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The Loss of Outer Capsid Protein P2 Results in Nontransmissibility by the Insect Vector of Rice Dwarf Phytoreovirus

MASATOSHI TOMARU,[1]† WAKAKO MARUYAMA,[1] AKIRA KIKUCHI,[1] JIN YAN,[1] YAFENG ZHU,[1]‡ NOBUHIRO SUZUKI,[2] MASAMICHI ISOGAI,[1]§ YUZURU OGUMA,[3] IKUO KIMURA,[1] AND TOSHIHIRO OMURA[1]*

National Agriculture Research Center[1] and Institute of Biological Sciences, University of Tsukuba,[3] Tsukuba, Ibaraki 305, and Biotechnology Institute, Akita Prefectural College of Agriculture, Ohgata, Akita 010-04,[2] Japan

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A transmission-defective (TD) isolate of rice dwarf phytoreovirus lacked the ability to infect cells when derived from the virus-free insect vector *Nephotettix cincticeps*. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified virus showed that among six structural proteins, the P2 outer capsid protein (encoded by genome segment S2) was absent from the TD isolate, whereas all six proteins were present in the transmission-competent (TC) isolate. P2 was not detected on immunoblots of rice plants infected with the TD isolate. Genome segment S2 and its transcript were detected in both TD and TC isolates. Sequence analysis of the S2 segment of the TD isolate revealed the presence of a termination codon due to a point mutation in the open reading frame, which might explain the absence of P2 in the TD isolate. These results demonstrate that the P2 protein is one of the factors essential for infection by the virus of vector cells and, thus, influences transmissibility by vector insects.

Rice dwarf phytoreovirus (RDV) is an icosahedral double-shelled particle of approximately 70 nm in diameter (3, 5). The core particle is composed of 12 segments of double-stranded RNA (dsRNA) and four different proteins, and it is enclosed by a capsid that consists of the P2 and P8 proteins (27). RDV is acquired by vector insects from infected plants; it replicates within the insect vector and is transmitted to healthy plants. The P2 outer capsid protein of 123 kDa (127 kDa in the O strain of RDV; accession no. AB001579) is considered to be essential for infection by the virus of vector cells, in view of the fact that the extent of infection by the virus of vector cell monolayers (VCM) is proportional to the amount of P2 protein in the virus particles (27). P2-free particles fail to propagate in the vector insect, and they are not transmitted to healthy plants by insects. These results suggest that the P2 protein is required for viral transmissibility by vector insects.

Transmission-defective (TD) isolates of phytoreoviruses have been reported for both wound tumor virus (20, 21) and RDV (6). In view of the fact that transmissibility via the insect vector was lost by virus particles without the P2 protein, which had been artificially eliminated by chemical treatment (27), we were interested to determine whether the P2 protein was present in a TD isolate of RDV that had been derived from a transmission-competent (TC) isolate, which itself had been maintained in a greenhouse for several years without passage through vector insects. This study was performed in an attempt to identify the molecular basis of the transmissibility of phytoreoviruses.

The O strain of RDV (8) that we used as a TC isolate has been maintained at our facility by inoculation of healthy rice seedlings at least once a year. The TD isolate of RDV was obtained by the method described by Kimura (6). In 1983, seedlings of rice plants were inoculated with RDV by using the viruliferous insect *Nephotettix cincticeps* (leafhopper). One infected seedling was selected and grown in a greenhouse. Three months after inoculation, tillers were separated from vegetatively propagated plants and propagated separately. After the first winter season, one plant was selected from a set of vegetatively propagated infected plants and used for vegetative propagation the following year. The same cycle of selection and vegetative propagation was repeated for 12 years. The experiments described herein were performed from 1994 to 1996.

RDV from such plants was neither acquired by insects nor transmitted to healthy rice plants, in contrast to the high frequency of transmission for the isolate used for inoculation in 1994 (Table 1), an indication that the isolate used for inoculation in 1983 had become TD. We used this virus as the TD isolate in the following studies.

To characterize the TD isolate in further detail, we examined the ability of the TD isolate to infect VCM. The fluorescent antibody focus-counting technique using VCM (7, 17, 27) was used to assay the infectivity of RDV. The crude sap prepared by the method of Kimura (7) from TD-infected plants failed to infect VCM, in contrast to the high infectivity titer of the TC isolate (Fig. 1). Furthermore, the virus purified by the method reported earlier (27) from the TD isolate had no infectivity, whereas the TC isolate was highly infectious. These results suggest that the inability of the TD isolate to infect vector cells was the cause of the defect in transmission.

In a previous report, we showed that the artificial removal of P2, one of the outer capsid proteins in TC isolates, is associated with the loss of the ability to infect vector cells (27). We

* Corresponding author. Mailing address: National Agriculture Research Center, Tsukuba, Ibaraki 305, Japan. Phone: 81-298-38-8932. Fax: 81-298-38-8929. E-mail: toomura@narc.affrc.go.jp.

† Present address: Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan.

‡ Present address: DNAVEC Research Inc., Kannondai, Tsukuba, Ibaraki 305, Japan.

§ Present address: Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan.

TABLE 1. Percentage of individual leafhoppers[a] that acquired and transmitted RDV after acquisition of the virus from rice plants infected with TD and TC isolates

Replication expt	% Insects[a] that transmitted RDV from plants infected with:			
	TD isolate		TC isolate	
	Propagation[b]	Transmission[c]	Propagation[b]	Transmission[c]
I	0	0	94	35
II	0	0	82	52

[a] Eighty insects were used in each experiment. Sixteen non-virus-exposed insects, used as healthy controls in each experiments, did not give positive results.
[b] Insects that gave positive results in the enzyme-linked immunosorbent assay (24).
[c] Insects that transmitted RDV to rice seedlings by the method of Yan et al. (27).

postulated that the loss of infectivity of the TD isolate observed in the present study (Fig. 1) might have been due to the lack of some structural protein(s) of RDV. Among the six known structural proteins, only the P2 protein was not detected after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (13) of the purified TD isolate (Fig. 2). By contrast, the TC isolate contained all six proteins. These results demonstrate that the lack of the P2 protein was associated with loss of the ability to infect vector cells, as observed in our previous study (27).

To clarify whether the lack of P2 protein in the purified TD isolate was due to failure to synthesize the P2 protein or to failure of the integration of P2 into viral particles, we examined the presence of five major structural proteins (P1, P2, P3, P7, and P8) of RDV in rice plants that had been infected with TD or TC isolates by immunoblotting. The vector *N. cincticeps*, viruliferous for the TC isolate, and VCM infected with the isolate were used as references.

Fresh rice leaves infected with a TC or TD isolate (0.2 g) were homogenized in 0.6 ml of 0.1 M histidine that contained

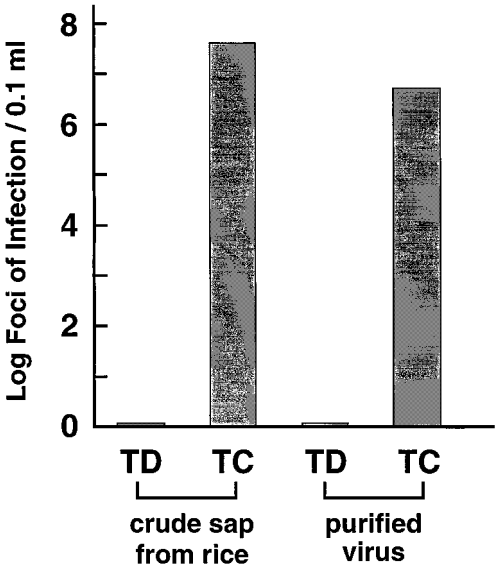

FIG. 1. Infection of vector cell monolayers by preparations of RDV. Crude sap prepared from a rice plant infected with the TD or TC isolate and RDV purified from rice plants infected with the TD or TC isolate were appropriately diluted and used to inoculate monolayers of cells from the vector *N. cincticeps*. Then the numbers of infected cells were determined.

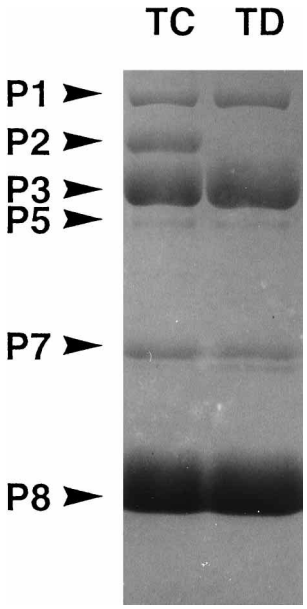

FIG. 2. Analysis of protein components of TC and TD isolates of purified RDV by SDS-PAGE (10% polyacrylamide). Proteins on the gel were stained with Coomassie brilliant blue R-250. Positions of the various proteins are indicated on the left.

0.01 M $MgCl_2$, pH 6.2 (His-Mg), and extracts were centrifuged for 10 min at 1,700 × *g*. Each supernatant was subjected to serial twofold dilutions of from 1 to 5 to 1 to 10,240. Ten adult leafhoppers (ca. 10 mg) were homogenized in 300 μl of His-Mg in a microtube. After centrifugation for 10 min at 1,700 × *g*, the supernatant was diluted to appropriate concentrations. VCM were cultured in 40-ml culture flasks (25 cm^2) at 25°C, with transfer to fresh flasks every 8 to 12 days. Seven to 10 days after transfer, they were inoculated with purified virus (A_{260} = 2.5×10^{-2}) in His-Mg for 2 h. The inoculum was 100% infectious, as determined by the fluorescent antibody focus technique in a separate experiment. After incubation for 2 days at 25°C, cells were harvested and suspended in 50 μl of His-Mg per culture flask. His-Mg without RDV was used to prepare a mock-infected control.

Samples in dissociation buffer (13) were boiled for 3 min and loaded on a 17.5% polyacrylamide gel (acrylamide-bisacrylamide, 30:0.125; 0.1% SDS). After electrophoresis, the polypeptides in the gel were transferred electrophoretically to a polyvinylidene difluoride membrane (Immobilon-P; Millipore). The P1, P2, P3, P7, and P8 proteins were detected with respective antisera and visualized by chemiluminescence (ECL-Western blotting detection reagents; Amersham). Antisera against viral proteins were prepared as described earlier (16). After SDS-PAGE (10% polyacrylamide) of purified RDV, the Coomassie brilliant blue-stained bands of P1, P2, P3, P7, and P8 proteins were cut out. Each protein was electroeluted in 10-fold-diluted Tris-glycine-SDS buffer (13) in a protein-extraction unit (Nihon Eido) for 3 h at 1 W and then injected into rabbits.

Among the proteins, P2 was not detected in the TD isolate, whereas all five proteins were detected in the TC isolate (Table 2). The reciprocal of the dilution end point for each protein was lower in the TD than in the TC isolate. All five viral proteins were also detected in vector insects that carried the TC virus and in VCM inoculated with the TC isolate. These results suggest that P2 was not synthesized in rice plants that

TABLE 2. Immunodetection of the major structural proteins of RDV synthesized in vivo

Viral protein	RDV-infected plants[a]			Vector insect (5-fold dilution)		VCM (5-fold dilution)	
	TD	TC	Healthy	Virus infected	Healthy	Infected	Mock infected
P1	80	320	<5	+	−	+	−
P2	<5	640	<5	+	−	+	−
P3	160	640	<5	+	−	+	−
P7	40	640	<5	+	−	+	−
P8	320	320	<5	+	−	+	−

[a] Values are the reciprocals of dilution end points (gram per milliliter) that gave positive reactions.

had been infected with the TD isolate, explaining the absence of P2 protein in the virus particles.

One possible mechanism for the failure to synthesize P2 protein is the internal deletion of genome segment S2, as in the case of S5 of wound tumor virus (1). To examine this possibility, we examined genome segments of purified TD and TC isolates by SDS-PAGE. RNAs were released from virus particles by the addition of 1% SDS and 0.1% EDTA to solutions of purified virus. As shown in Fig. 3, all 12 anticipated segments were detected in equimolar amounts in the purified TD and TC isolates. No significant difference in the size of S2 segments was detected between the isolates. Apparent differences in migration were observed for genome segments S4 and S11, and the biological implications of this observation remain to be clarified.

The reduced efficiency of transcription of S2 is another explanation of the failure to synthesize the P2 protein. To explore this hypothesis, we quantified the positive- and negative-sense RNAs produced in vivo.

Nucleic acids were extracted by the method of Shure et al. (22). Rice leaves were homogenized in liquid nitrogen and mixed with extraction solution (0.05 M Tris-HCl [pH 7.5], 5.0 M urea, 0.35 M NaCl, 0.02 M EDTA, 2% sarcosyl, and 5% phenol). SDS was added to 0.2% (wt/vol) and mixed for 10 min. The mixture was extracted three times with a mixture of phenol and chloroform (3:1 [vol/vol]) and centrifuged for 10 min at 2,000 × *g*. The aqueous phase was incubated overnight at 4°C with LiCl at 2 M. Insoluble single-stranded RNA (ssRNA) was collected by centrifugation for 15 min at 15,000 × *g*. The ssRNA was subjected to a second round of precipitation with 2 M LiCl. Viral dsRNA and plant genomic DNA were precipitated from the LiCl supernatant with 2.5 volumes of ethanol. After treatment with RNase A, the remaining plant genomic DNA was quantitated in terms of absorbance at 260 nm.

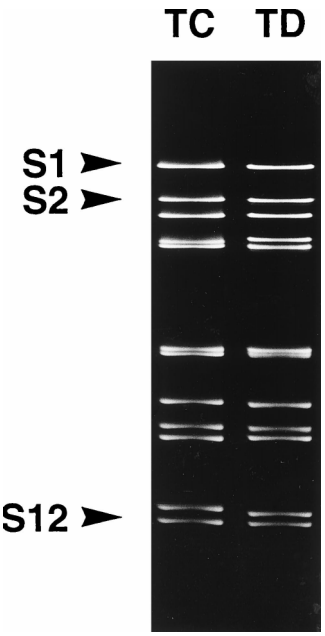

FIG. 3. Analysis of genomic dsRNAs stained with ethidium bromide of TC and TD isolates of RDV by SDS-PAGE (10% polyacrylamide). Positions of some segments of the genome are indicated on the left.

Probe DNAs were labeled by the following methods. cDNA of the S2 segment of the RDV genome was prepared by reverse transcription-PCR, cloned into pT7Blue T-Vector (Novagen), and digested with the restriction endonucleases *Spe*I and *Eco*RV. The resulting 584-bp DNA fragment (nucleotides [nt] 1237 to 1821 of S2) (25) was cloned into pBluescript II vector (Stratagene). To avoid detection of minor contaminants, two types of probe were prepared: sequences complementary to ss positive-sense RNA [ss(+)RNA] or those complementary to negative-sense ss(−)RNA of the S2 segment. Fifty nanograms of template DNA, 10 pmol of primer (T3 and T7), 20 pmol each dinucleoside triphosphate except for dCTP (10 pmol), and 11.1 MBq of radiolabeled dCTP (PB10205; Amersham) was mixed and heated for 2 min at 94°C. The extension reaction was performed by the addition of 1 U of *Taq* polymerase (50°C for 2 min, 72°C for 5 min, and 99°C for 5 min). After purification of labeled ssDNAs on a Sephadex superfine G25 column (Pharmacia), they were used as ss(+)RNA- or ss(−)RNA-specific probes.

RNA blot analysis was performed as follows. Equal amounts of ssRNAs, standardized relative to the amount of plant genomic DNA, were lyophilized to dryness and suspended in 10 μl of 2.2 M formaldehyde in 50% formamide and denatured for 10 min at 65°C. The denatured RNAs were subjected to electrophoresis in a 1.5% agarose gel that contained 2.2 M formaldehyde in 1× morpholinepropanesulfonic acid (MOPS) buffer (pH 7.0), and then RNAs were transferred to a Hybond-N^+ filter (Amersham) in 20× SSC (0.3 M NaCl, 0.03 M sodium citrate) as the transfer medium. Blots were soaked in 0.05 N NaOH for 5 min, rinsed with 2× SSC, and then air dried. Prehybridization and hybridization reactions were performed in a solution of 1 M NaCl, 1% SDS, and 10% dextran sulfate for 16 h at 60°C with 1×10^5 to 4×10^5 cpm/ml of heat-denatured ssRNA probe. The blots were washed twice for 5 min each with 2× SSC at room temperature, twice with 2× SSC that contained 1% SDS for 30 min each at 60°C, and twice with 0.1× SSC that contained 0.1% SDS for 5 min each at room temperature. The signals were quantitated with an image analyzer (BAS-2000; Fuji Photo Film).

As shown in Fig. 4, RNA blot analysis revealed the presence of both positive- and negative-sense RNAs in rice plants infected with the TC and TD isolates in vivo and their sizes did not differ from that of the viral genome. We assumed that if S2 were transcribed efficiently more ss(+)RNAs should be detected than ss(−)RNAs. The intensities of signals from both ss(+)RNAs and ss(−)RNAs were quantitated relative to levels of purified dsRNAs as controls, and the ratio of ss(+)RNAs to ss(−)RNAs was calculated for both isolates. The ratios were 4.9 and 2.5 for the TC and TD isolate, respectively, indicating that the transcripts of S2 were present in both cases.

To address the possibility that a mutation had occurred within the open reading frame (ORF) of S2, we analyzed the nucleotide sequence of the S2 segment in TD and TC isolates.

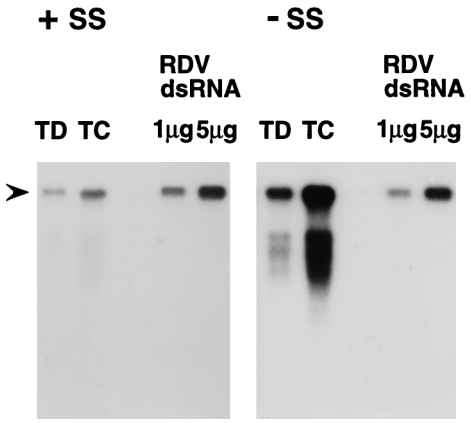

FIG. 4. Northern blot analysis of RDV RNAs. Total RNA of TD and TC isolates was subjected to electrophoresis on a 1% agarose gel, blotted, and allowed to hybridize with a ^{32}P-labeled positive-sense RNA (+ss) or negative-sense RNA (−ss) that corresponded to genome segment S2 of RDV, expressed after cloning of the cDNA into pBluescript II. One and five micrograms of genomic dsRNA of RDV was processed in the same manner as controls. Arrowhead, position of the full-sized transcript (3.5 kb).

cDNA fragments of TC and TD isolates, corresponding to nt 3 to 3365 of the S2 genome segment of the RDV H strain (RDV-H) (25), were produced by reverse transcription-PCR from total genomic dsRNA. Primers P5B-2, 5′ CAGGATC CCGCGATGGCTTATCCT 3′ (corresponding to nt 3 to 26), and P3B-1, 5′ CAGGATCCCGACCACATCATGCGC 3′ (complementary to nt 3358 to 3381), corresponded to the sequences on either side of the ORF (nt 15 to 3365) of RDV-H. P5B-2 and P3B-1 included *Bam*HI sites (underlined) as a result of a few base changes. The PCR products were cloned by using the pT7Blue T-Vector. The *Bam*HI-digested inserts were recloned into the plasmid vector pBluescript II to facilitate deletions. Deletions in the cDNAs were made at intervals of about 300 bp by digestion with exonuclease III (Takara Shuzo) from both ends. The nucleotide sequences of the inserts were determined on both strands by dideoxy sequencing with a Dye Primer Cycle Sequencing Kit (Perkin-Elmer Applied Biosystems) and automated sequencers (models 373A and 377; Perkin-Elmer Applied-Biosystems).

Rapid amplification of cDNA ends (RACE)-PCR was performed for cloning of the untranscribed 5′- and 3′-end regions of the S2 of the TC and TD isolates. The dsRNA extracted from purified virus was denatured and polyadenylated by using ATP in poly(A) buffer (125 mM Tris-HCl [pH 7.5], 500 mM NaCl, 25 mM $MgCl_2$, 5 mM $MnCl_2$, 2.5 mM dithiothreitol, 5 mM ATP) and poly(A) polymerase (Takara Shuzo). Oligonucleotide 5′ GGCCACGCGTCGACTAGTAGTAC(T)17, supplied with the 3′ RACE system (Gibco BRL), served to prime the synthesis of cDNA from the tailed template. After digestion of the RNA template by RNase H, 5′- and 3′-end fragments were generated by PCR, in which oligonucleotide 5′ GGCCACGCGTCGACTAGTAC and the internal sense primer for the 3′ end (5′ TGATGTCCTGCTTCTGCCTG GG, nt 3249 to 3270) or the antisense primer for the 5′ end (5′ AGCTGCTAAGACTGCGGCACGG, nt 269 to 290) were utilized, respectively. The resulting dsDNA was subcloned into the pT7Blue T-Vector, and the inserts were sequenced as described above. DNA sequencing data were assembled and analyzed with the DNASIS (Hitachi Software Engineering) and GENETYX (Software Development) software packages.

The segment sizes of the S2 of the TD and TC isolates were exactly the same, but approximately 0.6% of the nucleotides in all S2 segments from the TD isolate had point mutations. A change at nt 47 in the TD isolate generated a termination codon. Thus, the ORF ended at amino acid residue 11 of the predicted 127-kDa polypeptide encoded by the S2 segment in the TC isolate of the O strain of RDV (Fig. 5).

The virus particles in the TD isolate of RDV, which lacked the P2 protein (Fig. 2), did not infect VCM (Fig. 1). The virus was not propagated in vector insects (Table 1). These results resembled the results for a TC isolate from which the P2 protein had been eliminated by chemical (CCl_4) treatment (27). This coincidence suggests that a similar mechanism caused the loss of virus infectivity and failure in transmission of the virus in each case. P2 protein was missing from the TD isolate among the five major structural proteins detected by immunoblots (Table 2). Thus, the correlation between the presence of the P2 protein in viral particles and the ability of the virus to infect vector cells was apparent for two distinct materials: a TD isolate generated from a living plant and a TC isolate from which the P2 protein had been eliminated by treatment with CCl_4. These results suggest that the virus in the TD isolate was not transmissible by the vector because it had lost the ability to infect insect vector cells as a result of the absence of the P2 protein in virus particles. The results support the hypothesis that the P2 protein is one of the factors that is essential for infection of insect vector cells by RDV (27).

Our inability to detect the P2 protein in the TD isolate in vivo (Table 2) suggests that the absence of the P2 protein from the purified preparation was due to failure to synthesize P2 and not to the failure of the P2 protein to be integrated into viral particles. The S2 genome segment of apparently normal size was detected in equimolar amounts relative to other segments in the TD and TC isolates (Fig. 3), suggesting that the loss of the P2 protein encoded by S2 was due to a defect either in transcription or in translation. Since the multiplication cycle of phytoreovirus appears to be similar to that of other viruses that belong to the *Reoviridae* (15, 23, 26), the ss(+)RNAs, which are transcripts of RDV genes, are considered to function as mRNAs in the cytoplasm of host cells and to be translated into polypeptides (4, 14, 18, 28). Therefore, the presence of the ss(+)RNA transcript of S2 in TD-infected rice (Fig. 4) suggests that failure of translation resulted in the absence of P2 protein and that the mutation within the S2 ORF does not

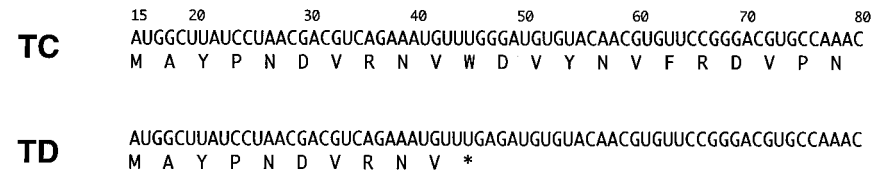

FIG. 5. Part of the ORF of plus-strand genome segment S2 and the predicted amino acid sequences for the TC and TD isolates. Nucleotides are numbered from the 5′ end. Amino acids encoded by each segment are indicated below the sequence in the single-letter code. A termination codon is indicated by an asterisk.

affect the efficiency of transcription and replication of the segment.

The nucleotide sequence of the S2 genome segment of the TD isolate revealed a termination codon at a position that corresponded to nt 47 in the ORF of the TC isolate (Fig. 5). Accordingly, the major ORF (nt 141 to 3461) of the S2 segment of the TD isolate, which is 142 bp shorter than the authentic S2 ORF, is preceded by a minicistron (bases 15 to 44). The large ORF of such an mRNA has been reported to be translated on occasion (9). However, this is not considered to be the case for the S2 segment of the TD isolate. If the downstream major ORF is to be translated, the AUG codon of the major ORF must be recognized by directly entering ribosomes that are guided by an internal ribosome entry-site sequence (19), by ribosomes that bypass the first AUG and search for a downstream AUG triplet (12), or by reinitiation-competent ribosomes (10, 12). No internal ribosome entry-site sequence was found in the 5′-terminal region of the major S2 ORF of the TD isolate. The first AUG (nt 15 to 17) is unlikely to be leaky since it is a strong initiator, according to Kozak's rule (11). Although reinitiation might be possible in view of Kozak's observations with a mammalian system (10), such is not the case for expression of S2 of the TD isolate in plants, as shown in Fig. 5. It is unknown whether the minicistron is expressed in TD-infected rice cells.

The P2 protein of RDV is located in the outer capsid of the viral particle and is considered to play an important role in some step(s) from adsorption of the virus to the insect cell to the initial onset of multiplication of the virus within the cell (27). The virus in infected rice plants is acquired by an insect mouth stylet. It probably infects the intestinal tract, multiplies in intestinal cells, and is distributed to various organs (2). The insect starts to transmit the virus after a latent period of 10 to 20 days (2, 3). The absence of P2 protein in the TD isolate might result in failure of the virus to infect the cells of the intestinal tract of the vector insect, with a consequent defect in transmission of the isolate.

Loss of the ability to synthesize P2 protein by the TD isolate that had been maintained in plants without passage through vector insects suggests that the P2 protein might not be required for virus propagation in rice plants. Therefore, the S2 genome segment might be free from the pressure of natural selection in such an environment, and S2 with a mutation might have a chance to spread throughout the host plant, as well as the normal segment. Clarification of the molecular mechanism of viral multiplication in distinct hosts (insects and plants) should bring new insight to the roles of proteins that function in virus-host interactions.

Nucleotide sequence accession numbers. The nucleotide sequences reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with accession no. AB001579 (TC genome segment S2) and AB001580 (TD genome segment S2).

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REFERENCES

1. **Anzola, J. V., Z. Xu, T. Asamizu, and D. L. Nuss.** 1987. Segment-specific inverted repeats found adjacent to conserved terminal sequences in wound tumor virus genome and defective interfering RNAs. Proc. Natl. Acad. Sci. USA **84:**8301–8305.
2. **Black, L. M.** 1984. The controversy regarding multiplication of some plant viruses in their insect vectors. Curr. Top. Vector Res. **2:**1–29.
3. **Boccardo, G., and R. G. Milne.** 1984. Plant reovirus group. CMI/AAB Descriptions of plant viruses, no. 294. Commonwealth Mycology Institute and Association of Applied Biology, Kew, Uited Kingdom.
4. **Eaton, B. T., A. D. Hyatt, and S. B. Brookes.** 1990. The replication of bluetongue virus. Current Top. Microbiol. Immunol. **162:**89–117.
5. **Iida, T., A. Shinkai, and I. Kimura.** 1972. Rice dwarf virus. CMI/AAB Descriptions of plant viruses, no. 102. Commonwealth Mycology Institute and Association of Applied Biology, Kew, United Kingdom.
6. **Kimura, I.** 1976. Loss of vector-transmissibility in an isolate of rice dwarf virus. Ann. Phytopath. Soc. Jpn. **42:**322–324.
7. **Kimura, I.** 1986. A study of rice dwarf virus in vector cell monolayers by fluorescent antibody focus counting. J. Gen. Virol. **67:**2119–2124.
8. **Kimura, I., Y. Minobe, and T. Omura.** 1987. Changes in a nucleic acid and a protein component of rice dwarf virus particle associated with an increase in symptom severity. J. Gen. Virol. **68:**3211–3215.
9. **Kozak, M.** 1986. Bifunctional messenger RNAs in eukaryotes. Cell **47:**481–483.
10. **Kozak, M.** 1987. Effects of intercistronic length on the efficiency of reinitiation by eucaryotic ribosomes. Mol. Cell. Biol. **7:**3438–3445.
11. **Kozak, M.** 1989. The scanning model for translation: an update. J. Cell Biol. **108:**229–241.
12. **Kozak, M.** 1991. An analysis of vertebrate mRNA sequences: intimations of translational control. J. Cell Biol. **115:**887–903.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
14. **McCrae, M. A.** 1986. The reovirus family at the molecular level, p. 327–353. *In* R. P. Bercott (ed.), Molecular basis of viral replication. Plenum Press, New York, N.Y.
15. **Nuss, D. L., and D. J. Dall.** 1990. Structural and functional properties of plant reovirus genomes. Adv. Virus Res. **38:**249–306.
16. **Omura, T., Y. Minobe, M. Matsuoka, Y. Nozu, T. Tsuchizaki, and Y. Saito.** 1985. Localization of structural proteins in particles of rice gall dwarf virus. J. Gen. Virol. **66:**811–815.
17. **Omura, T., and I. Kimura.** 1994. Leafhopper cell culture for virus research, p. 91–107. *In* K. Maramorosch and A. H. McIntosh (ed.), Arthropod cell culture systems. CRC Press Inc., Boca Raton, Fla.
18. **Patton, J. T.** 1994. Rotavirus replication. Curr. Top. Microbiol. Immunol. **185:**107–127.
19. **Pelletier, J., and N. Sonenberg.** 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature **334:**320–325.
20. **Reddy, D. V. R., and L. M. Black.** 1974. Deletion mutations of the genome segments of wound tumor virus. Virology **61:**458–473.
21. **Reddy, D. V. R., and L. M. Black.** 1977. Isolation and replication of mutant population of wound tumor virions lacking certain genome segments. Virology **80:**336–346.
22. **Shure, M., S. Wessler, and N. Fedoroff.** 1983. Molecular identification and isolation of the waxy locus in maize. Cell **35:**225–233.
23. **Suzuki, N.** 1995. Molecular analysis of the rice dwarf virus genome. Semin. Virol. **6:**89–95.
24. **Takahashi, Y., T. Omura, K. Shohara, and T. Tsuchizaki.** 1991. Comparison of four serological methods for practical detection of ten viruses of rice in plants and insects. Plant Dis. **75:**458–461.
25. **Uyeda, I., N. Suda, N. Yamada, H. Kudo, K. Murao, H. Suga, I. Kimura, E. Shikata, Y. Kitagawa, T. Kusano, M. Sugawara, and N. Suzuki.** 1994. Nucleotide sequence of rice dwarf phytoreovirus genome segment 2: completion of sequence analysis of rice dwarf virus. Intervirology **37:**6–11.
26. **Uyeda, I., and R. G. Milne.** 1995. Introduction: genomic organization, diversity and evolution of plant reoviruses. Semin. Virol. **6:**85–88.
27. **Yan, J., M. Tomaru, A. Takahashi, I. Kimura, H. Hibino, and T. Omura.** 1996. P2 protein encoded by genome segment S2 of rice dwarf phytoreovirus is essential for virus infection. Virology **224:**539–541.
28. **Zarbl, H., and S. Millward.** 1983. The reovirus multiplication cycle, p. 107–196. *In* W. K. Joklik (ed.), The Reoviridae. Plenum Press, New York, N.Y.