Key words: TBRV/nepoviruses/RNA sequence homologies

Nucleotide Sequence Homologies among RNA Species of Strains of Tomato Black Ring Virus and Other Nepoviruses

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SUMMARY

Nucleotide sequences of the genome RNA species of four strains of tomato black ring virus (TBRV), two each from the Scottish and German serotypes, were compared by hybridization with complementary DNA copies. Within each serotype 90% or more homology was observed between the corresponding RNA species of each pair of strains. Between serotypes greater differences were found, with 30 to 40% of RNA-1 sequences and only about 10% of RNA-2 sequences being common to all four strains. Hybridization reactions between RNA species and complementary DNA preparations representing different serotypes proceeded more slowly, and the hybrids melted at lower temperatures than those between homologous combinations, indicating that the apparently conserved sequences are similar, but not identical, in the two serotypes. Satellite RNA species from two TBRV strains in different serotypes had only about a quarter of their sequences in common. Limited nucleotide sequence homology was found between the genome RNA species of TBRV and those of the serologically distantly related grapevine chrome mosaic, cacao necrosis and artichoke Italian latent viruses, but not between TBRV RNA species and those of seven serologically unrelated nepoviruses.

INTRODUCTION

Tomato black ring virus (TBRV), a member of the nepovirus group, has a bipartite single-stranded RNA genome. RNA-1 has a mol. wt. of 2.69 x 10^6 and RNA-2 a mol. wt. of 1.66 x 10^6, estimated by agarose gel electrophoresis under denaturing conditions (Murant et al., 1981). Both RNA species are necessary for the production of infective virus particles, and work with pseudo-recombinant isolates has assigned genetic markers to either RNA-1 or RNA-2 (Randles et al., 1977; Harrison & Murant, 1977; Hanada & Harrison, 1977). In isolated protoplasts, RNA-1 is capable of replicating independently of RNA-2, whereas RNA-2 is synthesized only in the presence of RNA-1 (Robinson et al., 1980). Both RNA species are polyadenylated at the 3' end (Mayo et al., 1979) and possess a covalently linked protein (VPg), probably at the 5' end (Mayo et al., 1982; Koenig & Fritsch, 1982). RNA extracted from virus particles is infective, provided VPg remains attached (Harrison & Barker, 1978), and can be translated in vitro, indicating it is of positive polarity (Fritsch et al., 1980). Hybridization with complementary DNA (cDNA) copies detected no nucleotide sequences common to RNA-1 and RNA-2 of strain A (Robinson et al., 1980) or of strain S (Robinson, 1982).

Some strains of TBRV contain an additional RNA species of mol. wt. 4.8 x 10^5 which is not required for infectivity. This satellite RNA is not synthesized in plants inoculated with RNA-1 and RNA-2 only, and contains nucleotide sequences which are not present in RNA-1 or RNA-2 (Robinson, 1982).

Serological studies have shown that strains of TBRV can be divided into two serotypes, the Scottish serotype and the German serotype (Harrison, 1958b). Within each serotype, strains are serologically closely related, whereas members of different serotypes show a more distant serological relationship.

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In the present study we have prepared cDNA copies of each genome RNA species, from each of four strains (two from each serotype), and used them in hybridization experiments to confirm that lack of common sequences in RNA-1 and RNA-2 is a general property of this virus, and to examine the extent of sequence homology between strains. We have also compared the sequences of satellite RNA species (RNA-3) from two of the strains. In addition, hybridization experiments have been performed to compare the genome nucleotide sequences of several other nepoviruses with those of TBRV.

**METHODS**

**Virus isolates.** The following viruses were used: TBRV strains A, S and S12 (Scottish serotype), strains G and G12 (German serotype) (Hanada & Harrison, 1977) and strain C (celery yellow vein strain; Hollings, 1965), another member of the German serotype (A.F. Murant, personal communication); grapevine (Hungarian) chrome mosaic virus (GCMV), type strain from grapevine (Martelli & Quaquarelli, 1972); cacao necrosis virus (CNV), Ghanaian isolate (Kenten, 1972); artichoke Italian latent virus (AILV), grapevine isolate (Savino et al., 1977); raspberry ringspot virus (RRV), the type culture of the English strain originally obtained from Rubus procerus (Cadman, 1960); tobacco ringspot virus (TobRV), an isolate from blueberry (Lister et al., 1963); tomato ringspot virus (TomRV), type strain supplied by R. W. Fulton (Murant & Taylor, 1978); arabis mosaic virus (AMV), an isolate obtained from raspberry (Harrison, 1958a); myrobalan latent ringspot virus (MLRV), type isolate (Dunez et al., 1976); mulberry ringspot virus (MRV), from mulberry (Tsuchizaki et al., 1971); strawberry latent ringspot virus (SLRV), type strain from strawberry (Lister, 1964). TBRV-G, GCMV, CNV, AILV, TobRV, TomRV, MRV and SLRV were held under licence from the Department of Agriculture and Fisheries for Scotland.

TBRV, RRV, TobRV, TomRV and AMV were propagated in Nicotiana clevelandii, AILV, GCMV, MLRV, MRV and SLRV in Chenopodium quinoa, and CNV in Phaseolus vulgaris.

**Virus purification.** MRV particles were purified by the method of Tsuchizaki et al. (1971). Particles of all other nepoviruses were purified as described for TBRV (Mayo et al., 1979). Middle and bottom nucleoprotein components of TBRV were separated by sucrose density gradient sedimentation (3-25 h at 26000 r.p.m. in 10 to 40% sucrose gradients in 0-07 M-phosphate buffer, pH 7-0). Middle component was further purified by another cycle of sucrose density gradient sedimentation, and bottom component by sedimentation to equilibrium in CsCl solution (Harrison & Barker, 1978).

**Preparation of RNA samples.** RNA-1 and RNA-2 species of TBRV were extracted from bottom and middle components respectively of strains A, S12, G12 and C, as described by Mayo et al. (1979), except that the nucleoprotein components were incubated at 60 °C for 15 min in 2% SDS, 0-01 M-Tris-HCl pH 9-0, 0-5 mM-EDTA, before treatment with phenol. Further purification of RNA species was as described by Robinson et al. (1980). RNA-3 was prepared from strains S and G as described by Robinson (1982). For all other nepoviruses, total RNA was extracted from preparations of virus particles using the same procedure.

**Preparation and characterization of complementary DNA (cDNA).** 3-H-labelled copies of TBRV RNA species were prepared by the method of Taylor et al. (1976), using the reaction conditions of Robinson et al. (1980). The size distribution of 3-H-labelled cDNA was assessed by electrophoresis in 3% agarose gels, under denaturing conditions (Murant et al., 1981). Approximately 1 × 10^6 c.p.m. cDNA was precipitated with ethanol at −18 °C, in the presence of 5 μg Escherichia coli RNA (Murant & Taylor, 1978) as a carrier, and denatured with glyoxal (Murant et al., 1981). Similarly denatured E. coli RNA markers were run in parallel gels, and bands located by toluidine blue staining. Gels containing cDNA were cut into slices 1 mm thick, and melted in 0-75 ml H_2O for 5 min at 70 °C; 10 ml scintillation fluid [toluene-Triton X-100 (3:1) + 0-5% diphenyloxazole] was then added to each fraction and radioactivity measured in an Intertechnique SL30 liquid scintillation counter.

**RNA–cDNA hybridization analysis.** Hybrids were allowed to form in 0-18 M-NaCl, 0-01 M-Tris-HCl pH 7, 1 mM-EDTA, 0-05% SDS, and assayed using S1 nuclease as described by Robinson et al. (1980). The equation for pseudo-first order kinetics was fitted using the RE'OLV programme of Koepppe & Hamann (1980), and plots were scaled to give asymptotes of 0% and 100% cDNA hybridized in homologous reactions. R_0,t values (RNA concentration × time, for 50% hybridization) were estimated by interpolation.

Homologous and heterologous hybrids, prepared by incubation to a R_0,t value ensuring completion of hybrid formation, were used to construct thermal denaturation curves. Duplicate samples were incubated for 5 min at temperatures ranging from 60 to 100 °C, chilled on ice, and the percentage of hybrid remaining was assayed using S1 nuclease.

To compare the genome RNA sequences of other nepoviruses with those of TBRV, cDNA copies of TBRV RNA-1 and RNA-2 (both serotypes) were incubated with unfraccionated RNA of each virus, to a R_0,t value of 1-8 mol s/l, thus ensuring hybridization of homologous sequences.

**Serological methods.** Immunosorobent electron microscopy (ISEM) was performed as described by Roberts & Harrison (1979). Antiserum-coated grids were floated on 10 μl drops of purified AILV particles, diluted in
**RESULTS**

**Characterization of $^3$H-labelled cDNA**

The size distribution of cDNA transcribed from RNA-1 of TBRV-C is shown in Fig. 1. A broad range of transcript sizes was observed, with an average chain length of approximately 200 nucleotides. Similar results were obtained for cDNA transcribed from other TBRV RNA species.

**Homology between RNA-1 and RNA-2 species**

It has previously been reported that no sequences common to both RNA-1 and RNA-2 of TBRV-A or TBRV-S (Scottish serotype) can be detected by hybridization analysis (Robinson et al., 1980; Robinson, 1982). Using the same technique, no sequence homology between the genome components of TBRV-G or TBRV-C (German serotype) was observed. It is estimated that common sequences comprising 5% or more of either genome RNA species would be reliably detected by this method.

**Homology between corresponding genome RNA species of different strains**

Fig. 2(a) shows the kinetics of hybridization of cDNA transcribed from RNA-1 of TBRV-A with its homologous RNA species (upper curve), and with RNA-1 of TBRV-C (lower curve). Both homologous and heterologous reactions proceeded with pseudo-first order kinetics. The extent of reaction in the heterologous combination indicated the presence of common sequences, totalling about 30%, between the RNA-1 species of TBRV-A and TBRV-C.

Fig. 2(b) shows the kinetics of hybridization of cDNA transcribed from RNA-2 of TBRV-A with its homologous RNA species (upper curve) and with RNA-2 of TBRV-G (lower curve). In this instance the extent of reaction indicated that only about 9% of the nucleotide sequences are common to these two RNA species.
Fig. 2. Kinetics of hybridization of (a) TBRV-A cDNA-1 with TBRV-A RNA-1 (●) and TBRV-C RNA-1 (▲), and (b) TBRV-A cDNA-2 with TBRV-A RNA-2 (●) and TBRV-G RNA-2 (▲).

<table>
<thead>
<tr>
<th>Table 1. Estimated percentage sequence homology between TBRV RNA-1 species (a) and TBRV RNA-2 species (b)</th>
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</thead>
<tbody>
<tr>
<td>(a) RNA-1 of strain</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>S</td>
</tr>
<tr>
<td>G</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>(b) RNA-2 of strain</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>S</td>
</tr>
<tr>
<td>G</td>
</tr>
<tr>
<td>C</td>
</tr>
</tbody>
</table>

* Estimated percentage sequence homology. Mean of values obtained in three separate experiments each comprising two or more replicate determinations (± standard deviation). Hybridizations were performed at 60 °C at R₀ values between 0.35 and 2.25 mol·s/l.
† ND. Not determined.

Similar experiments were performed using all combinations of cDNA-1 and RNA-1 and the results are summarized in Table 1 (a). Reciprocal determinations of percent homology were done simultaneously; the values given each represent the mean of three independent determinations. Within each serotype a high degree of sequence homology, about 90%, was observed, whereas between serotypes more difference was noted, only 30 to 40% of sequences being common to all four strains. Table 1 (b) shows similar estimates of percentage sequence homology between the RNA-2 species of the four strains. Again, a high degree of sequence similarity was found within each serotype, the RNA-2 species of the Scottish serotype cross-hybridizing almost completely, and those of the German serotype differing by about 10%. About 10% of the RNA-2 sequences are common to all four strains.
**TBRV nucleotide sequence homologies**

Table 2.\( R_{0t}\) values for hybridizations comparing TBRV RNA-1 (a) and TBRV RNA-2 (b) species

<table>
<thead>
<tr>
<th></th>
<th>cDNA-1 of strain</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>S</td>
<td>G</td>
<td>C</td>
</tr>
<tr>
<td>A</td>
<td>1.0*</td>
<td>1.0</td>
<td>5.6</td>
<td>3.0</td>
</tr>
<tr>
<td>S</td>
<td>0.99</td>
<td>0.83</td>
<td>2.5</td>
<td>ND†</td>
</tr>
<tr>
<td>G</td>
<td>3.3</td>
<td>ND</td>
<td>1.5</td>
<td>1.9</td>
</tr>
<tr>
<td>C</td>
<td>4.9</td>
<td>ND</td>
<td>2.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>cDNA-2 of strain</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.53*</td>
<td>0.93</td>
<td>2.5</td>
<td>2.9</td>
</tr>
<tr>
<td>S</td>
<td>0.73</td>
<td>0.73</td>
<td>2.4</td>
<td>ND</td>
</tr>
<tr>
<td>G</td>
<td>3.6</td>
<td>1.4</td>
<td>0.7</td>
<td>1.4</td>
</tr>
<tr>
<td>C</td>
<td>2.3</td>
<td>ND</td>
<td>0.89</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* \( R_{0t}\) values (mol·s/l \times 10^2)
† ND, Not determined.

Table 3. Melting temperatures of hybrids between TBRV RNA-1 species (a) and TBRV RNA-2 species (b) and their cDNA copies

<table>
<thead>
<tr>
<th></th>
<th>cDNA-1 of strain</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>84.0*</td>
<td>72.0</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>84.0</td>
<td>ND†</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>74.0</td>
<td>85.5</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>cDNA-2 of strain</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>82.5</td>
<td>77.5</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>76.5</td>
<td>82.0</td>
<td></td>
</tr>
</tbody>
</table>

* Melting temperature (°C) of hybrids, defined as the temperature at which 50% of the \(^3\)H-labelled cDNA in the hybrid remained insensitive to S1 nuclease digestion.
† ND, Not determined.

A reaction between cDNA copies of an RNA and any RNA species of the same sequence complexity is expected to proceed at the same rate, measured as \( R_{0t}\), whatever the extent of the reaction. However, inspection of Fig. 2 clearly shows that heterologous reactions proceeded several-fold slower than homologous ones.

Table 2 shows \( R_{0t}\) values for a series of kinetic analyses, comparing either RNA-1 or RNA-2 species. The values for homologous and heterologous hybridizations between members of the Scottish serotype are similar to one another, but those for heterologous hybridizations involving comparisons between serotypes, and to a lesser extent between members of the German serotype, are consistently higher.

To investigate this further, melting profiles of homologous and heterologous hybrids were compared. The melting profiles of hybrids formed between cDNA-1 of TBRV-S and the RNA-1 species of TBRV-A or TBRV-S were indistinguishable (Fig. 3). Both showed a sharp thermal transition, and a high melting temperature of 84 °C. In contrast, hybrids formed between the same cDNA preparation and the RNA-1 species of TBRV-G exhibited a broader melting curve and a melting temperature of 74 °C. The melting temperatures of different RNA-1 : cDNA-1 and RNA-2 : cDNA-2 hybrids are given in Table 3(a) and 3(b) respectively. Lower thermal stability of hybrids was consistently associated with those combinations exhibiting lower rates of hybrid formation.
Table 4. Estimated percentage sequence homology between satellite RNA (RNA-3) species from two TBRV strains

<table>
<thead>
<tr>
<th>RNA-3 from strain</th>
<th>cDNA to RNA-3 from strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>S 100</td>
</tr>
<tr>
<td>G</td>
<td>G 22 100*</td>
</tr>
</tbody>
</table>

* Estimated percentage sequence homology. Hybridizations were performed at 60 °C to a R₀t value of 0·35 mol·s⁻¹.

Homology between satellite RNA species from TBRV strains S and G.

Mixtures containing RNA-3 from TBRV-S or TBRV-G together with their cDNA copies in homologous and heterologous combinations were incubated to a R₀t value of 0·35 mol·s⁻¹, sufficient to ensure completion of hybrid formation. The results (Table 4) indicate that these two satellite RNA species have about 25% of their sequences in common.

Comparison of TBRV genome RNA sequences and those of other nepoviruses

The results of hybridization experiments comparing TBRV RNA species with those of other nepoviruses are shown in Table 5. The slight S₁ nuclease resistance seen for example with combinations including TobRV RNA is not considered significant. No sequence homology could be detected between TBRV RNA and RNA from RRV, TobRV, TomRV, SLRV, MRV, MLRV or AMV. GCMV was found to have about 20% of its RNA-2 sequences and about 7% of its RNA-1 sequences in common with TBRV (German serotype) but little if any sequence in common with the Scottish serotype. A small amount of hybridization was also detected between CNV RNA and cDNA transcribed from TBRV RNA species. Approximately 20% sequence homology was detected between AILV RNA and RNA-1 from TBRV strains belonging to either serotype. AILV RNA was also found to contain about 30% of the sequences of RNA-2 from the Scottish serotype of TBRV, but a smaller proportion of sequences of RNA-2 from the German serotype. Interestingly, the homology between AILV RNA and TBRV-S RNA-2 is greater than that between the RNA-2 species of the two TBRV serotypes. These amounts of homology suggest that AILV is more closely related to TBRV than is either CNV or GCMV.

Serological relationship between AILV and TBRV

Although no serological relationship has previously been detected between AILV and TBRV, the discovery of common sequences in the RNA-2 species of these two viruses suggested that a distant serological relationship might exist. In ISEM tests (Table 6) coating of grids with AILV antiserum produced 100- to 200-fold increases in the number of AILV particles attached, compared with uncoated control grids. The TBRV antisera produced increases of between 5- and 35-fold in particle attachment.

Antibody coating was observed when AILV particles attached to AILV antiserum-coated grids were exposed either to AILV antiserum or to TBRV-S antiserum. The dilution endpoints for coating of particles were 1 : 2048 with AILV antiserum and 1 : 256 with TBRV-S antiserum. Both antisera coated all particles evenly, suggesting that the preparation contained virus particles of only one antigenic type.

In gel precipitin tests, no reaction was observed between sap from AILV-infected C. quinoa and TBRV-S antiserum, or between sap from TBRV-S-infected N. clevelandii and AILV antiserum. However, in tests using purified virus preparations, TBRV-S antiserum reacted with both TBRV-S and AILV (Fig. 4). The heterologous reaction was not abolished by absorption of the TBRV-S antiserum with sap from uninfected C. quinoa, and the homologous and heterologous precipitin lines joined and a spur was formed.

Taken together these experiments demonstrate that AILV and TBRV are distantly related serologically.
Fig. 3. Melting curves of hybrids between cDNA-1 of TBRV-S and RNA-1 of TBRV-S (■), RNA-1 of TBRV-A (▲) and RNA-1 of TBRV-G (■).

Fig. 4. Gel precipitin test. Centre well contained TBRV-S antiserum absorbed with healthy C. quinoa sap (final antiserum dilution 1/10). Odd-numbered wells contained purified TBRV-S particles (0-9 mg/ml). Purified AILV particles were contained in wells 2 and 4 (1-6 mg/ml) and wells 6 and 8 (60 μg/ml).

Table 5. Percentage hybridization between RNA of various nepoviruses and cDNA transcribed from TBRV RNA species

<table>
<thead>
<tr>
<th>Source of RNA</th>
<th>RNA used for cDNA preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RNA-1 (S)</td>
</tr>
<tr>
<td>RRV</td>
<td>0*</td>
</tr>
<tr>
<td>TobRV</td>
<td>1</td>
</tr>
<tr>
<td>TomRV</td>
<td>0</td>
</tr>
<tr>
<td>MRV</td>
<td>0</td>
</tr>
<tr>
<td>SLRV</td>
<td>0</td>
</tr>
<tr>
<td>AMV</td>
<td>0</td>
</tr>
<tr>
<td>MLRV</td>
<td>0</td>
</tr>
<tr>
<td>GCMV</td>
<td>0</td>
</tr>
<tr>
<td>CNV</td>
<td>3</td>
</tr>
<tr>
<td>AILV</td>
<td>12</td>
</tr>
<tr>
<td>Homologous</td>
<td>60†</td>
</tr>
</tbody>
</table>

* S1 nuclease resistance of cDNA after hybridization with RNA to a R₀ value of 1-8 mol·s/l. Values corrected for S1 nuclease resistance of cDNA after incubation in the absence of RNA.
† Corrected S1 nuclease resistance of cDNA after hybridization with homologous RNA to a R₀ value of 0-35 mol·s/l.

DISCUSSION

Hybridization analysis has shown that a high degree of sequence similarity exists between corresponding genome RNA species of TBRV strains belonging to the same serotype. Members of different serotypes have some sequences in common in both RNA species, but are much less alike. Approximately 30% of the RNA-1 sequences and 10% of the RNA-2 sequences appear to be common to all four strains.
Table 6. Tests for a relationship between AILV and TBRV by ISEM

<table>
<thead>
<tr>
<th>Coating antiserum</th>
<th>Homologous titre*</th>
<th>Expt. 1†</th>
<th>Expt. 2†</th>
</tr>
</thead>
<tbody>
<tr>
<td>AILV</td>
<td>1:512</td>
<td>116000</td>
<td>49580</td>
</tr>
<tr>
<td>TBRV-S</td>
<td>1:500</td>
<td>17227</td>
<td>6270</td>
</tr>
<tr>
<td>TBRV-A</td>
<td>1:1024</td>
<td>8613</td>
<td>3230</td>
</tr>
<tr>
<td>TBRV-G</td>
<td>1:2048</td>
<td>11527</td>
<td>2478</td>
</tr>
<tr>
<td>Control (no serum)</td>
<td>–</td>
<td>498</td>
<td>468</td>
</tr>
</tbody>
</table>

* Determined in gel precipitin tests.
† In expt. 1, AILV particles were diluted to 100 µg/ml, and in expt. 2, to 20 µg/ml.

Examination of the thermal stability of cDNA/RNA hybrids revealed that heterologous hybrids, particularly between representatives of different serotypes, were less temperature-stable than homologous hybrids. Wetmur (1976) suggested that the melting temperature of hybrids is reduced by 1 °C per 1% mismatching. This implies that heterologous hybrids between RNA-1 and cDNA-1 representing different serotypes (Table 3a) contain approx. 10% mismatched base pairs. Mismatching in the regions of common sequence of the RNA-2 species of strains G and C, to the extent of about 5%, is also implied by the slightly reduced melting temperatures of heterologous hybrids (Table 3b). Thus, the sequences apparently common to all four strains are similar, but not identical, in the two serotypes.

Hybrids with low temperature stability also exhibit reduced rates of formation (Table 2). The presence of mismatches destabilizes regions of base pairing and causes a net decrease in the rate of hybrid formation, reflected by high R_0t values. In these experiments, estimates of percentage homology have been made using relatively low salt concentrations, both during hybridization and in the subsequent S1 nuclease assay. Gonda & Symons (1978) have shown that hybrid stability is highly dependent on salt concentration, especially in the S1 nuclease assay. At lower salt concentrations, hybrids must contain a greater number of correctly matched base pairs to be stable; therefore, in the present study mismatching had a noticeable effect on the net rate of hybridization.

Reciprocal determinations of percentage homology should give the same value, within experimental error. In most instances no significant difference was seen; however, in some comparisons, notably those of the RNA-1 species of strains C and A, values obtained using cDNA transcribed from RNA-1 of TBRV-C were considerably higher than those using cDNA transcribed from RNA-1 of TBRV-A. It is not known whether this is of any real importance, but it may reflect a difference in cDNA representivity. Although representivity has not been investigated here, Gould & Symons (1977), using essentially the same conditions, demonstrated that cDNA preparations transcribed from the four major RNA species of cucumber mosaic virus were fully representative.

At present, it is only possible to speculate whether the conserved sequences in TBRV have any functional significance. As there is a distant serological relationship between members of the Scottish and German serotypes (Harrison, 1958b), it is possible that the sequences conserved in the RNA-2 species code for antigenic determinants of the coat protein. It is also possible, because of the degeneracy of the genetic code, that a greater degree of similarity exists between the amino acid sequences of the polypeptides coded for by these RNA species. However, it is interesting to note that there is no evidence for any sequence common to all members of the nepovirus group. Indeed of those nepoviruses examined, the only ones to show any sequence homology with TBRV were GCMV, CNV and AILV. In this respect nucleic acid hybridization reveals the same pattern of relationships as do serological methods; GCMV and CNV have previously been shown to be serologically related to both serotypes of TBRV (Murant, 1981; Murant & Raschke, 1981; Kenten, 1972) and immunoelectron microscopy and gel diffusion precipitin tests reported in this paper show that a distant serological relationship also exists between TBRV and AILV. These results, together with transmissibility by nematodes in the
TBRV nucleotide sequence homologies

genus Longidorus, and the similar biophysical properties of AILV and TBRV, provide a strong case for including AILV in the same nepovirus subgroup as TBRV.

It was previously shown (Robinson, 1982) that little or none of the nucleotide sequence of the satellite RNA of TBRV-S was represented in the genome RNA species. The present work shows further that the satellite RNA species of TBRV-S and of TBRV-G are substantially different, although they share about 25% of their sequences. This sequence difference is reflected in the different products obtained by translation in vitro of the satellite RNA species (Fritsch et al., 1984). Moreover, there are functional differences between the two satellites, for each satellite is dependent on RNA-1, which must come from a TBRV strain of the same serotype as that with which the satellite naturally occurs, and not from a strain of the other serotype (Murant & Mayo, 1982).

The overall pattern of homologies between the two serotypes of TBRV suggests that the three RNA species have evolved together, albeit to somewhat different extents. The functional significance of the sequences that are apparently conserved between strains of TBRV may become clearer as a result of direct sequencing studies which are now underway in several laboratories.

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