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Rice Dwarf Phytoreovirus Segment S12 Transcript Is Tricistronic in Vitro

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Sequence analysis revealed that rice dwarf phytoreovirus segment S12 is 1066 nucleotides long with a small out-of-phase, overlapping open reading frame (ORF) as well as a major ORF. The large ORF (positions 42 to 960) encodes 312 amino acids, while the small one (bases 313 to 591) encodes 92 amino acids with an additional in-frame AUG codon (positions 337-339) 24 nucleotides downstream from the first one. Transcripts from a full-length cDNA directed the in vitro synthesis of three polypeptides of 33 (considered to be translated from the long ORF), 8, and 7 kDa. Alteration of each of the two ATG codons on the small ORF demonstrated their involvement in the generation of the 8- and 7-kDa polypeptides. Although it is still unknown whether these proteins are expressed in vivo, the small ORF is shown to be conserved in S9s of two other members of the genus Phytoreovirus, rice gall dwarf virus and wound tumor virus, suggesting its common, important function.

Eukaryotic mRNAs are generally monocistronic, with 5'-proximal AUGs which are used as start sites for translation. However, examples of mRNAs which initiate at more than one AUG have been described. These mRNAs are translated into two (or more) completely different proteins or long and short protein isoforms (1). Most cases are limited to mRNAs of viruses including influenza B virus (2), infectious bronchitis coronavirus (3), reovirus (4, 5), rotavirus (6), barley yellow dwarf luteovirus (7), turnip yellow mosaic tymovirus (8) and cucumber necrosis tombusvirus (9). Rice dwarf phytoreovirus (RDV) (10, 11), a member of the family Reoviridae, has a genome composed of 12-segmented double-stranded (ds) RNA (S1–S12). Each segment reported so far has been shown to have a single long open reading frame (ORF) (12–21). However, during the course of sequence analysis of RDV S12, we have found that the segment has an out-of-frame, overlapping small ORF as well as a large ORF. In the current study, we determined the complete nucleotide sequence of RDV S12 and showed that transcripts from a full-length cDNA of S12 specified the large ORF-encoded polypeptide and long and short versions of the small ORF-coded proteins in an in vitro wheat germ translation system. Furthermore, we found that the corresponding small ORF is conserved in S9s of two other members of the genus Phytoreovirus, rice gall dwarf virus (RGDV) (22) and wound tumor virus (WTV) (23).

From the cDNA library of the KUV mRNAs constructed previously (16), two S12 cDNA clones (p12RD1, p12RD2) were newly selected. p12RD2 was recloned into the pUC18 polylinker site (24) in the orientation opposite to that of the original plasmids. Deletion plasmids, at intervals of about 200 bp, were produced from the original and recloned cDNA inserts by digestion from one end with exonuclease III (25). The deleted cDNAs were sequenced by the dideoxynucleotide chain-termination method (26) using a Sequenase Version 2.0 DNA sequencing kit (United States Biochemical Corp. Cleveland, OH), in which 7-deaza-dGTP was utilized instead of dGTP (27). The entire sequence was analyzed in both directions.

It was ascertained as in the case of S4 (10) that p12RD2 covered the 3' terminus of the RDV S12 transcript. p12RD1 and p12RD2 covered bases 24–1064 and bases 39–1066. Bases 1–23 were determined by sequencing S12 mRNA, in which the oligodeoxynucleotides complementary to bases 70–88 was used as a primer in the presence of dideoxynucleoside triphosphate (28). RDV S12 has 1066 base pairs and possesses large and small ORFs in different frames extending for 936 nucleotides from bases 42–977 and 276 nucleotides from bases 313–588, respectively. The nucleotide and deduced amino acid sequences are shown in Fig. 1. The long ORF encodes 312 amino acids with a M, of 33,919, while the other encodes 92 amino acids with a M, of 10,551. The small ORF additionally contains a downstream in-frame ATG codon at positions 337–339.

Wild type, deletion mutant, and site-directed mutant cDNAs were made and used for in vitro transcription...
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TABLE 1

| Name of clone           | Nucleotides around the ATG codon at position | Production of polypeptides of |
|-------------------------|---------------------------------------------|-------------------------------|
|                         | 313                                         | 33 kDa (P12) | 8 kDa (P120Pa) | 7 kDa (P120Pb) |
| pRS12 (Wild type, bases 1–1066) | TTAATGC                                   | +               | +               | +               |
| pRS12M1 (bases 1–1066)        | Wild type                                 | +               | +               | +               |
| pRS12OP (bases 310–1066)     | Wild type                                 | +               | +               | +               |
| pRS12OPM1 (bases 310–1066)   | Wild type                                 | +               | +               | +               |
| pRS12OPM2 (bases 310–1066)   | TTAATGG                                   | +               | +               | +               |

Note. ATG codons are indicated in boldface. Asterisks refer to nucleotide changes from the wild type.

The genus Phytoreovirus has three members, namely, WTV, RGDV, and RDV (11). The previous sequence analyses (13, 16, 18, 23, 32) showed the corresponding segment assignment of WTV and RDV, and suggested that RDV S12 and WTV S9 remain to be assigned on the basis of size similarity. However, no amino acid sequence homology with a score of more than 33 (33) was found between RDV P12 and the WTV S9-encoded nonstructural protein (Pns10, 345 amino acids). With regard to RGDV, RGDV S9 has been shown to have a 5'-noncoding sequence virtually identical to that of WTV S9 (22), although the RGDV S9-encoded polypeptide (323 amino acids) has no significant sequence homology with WTV Pns10 or any RDV-coded proteins. Surprisingly, however, careful inspection revealed that both WTV S9 and RGDV S9 also encode small polypeptides (P9OPs) which have significant sequence homologies, scores of which are more than 100, with RDV P120P. The small ORFs of WTV S9 and RGDV S9 were previously unrecognized. The RGDV S9 small ORF encoding 76 amino acids (P9OP) starts with an ATG codon at bases 234–236 and ends with a termination codon at bases 462–464, while the WTV S9 ORF extends from bases 240–482, which encodes 80 amino acids (P9OP). The sequence alignment and identity among the three viruses are shown in Fig. 3. The amino acid sequence similarities between RDV P120Pa and RGDV P9OP, between RDV P120Pa and WTV P9OP, and between RGDV P9OP and WTV P9OP...
and WTV P9OP are 31.6, 29.3, and 25.6%, respectively. Two regions (residues 33 to 39 and residues 57 to 64 on RDV P120Pa) are well conserved. Thus the three segments seem to have the same ancestral origin, and the small ORF might have been more conserved than the large one during the course of evolution.

No significant sequence homology was found between either RDV P12 or RDV P120Pa and proteins from the National Biomedical Research Foundation protein sequence library (Release 31), even between RDV P120Pa and the out-of-phase, small ORF-coded proteins of other reoviruses and plant viruses.

In the present study, RDV S12 proved to specify the in vitro expression of three polypeptide products. P12 (33 kDa) was assumed to be translated from the long AUG triplet between the first AUG codons of the two ORFs. The conservation of the internal small ORF in both positions -3 and +4, and no frame contains an AUG triplet between either RDV S12 or RDV P120Pa and proteins of other reoviruses and plant viruses.

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REFERENCES

1. Kozak, M., J. Cell Biol. 115, 887-903 (1991).
2. Williams, M. A., and Lamb, R. A., J. Virol. 63, 28-36 (1989).
3. Liu, D. X., Cavanagh, D., Green, P., and Inglis, S. C., Virology 184, 531-544 (1991).
4. Ernst, H., and Shatkin, A., Proc. Natl. Acad. Sci. USA 82, 48-52 (1985).
5. Sankel, G., Pelleienn, J., Bassel-Duby, R., Jayasuriya, A., Fields, B. N., and Sonenberg, N., J. Virol. 54, 720-725 (1985).
6. Matton, N. M., Mitchell, D. B., Both, G. W., and Estes, M. K., Virology 181, 296-304 (1991).
7. Dinesh-Kumar, S. P., Braut, V., and Miller, W. A., Virology 187, 711-722 (1992).
8. Welbom, J. J., and Dreher, T. W., Nucleic Acids Res. 17, 4675-4687 (1989).
9. Rohen, D. M., and Johnston, J. C., Virology 181, 656-665 (1991).
10. Ida, T. T., Shimkai, A., and Kimura, I., "CN/CAA Descriptions of Plant Viruses," No. 102 (1972).
11. Boccardo, G., and Milne, R. G., "CN/CAA Descriptions of Plant Viruses," No. 194 (1984).
12. Fukumoto, F., Omura, T., and Minobe, Y., Arch. Virol. 107, 135-139 (1991).
13. Nakashima, K., Kakutani, T., and Minobe, Y., J. Gen. Virol. 71, 726-734 (1990).
14. Omura, T., Ishikawa, K., Hirano, H., Ugaki, M., Minobe, Y., Tsuchizaki, T., and Kato, H., J. Gen. Virol. 70, 2759-2764 (1989).
15. Omura, T., Minobe, Y., and Tsuchizaki, T., J. Gen. Virol. 69, 227-231 (1988).
16. Suzuki, N., Harada, M., and Kusano, T., J. Virol. 72, 2233-2238 (1991).
17. Suzuki, N., Tanimura, M., Watanabe, Y., Kusano, T., Kitagawa, Y., Suda, N., Kudo, H., Uyeda, I., and Shikata, E., Virology 190, 240-247.
18. Suzuki, N., Watanabe, Y., Kusano, T., and Kitagawa, Y., Virology 197, 446-454 (1990).
19. Suzuki, N., Watanabe, Y., Kusano, T., and Kitagawa, Y., Virology 79, 455-459 (1990).
20. Uyeda, I., Kudo, H., Takehashi, T., Sanoh, T., Ohshima, K., Matsumura, T., and Shikata, E., J. Gen. Virol. 70, 1297-1300 (1989).
21. Uyeda, I., Matsumura, T., Sanoh, T., Ohshima, K., and Shikata, E., Proc. Jpn. Acad. Series B 63, 227-230 (1987).
22. Koganezawa, H., Hino, H., Mutoyoshi, F., Kato, H., Noda, H., Ishikawa, K., and Omura, T., J. Gen. Virol. 71, 1861-1863 (1990).
23. Anzola, J. V., Dall, U., Xu, Z., and Nuss, U. L., Virology 171, 222-228 (1989).
24. Norrander, J., Kemb, T., and Messing, J., Gene 26, 101-106 (1983).
25. Yanisch-Perron, C., Vieira, J., and Messing, J., Gene 33, 103-119 (1985).
26. Sanger, F., Nicklen, S., and Coulson, A. R., Proc. Natl. Acad. Sci. USA 74, 5463-5467 (1977).
27. Mizusawa, S., Nishimura, S., and Seela, F., Nucleic Acids Res. 14, 1319-1324 (1986).
28. DeBorde, D. C., Navee, C. W., Herlocher, M. L., and Maassab, H. F., Anal. Biochem. 157, 275-282 (1986).
29. Saki, R. K., Gelband, D. H., Stoffe, S., Sharp, S. J., Huguchi, R., Horn, G. T., Mulis, K. B., and Ehrlich, H. A., Science 239, 487-491 (1988).
30. LASERLM, U. K., Nature (London) 227, 680-685 (1977).
31. Shirako, Y., and Ehara, Y., J. Gen. Virol. 67, 1237-1245 (1986).
32. Dall, D. I., Anzola, J. V., Xu, Z., and Nuss, U., Nucleic Acids Res. 17, 3599 (1989).
33. Lipman, D. I., and Pearson, P. W., Science 279, 1435-1441 (1990).
34. Kozak, M., Nucleic Acids Res. 15, 8125-8132 (1987).