The complete nucleotide sequence of RNA 3 of citrus leaf rugose and citrus variegation ilarviruses

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Complete sequence data for the RNA 3 of both citrus leaf rugose (CiLRV) and citrus variegation (CVV) ilarviruses have been determined. The RNAs are 2289 nt (CiLRV) and 2309 nt (CVV) in length and both contain the typical Bromoviridae arrangement of two open reading frames (ORFs) which, when translated, code for proteins that correspond to the Mr 32 000 (32K) putative movement proteins (ORF 1) and the coat proteins (ORF 2) of the respective viruses. The 3′ termini of both viruses can be folded to form a secondary structure similar to those reported for other ilarviruses. These are the first complete nucleotide sequences for RNA 3 of members of subgroup 2 of the ilarviruses. The two viruses share substantial homology in nucleic acid sequence, code for identically sized coat proteins and share high levels of identity in the translated products of both ORFs. Although related, these viruses differ sufficiently to be considered distinct. The RNA 3s of CiLRV and CVV appear to be distinct from those of other ilarviruses for which comparable sequence data are available and also from the closely related alfalfa mosaic virus.

Citrus leaf rugose virus (CiLRV) and citrus variegation virus (CVV) are two serologically related but biologically distinct ilarviruses that infect citrus (Garnsey, 1975). Genomes of ilarviruses consist typically of three RNA species of approx. 3.4 kb (RNA 1), 2.9 kb (RNA 2) and 2.2 kb (RNA 3). While RNA 1 and RNA 2 are both monocistronic, RNA 3 is bicistronic with ORF 1 coding for a putative movement protein and ORF 2 coding for the viral coat protein. The coat protein gene in RNA 3 is not expressed directly but as a subgenomic, highly effective message – RNA 4 – of approx. 0.9 kb (Symons, 1985). CiLRV and CVV both have coat proteins with an Mr of 26000 which are serologically related to each other (Garnsey, 1975). As groupings within the ilarviruses are based on serological relationships both are members of subgroup 2 which also includes elm mottle virus, Tulare apple mosaic virus and asparagus virus II (Francki et al., 1991).

There are 16 distinct members of the ilarvirus group (Mink, 1991) but for many years only limited sequence data [RNA 3 of tobacco streak virus (TSV) – the group’s type member and sole member of subgroup 1] have been available (Cornelissen et al., 1984). However, complete sequences for the RNA 3 of prune dwarf virus (PDV) – subgroup 4 – (Bachman et al., 1994), the RNA 4 of apple mosaic virus (ApMV) – subgroup 3 – (Sánchez-Navarro & Pállás, 1994; Alrefai et al., 1994) and the RNA 2 of CiLRV – subgroup 2 – (Ge & Scott, 1994) have been published recently. Even so, much additional information is needed before it becomes possible to make any assessment of the taxonomy of this group at the molecular level. As part of a programme to develop sequence data for ilarviruses with the aim of confirming or revising the taxonomy of the group, we have cloned and sequenced the genomic RNAs of a number of ilarviruses. Here we present the first complete sequence data for the RNA 3 of members of ilarvirus subgroup 2, namely CiLRV and CVV.

Except where stated, materials and methods used were essentially those described by either Sambrook et al. (1989) or Ge & Scott (1994). Purified CiLRV and CVV were kindly supplied by Dr S. M. Garnsey (USDA, Orlando, Fla., USA). Total viral RNA was polyadenylated with E. coli poly(A) polymerase and used for cDNA synthesis. Synthesis of cDNA for CVV was primed by using oligo(dT)$_{18}$ and synthesis of cDNA for CiLRV was primed by using a primer complementary...
Short communication

Fig. 1. For legend see opposite.
The complete sequence of RNA 3 of citrus leaf rugose ilarvirus. Putative translation products for the two ORFs are shown under the sequence. The G at position 1281 indicates the 5' terminus of the RNA 4. The AUGC motifs involved in the secondary structure at the 3' terminus are emphasized and underlined. GenBank no. U17390.

Fig. 1. Complete sequence of RNA 3 of citrus leaf rugose ilarvirus. Putative translation products for the two ORFs are shown under the sequence. The G at position 1281 indicates the 5' terminus of the RNA 4. The AUGC motifs involved in the secondary structure at the 3' terminus are emphasized and underlined. GenBank no. U17390.
Fig. 2. For legend see opposite.
Table 1. Percentage similarity between CiLRV RNA 3 and CVV RNA 3 determined using the GCG procedure GAP

<table>
<thead>
<tr>
<th>Region</th>
<th>CiLRV</th>
<th>CVV</th>
<th>Similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5' Non-translated</td>
<td>1–358</td>
<td>1–354</td>
<td>68.6</td>
</tr>
<tr>
<td>ORF 1</td>
<td>359–1213</td>
<td>355–1203</td>
<td>71.7</td>
</tr>
<tr>
<td>Intergenic</td>
<td>1214–1330</td>
<td>1204–1328</td>
<td>59.8</td>
</tr>
<tr>
<td>ORF 2</td>
<td>1331–1984</td>
<td>1329–1982</td>
<td>66.5</td>
</tr>
<tr>
<td>3' Non-translated</td>
<td>1985–2289</td>
<td>1983–2309</td>
<td>63.2</td>
</tr>
<tr>
<td>Total sequence</td>
<td>1–2289</td>
<td>1–2309</td>
<td>67.9</td>
</tr>
</tbody>
</table>

Comparisons of coat protein gene translation products of CVV and CiLRV with similar products for ApMV, PDV, TSV and AIMV showed essentially a similar pattern. CiLRV and CVV coat proteins are more closely related to each other (65.8% identity) than to other ilarvirus coat proteins (17.0–33.5% identity). However, in this comparison it was the relationship between CVV and the ApMV sequence of Alrefai et al. (1994) – 33.5% identity – that differed from the figures for identity in other comparisons (17.0–27.4%). The relationship between the ApMV sequence of Sánchez-Navarro & Pállas (1994) and CVV (20.7% identity) was less. The two sequences for ApMV are dissimilar. However, unpublished data from this laboratory, from R. W. Hammond and J. M. Crosslin (Beltsville, Md., USA), from Canada and from France for isolates of prunus necrotic ringspot ilarivirus (PNRSV) show high levels of similarity (> 95% identity) with the sequence of Sánchez-Navarro & Pállas. Thus the differences in the relationship between the coat proteins of the two citrus viruses and the different isolates of ApMV may be resolved should it be accepted that ApMV of Sánchez-Navarro & Pállas is, in fact, an isolate of PNRSV.

Clearly CiLRV and CVV are related to each other as they share a substantial level of identity for the translated proteins of both ORFs and code for almost identically sized coat proteins. However, the levels of identity are lower than have been typically shown for viruses of which more than one strain or isolate has been sequenced, viz. the 98% identity that can be calculated among the coat proteins of strains 425 (Barker et al., 1983), B (Langereis et al., 1986) and S (Ravelonandro et al., 1984) of AIMV. The work with potyvirus coat protein amino acid sequence homology (Shukla & Ward, 1988) shows similar levels of identity for strains of a
single virus. Comparing levels of identity reported for strains of the same virus (> 90%) with those reported between CiLRV and CVV (65-8%), the biological differences known for CiLRV and CVV, and the heterologous nature of the serological relationship (Garnsey, 1975) these two ilarviruses should continue to be considered distinct viruses. CiLRV and CVV are also distinct from other ilarviruses for which sequence data are available and are distinct from AIMV. These separations agree with the currently accepted taxonomy of the ilarvirus group where the two ilarviruses from citrus are included in subgroup 2 while other sequence information which is available relates to viruses in subgroup 1 (TSV), subgroup 3 (ApMV) and subgroup 4 (PDV) (Francki et al., 1991).

A ‘zinc-finger’ motif (Cys-X_{2}-Cys-X_{10}-Cys-X_{2}-His) has been identified in the coat protein of TSV (Sehnke et al., 1989) and putative ‘zinc-finger’ motifs have been identified in two isolates of ApMV (Sánchez-Navarro & Pállas, 1994; Alrefai et al., 1994). However, this motif is not present in the coat protein of either PDV or AIMV although both these viruses do have areas rich in basic residues near the amino terminus of their respective coat proteins, and with PDV an amphipathic helix can be formed with the 22 amino acids adjacent to the amino terminus of the protein (Bachman et al., 1994). Neither a ‘zinc-finger’ nor an area rich in basic residues is found in the coat protein of either of the two citrus viruses, a fact that would support the taxonomic separation of the citrus ilarviruses from other ilarviruses.

Lovisolo (1993) proposed a common geographical origin for CiLRV and CVV (the Mediterranean basin or north America). He suggests that ilarviruses originated in wild plants and moved to cultivated hosts through pollen. However, he considers the movement from wild to cultivated hosts via pollen to be a rare event with subsequent spread of the viruses being through vegetative propagation. The rarity of the event would suggest it is probable that all ilarviruses in citrus are the result of a single transfer event followed by divergence from a common ancestor. Both the extent of the similarity in the translation products from the RNA 3s of these two viruses and the known biological similarities and differences of the two viruses may support such conjecture. The similarity of the movement proteins might be expected as both viruses occur in the same host. That the coat proteins are less related and differ at the amino-terminal region might explain the differences in symptom expression of the two viruses. Neeleman et al. (1991) have shown that single mutations near the amino-terminus of the coat protein of AIMV are associated with a complete change in symptom expression.

It is also noteworthy that these two distinct viruses will cross-protect against each other in certain citrus hosts (Garnsey, 1975). If such cross-protection is mediated through the coat protein then this might offer additional support for the idea of divergence from a common ancestor with the conserved areas of the coat protein being responsible in some way for the protection.

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References


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