Complete nucleotide sequence of the *Nilaparvata lugens* reovirus: a putative member of the genus *Fijivirus*

Nobuhiko Nakashima,* Mika Koizumi, Hirofumi Watanabe and Hiroaki Noda

National Institute of Sericultural and Entomological Science, Owashi, Tsukuba, Ibaraki 305, Japan

The nucleotide sequences of all genome segments of the *Nilaparvata lugens* reovirus (NLRV), which is found in the brown planthopper *Nilaparvata lugens*, have been determined and some genes have been assigned to structural and functional proteins. The genome of NLRV consists of 28,699 nucleotides and contains at least 11 large open reading frames (ORFs). The genome of NLRV is the largest among viruses of the family Reoviridae reported to date. The deduced amino acid sequence of genome segment S1 contained the major motifs of RNA polymerase and that of S7 had the purine NTP-binding motif. Based on the molecular masses of the deduced proteins and the particle structure of NLRV, segments S1, S3 and S7 were assigned to the 160, 140 and 75 kDa proteins, respectively, that are located in the inner core. It was deduced that S2 codes for the 135 kDa protein (B spike), which is located on the surface of the inner core. Most reported ORFs of rice black streaked dwarf virus (RBSDV), which shares many properties with NLRV, had similarities with the corresponding ORFs of NLRV. An exception was S7 ORF2, which is found in RBSDV but not NLRV and may therefore be involved in multiplication of RBSDV in rice plants. These results and our previous observations indicate that NLRV should be classified in the genus *Fijivirus*.

**Introduction**

Planthoppers and leafhoppers are vectors of phytopathogenic viruses which are classified into three genera, *Phytoreovirus*, *Fijivirus* and *Oryzavirus*, in the family Reoviridae (Murphy et al., 1995). The brown plant hopper, *Nilaparvata lugens*, is one of the most important pests of the rice plant because of its sucking damage and its ability to transmit plant-pathogenic viruses (Sogawa, 1982; Hibino, 1989). We found a virus, *Nilaparvata lugens* reovirus (NLRV), in a healthy colony of the brown plant hopper. NLRV has 10 segmented dsRNAs, termed S1 to S10 based on their electrophoretic mobility in polyacrylamide gels (Noda et al., 1991a). The terminal nucleotide sequences of each genome segment of NLRV are similar to maize rough dwarf virus (MRDV) and rice black streaked dwarf virus (RBSDV), which are members of the genus *Fijivirus* (Noda et al., 1994). All fijiviruses described so far are phytopathogenic. However, NLRV does not multiply in rice plants, although the virus is transmitted from a viruliferous plant hopper to a non-viruliferous one through the rice plant (Nakashima & Noda, 1995). Some non-phytopathogenic reoviruses are also found in leafhoppers and planthoppers, such as leafhopper A virus (Boccardo et al., 1980; Ofori & Francki, 1985) and *Peregrinus maidis* virus (Falk et al., 1988). Non-pathogenic reoviruses in planthoppers and leafhoppers can help to explain the origin of plant pathogenic reoviruses because of their common characteristics such as genome organization, life-cycle and morphology (Nuss & Dall, 1990; Nault & Ammar, 1989).

In the genus *Phytoreovirus*, many nucleotide sequences of genome segments have been reported: all 12 genome segments of rice dwarf virus (RDV; Uyeda et al., 1995), S4 to S12 of wound tumor virus (Nuss & Dall, 1990) and S3 (Takahashi et al., 1994), S8 and S10 (Noda et al., 1991b) of rice gall dwarf virus (RGDV). On the other hand, in the genus *Fijivirus*, only S6 of MRDV (Marzachi et al., 1991) and S7, S8 (Azuhata et al., 1993) and S10 (Uyeda et al., 1990) of RBSDV have been fully sequenced. We previously reported the nucleotide sequences of genome segments S8 (Nakashima & Noda, 1994) and S10 (Noda et al., 1994) of NLRV; their deduced amino acid sequences were similar to those of RBSDV S10 and MRDV S6, respectively. However,

---

* Author for correspondence. Fax +81 298 38 6028.
e-mail nakajij@ss.nises.affrc.go.jp

The nucleotide sequence data reported in this paper for NLRV S1–S7 and S9 will appear in the GSDB, DDBJ, EMBL and NCBI databases with the accession numbers D49693–49700, respectively.
amino acid sequence similarity between NLRV and fijiviruses (about 18%) was much lower than that between MRDV and RBSDV (more than 85%; Azuhata et al., 1993).

Comparison of nucleotide and amino acid sequences of non-pathogenic and plant-pathogenic reoviruses is necessary for elucidating the functions of proteins such as those involved in multiplication in plants. This paper reports the nucleotide sequences of genome segments S1–S7 and S9 of NLRV, and the assignment of structural proteins to each segment. Morphological properties of NLRV are also described because A and B spikes, which have been observed in fijiviruses (Milne & Lovisolo, 1977), have not been confirmed in NLRV.

Methods

cDNA library and sequencing. The cDNA library of NLRV genome segments and the screening method used to identify the clones containing each genome segment were reported previously (Noda et al., 1994). A total of 153 clones containing each genome segment were identified (S1, 15 clones; S2, 36; S3, 39; S4, 24; S5, 9; S6, 11; S7, 13; S9, 6). Deletion mutants were derived from the cDNA clones of each genome segment and sequenced as described (Noda et al., 1994). A part of the S1 cDNA (residues 2640 to 3394) was absent in the library and only one clone containing residues 2434 to 3427 of the cDNA from S5 was available. Therefore, DNAs corresponding to these regions were amplified by RT–PCR from genomic dsRNA using synthesized primers based on the flanking sequences of the regions, according to the method reported previously (Nakashima & Noda, 1995). Each genome segment was entirely sequenced in both directions using at least two clones.

Computer analysis of the sequenced data. The obtained sequence data were processed to search for open reading frames (ORFs) with the software GENETYX (Software Development). To search for homologous sequences in databases, the determined nucleotide sequences of each genome segment were divided into 240 nucleotide blocks that overlapped by 60 nucleotides, and their deduced amino acid sequences were divided into 180 residue blocks that overlapped by 60 amino acids. These blocks were then used for a similarity analysis against the GenBank and PIR databases using the FASTA program (Pearson, 1990) installed in the DDBJ database.

Morphological observations of NLRV. An N. lugens from the viruliferous colony was dissected and the salivary glands removed. The dissected glands were homogenized in a few drops of 0.1 M-sodium phosphate buffer pH 7.0. The homogenate was then mounted on a grid and stained with 2% uranyl acetate for observation by an electron microscope.

Expression of a truncated S3 gene in Spodoptera frugiperda (SF) 9 cells using a baculovirus vector. A truncated ORF of segment S3 was expressed in SF9 cells using a baculovirus expression system (MaxBac Complete System Plus; Invitrogen) to obtain antisera against the S3 product and to assign the product to a structural protein of NLRV. The inserted DNA to be expressed was amplified with RT–PCR using synthesized primers based on the nucleotide sequence of S3 from residues 15 to 35 (forward) and from 1045 to 1025 (reverse). PstI and HindIII recognition sequences were added at the S′ terminus of the forward and reverse primers, respectively, in order to insert the truncated ORF into a transfer vector, pBlueBacHis. The sequences of the primers were 5′ TTTGCGAGATGGCAATACACGTTACAGGC 3′ (forward) and 5′ TTAAGCTTGCGATTCACGTTAAGACACTG 3′ (reverse). An artificial termination codon introduced in the reverse primer is shown in bold, and recognition sequences of the restriction enzymes are underlined. The transfer vector was designed to produce a metal-binding fusion protein to facilitate purification of the expressed protein using an immobilized metal affinity chromatography column. Production and purification of the expressed truncated protein were conducted according to the manufacturer’s recommendations.

Immunodetection of the truncated S3 product. The separated S3 product was injected into a mouse and antisera against the protein was obtained. Purified NLRV particles were electrophoresed in a 10% SDS–polyacrylamide gel and proteins in the gel were transferred to a PVDF membrane using a semi-dry blotting apparatus according to the method of Hirano & Watanabe (1990) with some modifications. To improve blotting efficiency of the high molecular mass proteins of NLRV, SDS (0.05% w/v) and 2-mercaptoethanol (0.08% v/v) were added to the blotting buffers and the methanol concentration was decreased to 10%. A part of the blotted membrane was stained with Coomassie brilliant blue R-250 (CBB) to determine the electroblotting efficiency. The rest of the blotted membrane was treated with 500-fold-diluted antisera directed against the truncated S3 product and proteins that reacted with the antisera were detected with peroxidase-labelled goat anti-mouse IgG (Bio-Rad).

Results

general features of the NLRV genome

The length, GC content and coding capacity of each genome segment of NLRV are summarized in Table 1. The largest genome segment, S1, had 4391 nucleotides and the smallest, S10, had 1430. The mean GC content in the whole sequence of NLRV was 34.8%. The most GC-rich segment was S5 (39.0%) and the segment with the lowest GC content was S2 (33.1%). The coding capacity of NLRV ORFs was 8916 amino acids. The non-coding regions at the 5′ and 3′ termini of each genome segment totalled 623 and 1239 nucleotides, respectively.

Reoviruses usually have one ORF in a genome segment. A large ORF was found in all segments except S9 which had two non-overlapped ORFs. The first ORF in S9 was located at nucleotides 53 to 925 and the second at 982 to 1602. MRDV S6 (Marzachi et al., 1991) and RBSDV S7 (Azuhata et al., 1993) also have two non-overlapped ORFs. In addition to the ORFs listed in Table 1, six small ORFs were found in the plus strands and 19 in the minus strands using a program for ORF prediction (Fickett, 1982). The longest possible polypeptides among them had 177 and 145 amino acids, respectively.

flanking nucleotide sequences of the initiation codons

Flanking sequences of the initiation codons of large ORFs are shown in Fig. 1. Two AUG codons were found at the 5′ terminus of S8 as described (Nakashima & Noda, 1994). ORFs 7, 8 and 9-1 had a suitable consensus sequence for translational initiation by eukaryotic ribosomes (Kozak, 1984, 1986); they had A and G at the –3
Table 1. Features of the NLRV genome segments and their deduced proteins

<table>
<thead>
<tr>
<th>Genome segment</th>
<th>Length (bp)</th>
<th>GC content (%)</th>
<th>Non-coding region (5'/3')</th>
<th>Number of codons</th>
<th>Molecular mass of deduced protein (kDa)</th>
<th>pI*</th>
<th>Molecular mass in SDS-PAGE (kDa)</th>
<th>Locations†</th>
<th>Remark</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_1$</td>
<td>4391</td>
<td>33.5</td>
<td>20/42</td>
<td>1442</td>
<td>165.9</td>
<td>8.66</td>
<td>160 Core RNA polymerase</td>
<td>D49693</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_2$</td>
<td>3732</td>
<td>33.1</td>
<td>21/111</td>
<td>1199</td>
<td>136.6</td>
<td>7.87</td>
<td>135 Outer shell B spike</td>
<td>D49694</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_3$</td>
<td>3753</td>
<td>34.2</td>
<td>14/67</td>
<td>1223</td>
<td>138.5</td>
<td>5.17</td>
<td>140 Core Major core capsid</td>
<td>D49695</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_4$</td>
<td>3560</td>
<td>33.8</td>
<td>75/86</td>
<td>1132</td>
<td>130.0</td>
<td>6.97</td>
<td>75 -</td>
<td>D49696</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_5$</td>
<td>3427</td>
<td>39.0</td>
<td>201/307</td>
<td>972</td>
<td>106.4</td>
<td>5.60</td>
<td>95 Non-structural</td>
<td>D49697</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_6$</td>
<td>2970</td>
<td>36.8</td>
<td>149/328</td>
<td>830</td>
<td>95.1</td>
<td>6.45</td>
<td>95 Non-structural</td>
<td>D49698</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_7$</td>
<td>1994</td>
<td>34.1</td>
<td>40/64</td>
<td>629</td>
<td>73.5</td>
<td>8.95</td>
<td>75 Core NTP binding</td>
<td>D49699</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_8$</td>
<td>1802</td>
<td>35.3</td>
<td>6/107</td>
<td>562</td>
<td>62.4</td>
<td>6.11</td>
<td>65 Outer shell Major outer capsid</td>
<td>D26127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_9$</td>
<td>1640</td>
<td>33.2</td>
<td>52/56/38</td>
<td>290</td>
<td>33.0</td>
<td>7.45</td>
<td>75 Non-structural</td>
<td>D49700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_{10}$</td>
<td>1430</td>
<td>35.2</td>
<td>45/89</td>
<td>431</td>
<td>49.4</td>
<td>5.89</td>
<td>75 Non-structural</td>
<td>D14691</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Isoelectric point.
† Modified from Noda et al. (1991a; Fig. 4).
‡ Two large non-overlapped ORFs were observed in $S_9$.

Fig. 1. Flanking nucleotide sequences of the initiation codons of NLRV ORFs. The predicted initiation codons and residues located at the -3 and +4 positions are shown in bold. Start and stop codons of minicistronic ORFs observed in these regions are underlined ($S_4$, $S_5$, $S_8$, $S_9$ and $S_{10}$). The predicted amino acid sequences are shown under the nucleotide sequence.

and +4 positions of their AUG codons, respectively. Other ORFs deviated from the consensus sequence. The largest ORFs started from the first AUG codon in $S_1$, $S_2$, $S_3$, $S_6$, $S_7$, $S_8$ and $S_9$, but started from the second in $S_4$, $S_5$ and $S_{10}$. The reading frames starting from the first AUG codon of $S_5$ and $S_{10}$ had termination codons before the second AUG codon, which started the larger ORFs. In $S_4$, a termination codon in-frame with the first AUG codon emerged nine nucleotides from the second AUG codon. Although two AUG codons were found between the termination codon of ORF9-1 and the AUG codon of ORF9-2, these codons do not seem to work because there was a stop codon (UAA) before the start codon of ORF9-2.

Computer analysis

The amino acid sequence coded by NLRV $S_1$ from residues 792 to 1028 showed 18.7% identity to a 227 amino acid sequence in $S_1$ of bluetongue virus serotype 10 (BTV-10; Roy et al., 1990) using the LFASTA program (data not shown). This region included the GDD motif, which is well known as a core motif of RNA-dependent RNA polymerase (Kamer & Argos,
Fig. 2. Conserved amino acid sequences specific for RNA-dependent RNA polymerases of reoviruses. The conserved motifs presented by (i) Poch et al. (1989), (ii) Bruenn (1991) and (iii) Koonin (1992) are shown at the top. Asterisks show highly conserved amino acids and asterisks in parentheses show mostly conserved amino acids. Numbers in parentheses on the left and right indicate the starting and finishing positions of the aligned sequences. Numbers between motifs indicate the number of residues that occur between the motifs. The sequences of RDV (Suzuki et al., 1992), rotavirus SA11 (Mitchell & Both, 1990), reovirus serotype 3 (Weiner & Joklik, 1989) and BTV-10 (Roy et al., 1988) were used for comparison.

Fig. 3. Comparison of the C termini of the deduced amino acid sequences coded by NLRV S7 and RBSDV S8 (Azuhata et al., 1993). The sequences were aligned by the LFASTA program (Pearson, 1990). Purine NTP-binding motif (Golbalenya & Koonin, 1989) are shown in bold type.

1984), indicating that NLRV S1 codes for this enzyme (Fig. 2). In the aligned residues, an asparagine (N) residue in motif V (Koonin, 1992) was substituted with a leucine (L) residue. Three nucleotide substitutions were required to achieve this.

The C terminus of the deduced amino acid sequence of NLRV S7 showed 16.2% identity in a 265 amino acid overlap with the deduced sequence of RBSDV S8 (Azuhata et al., 1993) (Fig. 3). The N-terminal regions of the two segments were not aligned by the LFASTA program although their hydrophobicity plots were similar (data not shown). By a motif search analysis using the program PROSITE (release 11.0), the purine NTP-binding motif A/GXXXXGKS/T (Golbalenya & Koonin, 1989) was found at residues 401 to 408 in the predicted amino acid sequence of NLRV S7. RBSDV S8 also possesses the same predicted motif at residues 355 to 362.

With regard to the nucleotide sequences and the deduced amino acid sequences coded by $2, $3, $4, $5, $6 and $9 of NLRV, no other remarkable similarities to the sequences deposited in the databases were found.

**A and B spikes of NLRV**

The members of the genus *Fijivirus* typically have two kinds of spikes on the virus capsid, A spikes on the surface of the outer capsid and B spikes on the inner
Genome of the reovirus NLRV

Fig. 4. Structure of NLRV particles. Particles were stained with 2% uranyl acetate. (a) An NLRV particle with attached A spikes (arrows) in the homogenate of the salivary glands of viruliferous N. lugens. (b) A purified NLRV particle obtained by the method of Noda et al. (1991a). (c) An inner core particle with attached B spikes. (d) NLRV particles with an area (arrow) in which the outer capsid proteins are partially stripped away. Bar represents 125 nm.

capsid (Milne & Loisolo, 1977). In our earlier study of NLRV (Noda et al., 1991a), we observed the double-shelled structure of the particle, but the A and B spikes were not detected.

Fig. 4 shows the particle morphology of NLRV. A spikes were observed when NLRV particles were prepared directly from the salivary glands of a viruliferous planthopper (Fig. 4a), but were not seen in the purified NLRV particles (Fig. 4b). A spikes seem to come off easily from the outer capsid of NLRV during purification from planthoppers. Repeated CsCl ultracentrifugations stripped particles of their outer capsid proteins (Fig. 4d). B spikes on the inner core were recognized in these particles (Fig. 4c). In an earlier study of NLRV, we treated purified NLRV particles with 1.9 M-MgCl₂ solution to remove the outer capsid (Noda et al., 1991a), but B spikes also seemed to be removed because of the high stringency of the solution.

Blotting analysis of the structural proteins of NLRV and immunodetection of the S3 product

The result of immunodetection of the S3 product is shown in Fig. 5. NLRV has seven structural proteins, three major (140, 135 and 65 kDa), three intermediate (160, 110 and 75 kDa) and one minor (120 kDa) (Noda et al., 1991a). In this experiment, to separate two close bands corresponding to the 140 and 135 kDa proteins, the amount of sample loaded on to the gel was reduced so that the 110 kDa protein was scarce and the 120 kDa protein was not detected at all (Fig. 5, lane 1). Blotting efficiency differed among the structural proteins of NLRV. The 140 and 65 kDa proteins were transferred to the membrane easily (Fig. 5, lane 3), but the 160, 75 and 135 kDa proteins remained in the gel after the electroblotting procedure (Fig. 5, lane 2). The truncated ORF coded by S3 was expressed in Sf9 cells and isolated with metal-binding affinity chromatography. A protein having the expected molecular mass (42.4 kDa) was then observed on an SDS-polyacrylamide gel (data not shown). Mouse antiserum raised against the 42.4 kDa protein reacted with the 140 kDa protein of NLRV (Fig. 5, lane 4) and with the 42.4 kDa protein in the lysate of Sf9 cells that had been infected with the recombinant baculovirus (Fig. 5, lane 5). These results indicate that S3 codes for the 140 kDa protein.

Assignment of the structural proteins of NLRV

Morphological observations of the NLRV particles suggested that at least four major structural proteins are required to assemble them, namely the A and B spike proteins and the outer and inner capsid proteins (Fig. 4). As shown in Fig. 4(b), A spikes came away from purified NLRV particles and therefore should not appear in SDS-PAGE profiles as a major structural protein. The purified NLRV particle has three major structural proteins of 140, 135 and 65 kDa (Noda et al., 1991a). The 65 kDa protein was the major outer capsid protein and was coded by S8 (Nakashima & Noda, 1994); the 140 kDa protein was a major inner core structural protein and was coded by S3 (Fig. 5, lane 4). The 135 kDa protein is apparently the B spike protein. This conclusion is in accordance with previous observations. When the inner core and the outer shell of purified NLRV particles were separated by treatment with a high concentration of MgCl₂, the inner core particles did not retain B spikes (Noda et al., 1991a; Fig. 2b). The 135 kDa protein was partly recovered from the outer shell fraction but not from the inner core (Noda et al., 1991a; Fig. 4a, lane 3).

Comparison of the calculated molecular masses of the predicted proteins coded by the genome segments of NLRV indicated that S1 codes for the largest protein (160 kDa) because no other genome segment had such a large coding capacity (Table 1). S7 seems to code for the 75 kDa protein because it is the only segment that had a coding capacity close to 75 kDa. The 160 and 75 kDa proteins remained in the polyacrylamide gel (Fig. 5, lane 2) after the electroblotting procedure. Basic proteins are rarely electroblotted from a polyacrylamide gel to a membrane because blotting buffers are basic (Van Seuningen & Davril, 1990). Indeed, the isoelectric points...
Fig. 5. Immunodetection of the structural protein coded by genome segment $3$ of NLRV. Structural proteins of NLRV were separated by $10\%$ SDS-PAGE and electroblotted onto a PVDF membrane for immunodetection of $3$ product using antiserum directed against the $3$ truncated ORF product. Lane 1, purified NLRV particles; lane 2, proteins remaining in the gel after electroblotting, stained with CBB; lane 3, electroblotted proteins on PVDF membrane stained with CBB; lane 4, immunodetection of $3$ product ($140\,\text{kDa}$) on the membrane; lane 5, immunodetection of the truncated $3$ product ($42.4\,\text{kDa}$) in Sf9 insect cells that had been infected with a recombinant baculovirus.

The second largest protein ($140\,\text{kDa}$) was coded by $3$ (Fig. 5, lane 4). Of the remaining genome segments, only $2$ had the capacity to code for the $135\,\text{kDa}$ protein, the $B$ spike protein. The $135\,\text{kDa}$ protein did not electroblot well onto the membrane compared to the $140\,\text{kDa}$ protein (Fig. 5, lanes 2 and 3). Again, the isoelectric point of the deduced protein coded by $2$ was basic. These observations suggest that $2$ codes for the $135\,\text{kDa}$ protein.

The predicted proteins coded by NLRV $S9$ and $S10$ seemed to be non-structural proteins because $S9$ and $S10$ do not have coding capacities that exceed the smallest structural protein, $65\,\text{kDa}$ (Table 1). We were unable to assign two structural proteins, the $120$ and $110\,\text{kDa}$ proteins, to coding segments because the molecular masses deduced from predicted amino acid sequences and those estimated from SDS–PAGE did not correspond well. However, one of the segments in $S4$, $S5$ and $S6$ must code for the $A$ spike protein and this could be the $120\,\text{kDa}$ protein or a protein that did not appear in SDS–PAGE of purified particles.

**Discussion**

The total number of nucleotides in the NLRV genome was $28,699$, which is the largest number reported so far for a member of the family *Reoviridae*. For comparison, the sizes in nucleotides of the genomes of other reoviruses are: reovirus type 3 (genus *Orthoreovirus*), $23,549$ (Weiner et al., 1989); rotavirus SA11 (*Rotavirus*), $18,555$ (Mitchell & Both, 1990); BTV-10 (*Orbivirus*), $19,218$ (Fukusho et al., 1989); and RDV (*Phyto*reovirus), $25,749$ (Uyeda et al., 1995).

The genome segments of NLRV were numbered according to their mobilities in $10\%$ polyacrylamide gels, with higher numbers corresponding to higher mobilities. Segments $S4$ and $S5$ do not separate under these conditions (Noda et al., 1994). The relatively low mobility of $S5$ in polyacrylamide gels was assumed to be due to its high GC content. Segment $S2$ was shorter than $S3$ by $21\,\text{bp}$. This reversed mobility, however, cannot be due to GC content because their GC contents were almost the same. There may be some additional factors affecting the relative mobilities of dsRNAs in polyacrylamide gels, as indicated by Fukumoto (1992).

MRDV $S6$ has nucleotide sequence variation at a level of $1.18\%$, and one reason for this is that the source viruses were collected from field-harvested plants (Marzachi et al., 1991). In the case of NLRV, the average percentage of heterogeneous nucleotides in all segments except $S5$ was $0.09\%$. This value seems to be due to false incorporation of nucleotides by the reverse transcriptase used for cDNA synthesis. NLRV $S5$ differed from the other segments in that it had a nucleotide heterogeneity of $2.5\%$. Although this high rate was probably partly due to false incorporation, the reason why $S5$ alone had so many heterogeneous residues is unknown because cDNA synthesis of all segments was conducted concurrently from the total genome of NLRV (Noda et al., 1994). The viruliferous *N. lugens* were collected from a field in 1987 and were then reared in our laboratory. The virus for cDNA cloning was purified in 1990. It is unlikely that the heterogeneity in $S5$ could have arisen in this 3 year period, since the rate of nucleic acid substitution, estimated at $2.2\times10^{-3}$ substitutions/site/year in BTV (Kowalik & Li, 1991) is very low. Thus, NLRV $S5$ appears to have already been heterogeneous when the viruliferous insects were collected.

Consensus motifs of RNA-dependent RNA polymerases have been shown in various kinds of viruses by Poch et al. (1989), Brunn (1991) and Koonin (1992).
Motifs A, B and C defined by Poch et al. (1989), motifs 1, 2 and 3 from Bruenn (1991) and motifs IV, V and VI from Koonin (1992) are strongly conserved in reoviruses (Fig. 2). Motif I defined by Koonin (1992) and motif D from Poch et al. (1989) also seem to be conserved, although these two motifs were not noted by Suzuki et al. (1992). Motifs 4–8 in the work of Bruenn (1991) and motif VII defined by Koonin (1992) were difficult to identify in reoviruses. Koonin (1992) suggested that the enzymes of reoviruses are monophyletic. However, alignment of their deduced amino acid sequences was difficult because, except for the conserved motif regions shown in Fig. 2, they are not well conserved. To align the sequences of the enzymes and to elucidate the phylogenetic relations of the members of the family Reoviridae, the sequence data of other groups (e.g. oryzaviruses, Phytoreovirus, Fijivirus and NLRV) is required.

The equivalent proteins of RDV and RGDV, which belong to the genus Phytoreovirus, are both encoded by segment S3 (Suzuki et al., 1990; Takahashi et al., 1994). However, the deduced amino acid sequence coded by NLRV S3 was not homologous to those of RDV S3 and RGDV S3. Hopper-borne reoviruses are classified into three genera, Phytoreovirus, Fijivirus and Oryzavirus. Phytoreoviruses and fijiviruses have a double-shelled structure (Shikata, 1989; Murphy et al., 1995). Members of the genus Fijivirus and NLRV both have A and B spikes, whereas phytoreoviruses have no obvious spikes. This morphological difference between the members of the genera Phytoreovirus and Fijivirus (or NLRV) seems to account for the low similarity of the deduced amino acid sequence of their structural proteins.

NLRV has many properties similar to those of RBSDV, including morphology, terminal nucleotide sequences of genome segments (Noda et al., 1994), deduced amino acid sequences (Nakashima & Noda, 1994), manner of transmission through rice plants and multiplication ability in the small brown planthopper, Laodelphax striatellus (Nakashima & Noda, 1995). The most distinct difference between NLRV and RBSDV is that RBSDV has the ability to multiply in rice plants while NLRV does not. Among the reported sequences of S7, S8 and S10 in RBSDV, only the deduced amino acid sequence of ORF2 of RBSDV S7 showed no similarity with any sequences of NLRV. The product of RBSDV S7 ORF2 may have a different function from the gene products of NLRV and may be involved in multiplication in rice plants.

As far as viruses are classified based on their comprehensive characteristics, we consider NLRV should be classified in the genus Fijivirus. However, if NLRV is excluded from the genus because of its inability to multiply in plants, a new genus composed of non-phytopathogenic hopper-borne reoviruses will be required in the family Reoviridae.

References


(Received 18 May 1995; Accepted 4 September 1995)