereas Marzachì et al. (1991) and Azuhata et al. (1993) have reported the presence

Sequence data have been deposited with the EMBL/GenBank Data Libraries under Accession Nos. L76560 (S10), L76561 (S8) and L76562 (S7).

Figure 1. Complete nucleotide sequence of the coding strand of MRDV S7, as determined on the plasmid pMS7-246. Conserved terminal genus-specific oligonucleotides are located at positions 1–9 and 1933–1936; oligonucleotides of inverted complementarity at positions 7–15 and 1915–1923. The sequence has been aligned with that of RBSDV S8 (Azuhata et al., 1993), of which only differing nucleotides are shown.

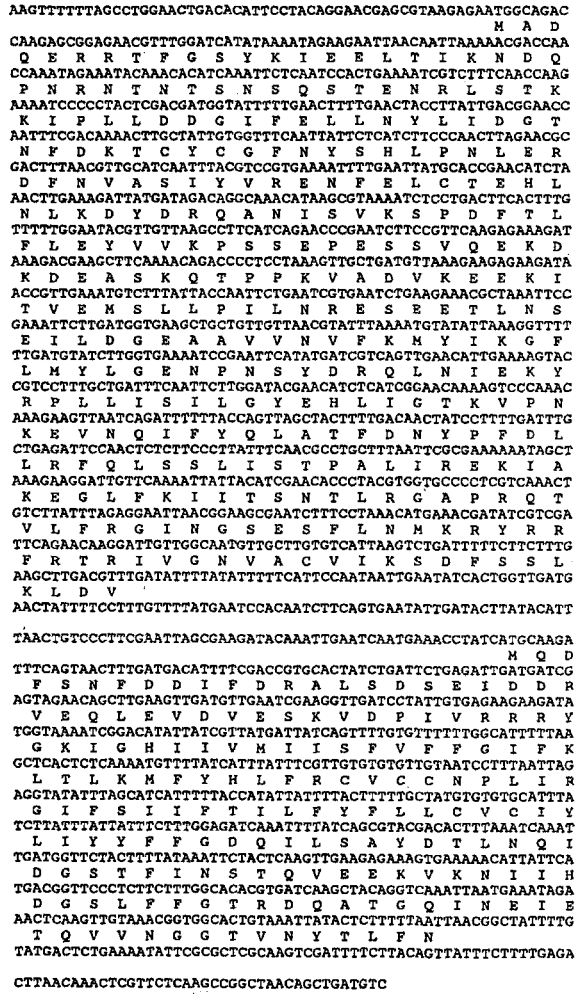

Figure 2. Complete nucleotide sequence of the coding strand of MRDV S8, as determined on plasmid pMS8-171. Conserved terminal genus-specific oligonucleotides are located at positions 1–9 and 1897–1900; oligonucleotides of inverted complementarity at positions 8–15 and 1882–1889.

of two large non overlapping open reading frames [ORFs] in different genome segments of MRDV and RBSDV. Similar configurations have been reported for wound tumour Phytoreovirus S12 (Asamizu et al., 1985), although the second ORF consisted of only 40 codons, and for the human reovirus segment encoding the hemagglutinin [S1 gene], where the two ORFs overlap (Ernst and Shatkin, 1985). In this paper, we report the sequences of MRDV segments S7, S8 and S10, obtained directly in both orientations on intact plasmids of the existing genomic library (Marzachì et al., 1991).

Segments S7 and S8 were found to be 1936 (Figure 1) and 1900 (Figure 2) bp long, respectively. They possessed the conserved, genus-specific, nucleotide sequences with adjacent regions of inverted complementarity [inverted repeats], as described by Marzachì

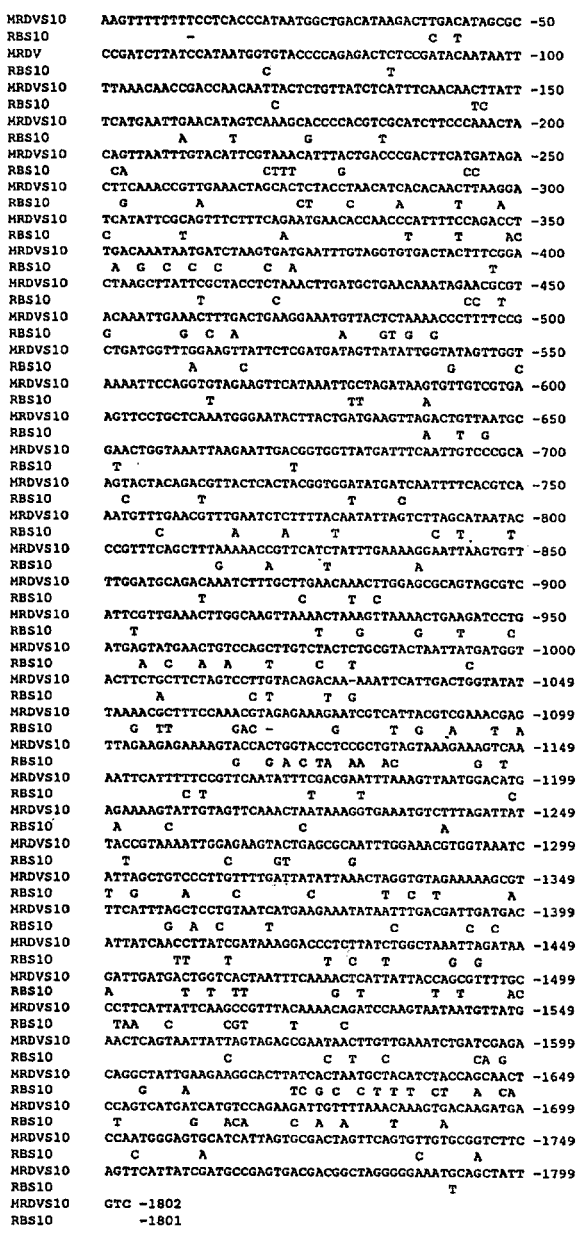

Figure 3. Complete nucleotide sequence of the coding strand of MRDV S10, as determined on the overlapping plasmids pMS10-525, -304 and -3, representing the 5′ terminal domain, the central region and the 3′ terminal domain, respectively. The sequence has been aligned with that of the corresponding segment of RBSDV (Uyeda et al., 1990), of which only differing nucleotides are shown. For locations of conserved oligonucleotides see the text.

et al. (1991). S10 was found to be 1802 bp long (Figure 3), and also contained the same genus-specific sequences at positions [+] 1–9 and 1799–1802 and the segment-specific adjacent inverted repeats at positions [+] 21–28 and 1792–1799. S7 and S10 had 5′ [+] proximal initiation codons [AUG] at positions 25–27 and 23–25, respectively, with consensus sequences containing A at −3, and G at +1 in both instances (Kozak, 1980). S7 and S10 each contained a single large ORF extending to the termination codons [UAA in S7, UGA in S10] at positions 1798–1800 and 1696–1698, respectively, followed by 3′ [+] non-coding regions of 135 and 103 nucleotides, respectively. The S7- and S10-encoded polypeptides had predicted molecular weights of 68,090 and 62,947 Da and approximate isoelectric points of 7.08 and 6.79, respectively. When the sequences of the coding strands of MRDV and RBSDV (Uyeda et al., 1990) S10 are aligned, overall homology between the two sequences is 87.5% (Figure 3). MRDV S10 has one nucleotide [U] insertion at position 11 and is therefore one base pair longer than the corresponding RBSDV segment. The insertion is located in the 5′ non-coding domain, and it does not interfere with the conserved genus-specific 9-mer and segment-specific inverted repeat which are the same, in length and sequence, for both viruses. In the best alignment, RBSDV S10 has one base insertion [G] at position 1029, which is compensated with one insertion [A] at position 1069 of MRDV S10. This gives rise, during gene expression, to a tract of amino acids in which only two are the same as MRDV. Sequence homology at the amino acid level is resumed at the second base insertion [position 1069] of MRDV. Alignment of the predicted peptides shows 90.1% homology, with few short stretches of non-conserved amino acids, especially located in the amino terminal domains. No relevant amino acid sequence homology has been found between other plant-infecting reovirus-encoded peptides and the proteins encoded by S10 of MRDV and RBSDV.

S8 was predicted to contain two large non-overlapping ORFs of different sizes. The first [ORF 1] began with the 5′ [+] proximal AUG codon at positions 52–54 [consensus sequence: A at −3, G at +1] and extended for 247 codons to a UGA terminator at positions 1093–1095. The encoded polypeptide had a predicted molecular weight of 39,959 Da and an approximate isoelectric point of 5.73. The second ORF began, after a 158 nucleotide intergenic region, with a different frame AUG codon at positions 1253–1255 with consensus sequence A at −3, C at +1, the most legitimate among the three putative opening codons downstream the first ORF, and ended with a UAA at positions 1787–1789. The encoded polypeptide had a predicted molecular weight of 24,189 Da and approximate isoelectric point of 4.43.

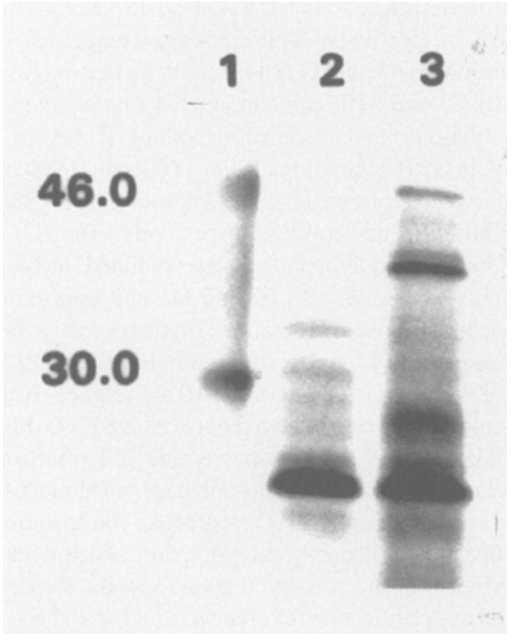

Figure 4. Electrophoretic fractionation of the *in vitro* (rabbit reticulocyte lysate) products of a synthetic transcript from a tailored transcription plasmid containing both S8 open reading frames [lane 3]. Translation products were labelled with ^{35}S-methionine. Lanes 1 and 2: Amersham protein molecular weight markers and – RNA control, respectively.

In vitro translation of polyacrylamide gel-isolated dsRNA segments, (Xu et al., 1989), was successful for S7 and S10, and resulted in the synthesis, for each segment, of one major polypeptide of estimated M*r* of 68 and 63 kDa, respectively, when analysed in 12.5% [w/v] polyacrylamide gels (Laemmli, 1970) [data not shown]. This is in good agreement with the prediction from sequence analysis and confirms the monocistronic nature of these dsRNAs. Genomic segment S8 was not translatable directly *in vitro*, so we used a tailored transcription plasmid containing all nucleotides of S8 from position [+] 1 to 1884. The *in vitro* translation products obtained (Figure 4) were two proteins of about 40 and 25 kDa, wich is again in good agreement with the predicted values. This differs from what has already been described for the other dicistronic segment of MRDV (S6, Marzachì et al., 1991), in which case the second ORF was not expressed *in vitro* under similar experimental conditions. The synthesis of two proteins from non-overlapping reading frames has been investigated in some detail (Kozak, 1986, 1987). It appeared that reinitiation at a downstream AUG codon does occur provided translation of the upstream frame has terminated, and that efficiency of reinitiation depends on the length of the intercistronic region. The intergenic regions of S6 and S8 are 52 and 158 nucleotide long, respectively, and this could be the reason of the different behaviour *in vitro*.

The sequence homology results reported in this paper support the hypothesis of considering MRDV and RBSDV as geographical races of a single species (Boccardo and Milne, 1984). Our data also confirm that Fijiviruses have a more complex genome organisation than Phytoreoviruses and that the presence of dicistronic segments is a common feature for MRDV. Further work is in progress to understand the mechanisms underlying *in vivo* expression of genes coded at the 3′ end of the genomic segments, their regulation and functions.

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