Evidence for a Satellite RNA Associated Naturally with the U5 Strain and Experimentally with the U1 Strain of Tobacco Mosaic Virus

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SUMMARY

Isolates of tobacco mosaic virus strain U5 (TMV-U5) from native Nicotiana glauca plants induced the accumulation of a dsRNA (mol. wt. 0.6 × 10^6) in infected plants that was more abundant than the replicative form (RF) dsRNA of TMV (mol. wt. 4.3 × 10^6). Some but not all subcultures of such a field isolate obtained from single local lesions on N. tabacum cv. Xanthi-nc, had lost the ability to induce the 0.6 × 10^6 mol. wt. dsRNA. Co-inoculation experiments in N. silvestris established that the dsRNA could accumulate in plants infected with TMV-U1, but to a lesser extent than when associated with TMV-U5. A ssRNA (mol. wt. 0.3 × 10^6) was isolated from plants containing the dsRNA. This was not infectious by itself but became so when associated with TMV-U5 or TMV-U1, and then induced the accumulation of the 0.6 × 10^6 dsRNA. Plants infected with TMV-U5 isolates which did or did not induce the 0.6 × 10^6 dsRNA had identical symptoms. The host range of the 0.3 × 10^6 ssRNA was the same as that of the TMV strain with which it was associated in each of the 20 experimental hosts tested. Complementary DNA transcribed from purified 0.6 × 10^6 dsRNA did not hybridize with RF and other dsRNAs of TMV-U5, TMV-U1, tobacco necrosis virus, potato virus X, citrus tristeza virus, and cucumber mosaic virus + CARNA 5, but the cDNA did hybridize with the 0.6 × 10^6 mol. wt. dsRNA and the 0.3 × 10^6 mol. wt. ssRNA found only in plants containing this dsRNA. The results indicate that the 0.6 × 10^6 dsRNA is the RF of a satellite RNA of TMV. Purified nucleoprotein from plants infected with TMV-U5 and the satellite RNA were infectious for the satellite RNA, but the nature of encapsidation of the satellite RNA has yet to be determined.

INTRODUCTION

Natural infections of Nicotiana glauca Grah. by tobacco mosaic virus (TMV) have been reported in California (Bald & Goodchild, 1960), India (Nariani & Singh, 1952), Israel (Bar-Joseph et al., 1983) and Australia (Randles et al., 1981). The U5 strain of TMV was originally described by Siegel & Wildman (1954), and was characterized further by Bald & Goodchild (1960) and Shalla et al. (1975). This strain was originally isolated from N. glauca from southern California and it can be diagnosed by its ability to induce necrotic local lesions on N. silvestris Speg. & Comes and by its failure to infect most cultivars of Lycopersicon esculentum Mill. Different degrees of nucleic acid homology were found to exist between various isolates of TMV from N. glauca in Australia (Randles et al., 1981), and Palukaitis & Symons (1980) concluded that some isolates of TMV-U5 and TMV-U1 shared appreciable nucleotide sequence homology. The genome of the U1 strain and other viral RNAs, including dsRNAs, that are present in extracts of infected plants have been well characterized (Dawson & Dodds, 1982; Palukaitis et al., 1983). One major dsRNA, mol. wt. 4.3 × 10^6 (the replicative form, RF), three other readily
detected dsRNAs (3.0 \times 10^6, 0.95 \times 10^6, \text{and} 0.42 \times 10^6), and several additional minor dsRNAs have been reported (Dawson & Dodds, 1982; Palukaitis et al., 1983; Zelcer et al., 1981).

During the course of study of the dsRNAs of several members of the tobamovirus group (Valverde et al., 1986), we obtained new isolates of TMV-U5 from local N. glauca plants. The presence of a prominent dsRNA (mol. wt. 0.6 \times 10^6) in the dsRNA profiles of these TMV-U5 isolates distinguished them from the isolate used by Bald & Goodchild (1960). In this paper we describe this prominent dsRNA associated with the new isolates of TMV-U5, and provide evidence that it is the dsRNA of a satellite RNA.

**METHODS**

**Virus isolates.** The U2 strain of TMV (TMV-U2), and the green tomato atypical mosaic virus (GTAMV) were supplied by A. Siegel. The U1 strain of TMV (TMV-U1) was obtained from the American Type Culture Collection (ATCC, PV-135). The purple strain of tomato mosaic virus (ToMV) was supplied by J. P. Fulton. One of six isolates of TMV-U5 obtained from naturally infected N. glauca plants found near Riverside, California was used in this study. This TMV-U5 isolate (isolate no. 2) was passaged through two single local lesion transfers on N. tabacum cv. Xanthi-nc and maintained in N. tabacum cv. Turkish. Other viruses used [potato virus X (PVX), cucumber mosaic virus (CMV), tobacco necrosis virus (TNV) and citrus tristeza virus (CTV)] were isolates presently available in our laboratory. With the exception of CTV, which was propagated in Citrus aurantifolia (Christm.) Swingle, viruses were maintained and propagated in N. tabacum cv. Turkish.

**Isolation of TMV-U5 from field-collected samples.** Leaf samples from 24 arbitrarily selected N. glauca plants were collected in the vicinity of Riverside, and dsRNA was extracted from them and analysed. Crude sap extracts from six selected samples known to contain TMV-U5 dsRNA were heat-treated (90 °C for 10 min), or stored at 22 °C for up to 3 months in order to eliminate CMV and CMV-associated RNA 5 (CARNA 5), which were present as contaminants. The isolates obtained following inoculation of N. tabacum cv. Turkish with treated sap were evaluated by dsRNA analysis, host reaction, and examination of particle morphology after negative staining.

**Isolation of subcultures of TMV-U5 free of the satellite dsRNA.** In efforts to obtain isolates of TMV-U5 that did not induce the satellite dsRNA, TMV-U5 was passaged through single lesion transfers on N. tabacum cv. Xanthi-nc and then maintained in N. tabacum cv. Turkish. In addition, crude sap from TMV-U5-infected plants was heated for 10 min at different temperatures (70, 80, 90 and 100 °C) prior to inoculation to N. tabacum cv. Turkish. Also, plants were inoculated with full-length genomic ssRNA of TMV-U5 electroeluted from a slice of an agarose gel through which ssRNA from purified virus had been electrophoresed.

**Host range and symptoms.** Seedlings of 20 plant species belonging to seven different families were mechanically inoculated with sap extracts from plants infected with TMV-U5/S+ (isolate that induced the satellite dsRNA), and TMV-U5/S− (isolate that did not induce the satellite dsRNA), obtained after passage of TMV-U5/S+ through single lesion transfers) in 0.02 M-NaHPO4 buffer pH 7.2. Symptoms were recorded and dsRNAs were analysed 8 and 14 days after inoculation.

**Purification of virions.** Virions of TMV-U1, TMV-U5/S+ and TMV-U5/S− were purified by polyethylene glycol precipitation (Gooding & Hebert, 1967) from infected N. tabacum cv. Turkish. Further purification was accomplished by alternate low (8000 g for 15 min) and high (90000 g for 120 min) speed centrifugations. Pellets obtained following high speed centrifugation were resuspended in sodium citrate buffer pH 8.0. PVX and CMV were purified by previously published procedures (Bercks, 1971; Dodds et al., 1985). CMV was further purified by sucrose density gradient centrifugation in 5 mM-EDTA pH 7.0.

**Purification of nucleic acids**

**DsRNA purification.** DsRNAs were purified from 7.0 g of leaf tissue by two cycles of fractionation on columns of CF-11 cellulose powder (Jordan et al., 1983). DsRNAs that eluted from the columns in ethanol-free STE (100 mM-NaCl, 50 mM-Tris–HCl, 1.0 mM-EDTA, pH 7.0) were precipitated with 3 vol. 95% ethanol and resuspended in 300 μl electrophoresis buffer (TAE buffer) (40 mM-Tris–HCl, 20 mM-sodium acetate, 1 mM-EDTA, pH 7.8) (Jordan et al., 1983).

**Purification of satellite dsRNA.** After electrophoretic fractionation of dsRNA in 1.2% agarose gels, the region of the gel containing the satellite dsRNA was excised and placed in a dialysis bag containing a minimal volume of 1/5th strength electrophoresis buffer. Elution of the dsRNA from the gel was carried out by electrophoresis (60 mA for 60 min). Eluted dsRNA was recovered in the solution from the bag and precipitated by addition of ethanol to 66%.

**SsRNA purification.** SsRNA was isolated from purified TMV-U1, TMV-U1/S+, TMV-U5/S+, TMV-U5/S−, PVX and CMV virions by the sodium perchlorate method (Wilcockson & Hull, 1974). SsRNA was extracted from 7.0 g of leaf tissue of uninfected N. tabacum cv. Turkish and from plants infected with TMV-U1, TMV-U1/S+, TMV-U5/S+ and TMV-U5/S−. Tissue was ground to a powder in liquid nitrogen and then
stirred for 30 min with 14 ml STE buffer, 18 ml STE-saturated phenol, 2 ml 10% SDS and 18 mg bentonite. The mixture was centrifuged (8000 g for 15 min) and the aqueous phase was adjusted to 2.0 M-LiCl and stored overnight at 4 °C. Samples were centrifuged (8000 g for 30 min), and pellets were washed with 2.0 M-LiCl and resuspended in 5 ml H₂O. Nucleic acids that were insoluble in 2.0 M-LiCl but soluble in H₂O (mostly ssRNA) were precipitated by addition of 15 ml 95% ethanol and 0.5 ml 3.0 M-sodium acetate pH 5.5, stored overnight at −20 °C, centrifuged (8000 g for 20 min) and resuspended in sterile electrophoresis buffer.

Gel electrophoresis and blotting of nucleic acids. DsRNA was electrophoresed through 6% polyacrylamide gels in a vertical slab gel apparatus (9 cm × 8 cm × 1-5 mm) in electrophoresis buffer at constant voltage (100 V) for 3 h. Molecular weight standards were the dsRNAs of CTV (mol. wt. 13.3 × 10⁶), TNV (mol. wt. 2.3, 0.78 and 0.65, all × 10⁶) and CMV (mol. wt. 2.0, 1.9, 1.30 and 0.55, all × 10⁶) (Valverde et al., 1986; Dodds & Bar-Joseph, 1983). SsRNA was electrophoresed through a 1:5% agarose gel containing 20 mM-HEPES pH 7.8, 1 mM-EDTA and 6% formaldehyde (Lehrach et al., 1977; Gustafson et al., 1982). Samples were heated to 65 °C for 4 min in electrophoresis buffer containing 50% deionized formamide, cooled on ice and electrophoresed for 2 h at 60 V. Alternatively, samples were run in 1.2% agarose gels under non-denaturing conditions using TAE buffer. Gels were stained with ethidium bromide, 20 ng/ml.

Prior to electroblotting, polyacrylamide gels were denatured with 50% DMSO, 1 M-glyoxal in 25 mM-sodium phosphate buffer pH 6.5, at 50 °C for 1 h. Non-denatured agarose gels were denatured in 0.05 M-NaOH for 15 min followed by neutralization in 1 M-Tris-HCl pH 7.4 for 30 min (Bar-Joseph et al., 1983). Gels were electroblotted to nylon membranes (Zeta-Probe, Bio-Rad) in the presence of 10 mM-Tris-HCl, 5 mM-sodium acetate, 0.5 mM-EDTA, pH 7.8. The Zeta-Probe membrane to which nucleic acids were transferred was baked in a vacuum oven for 2 h at 80 °C.

In order to determine whether the satellite RNA had homology with host nucleic acid (RNA and DNA) 2.5 µg of phenol-extracted total nucleic acid (denatured with formamide) from uninfected N. glauca and N. tabacum was spotted on a nylon membrane. Similar samples from infected plants were also spotted as controls. Membranes were baked for 2 h at 80 °C and stored at room temperature until ready for hybridization.

Complementary DNA synthesis and hybridization. Randomly primed cDNA was transcribed from RNA with avian myeloblastosis virus reverse transcriptase (Bethesda Research Laboratories) in the presence of [α-32P]dCTP (approx. 3000 Ci/mmol; Amersham) essentially as described by Taylor et al. (1976) and Gould & Symons (1977). The RNA used was either dsRNA denatured with formamide (in the case of the satellite dsRNA) or (in the case of TMV-U5/S- ssRNA extracted from virus particles. Prehybridization and hybridization were carried out according to procedures in the instructions for the use of Zeta-Probe membranes provided by the manufacturer (Bio-Rad).

Infectivity of the satellite RNA. Purified preparations (50 µg/ml) of the satellite dsRNA in sterile 1 mM-EDTA pH 7.4 were mechanically inoculated to N. tabacum cv. Turkish, alone or in combination with genomic ssRNA of TMV-U5/S- (50 µg/ml). Similar experiments were done using melted satellite dsRNA as described by Jackson et al. (1971). Satellite ssRNA was obtained by electroelution from the section of an agarose gel (used to fractionate 2.0 M-LiCl-insoluble ssRNAs) that contained RNA which hybridized with probe made from the satellite dsRNA. This RNA (approximately 50 µg/ml) was suspended in 1 mM-EDTA pH 7.4 and mechanically inoculated alone and in combination with TMV-U5/S-, TMV-U1, PVX or CMV ssRNA (100 µg/ml) to N. tabacum cv. Turkish. Healthy tobacco plants and plants previously infected with TMV-U5/S- or TMV-U1 were also inoculated with electroeluted satellite ssRNA. Ten days after inoculation 7.0 g of leaf tissue was harvested and used for dsRNA analysis.

Other attempts to transfer the satellite RNA from TMV-U5/S- to TMV-U1, PVX and CMV were made by mechanically inoculating plants of N. silvestris, which is a systemic host of the target viruses (TMV-U1, PVX and CMV) but is not a systemic host of TMV-U5. The inocula used consisted of sap (1:5 dilution in 1 mM-Na₂HPO₄ buffer pH 7.0) from mixed infections in N. tabacum cv. Turkish of TMV-U5/S+ and the respective target virus. Ten days after inoculation, 7.0 g of systemically infected leaf tissue was harvested and analysed for dsRNA.

RESULTS

DsRNAs of tobamoviruses

DsRNA analysis of several tobamoviruses is shown in Fig. 1. The dsRNAs of ToMV, GTAMV, TMV-U2 and TMV-U1, though similar, were differentiated by dsRNAs other than the RF. The U5 strain of TMV selected for the remaining work had an RF with a slightly faster electrophoretic mobility than the RF of the other tobamoviruses. A unique, prominent dsRNA (satellite dsRNA) with a mol. wt. of 0.6 × 10⁶ was present in dsRNA extracted from plants infected with TMV-U5/S+ (Fig. 1, lane 6) but not from those infected with TMV-U5/S- (Fig. 1,
Fig. 1. Polyacrylamide gel electrophoresis (PAGE) of dsRNAs from *N. tabacum* plants infected with ToMV-p (lane 1), GTAMV (lane 2), TMV-U2 (lane 3), TMV-U1 (lane 4), TMV-U1 + TMV-U5/S- (mixed infection) (lane 5), TMV-U5/S+ (lane 6), TMV-U5/S- (lane 7) and CMV + CARNA 5 (lane 8). ▲, RF of tobamoviruses (4.3 × 10⁶); ▲, satellite; a to c, other dsRNAs of TMV-U1 (a, 3.0 × 10⁶; b, 0.95 × 10⁶; c, 0.42 × 10⁶).

lane 7). This dsRNA was much more abundant than the RF of TMV-U5 and was not detected in extracts from plants infected with any of the other tobamoviruses analysed in this and in a previous study (Valverde *et al.*, 1986).

**Frequency of TMV-U5 in field-collected samples**

Of 24 *N. glauca* plants tested, six contained dsRNA of TMV-U5 (Fig. 2) (all six also had satellite dsRNA). Those six plants were also infected with CMV and CARNA 5 but these agents were eliminated from sap by heat treatment, or storage for up to 3 months, prior to using it as inoculum. DsRNA 4 of CMV co-electrophoresed with satellite dsRNA. All six isolates so obtained were confirmed to be TMV-U5 by particle morphology, host reaction (failure to infect *L. esculentum* cv. Rutgers, the induction of necrotic local lesions on *N. silvestris*, and mild mosaic symptoms on *N. tabacum* cv. Turkish), and their dsRNA banding pattern. Some satellite dsRNA associated with different TMV-U5 field isolates apparently had differences in electrophoretic mobilities.

**Subcultures of TMV-U5 free of the satellite dsRNA**

After single local lesion transfers of one TMV-U5/S+ isolate, 10 out of 30 of the isolates obtained were free of the satellite dsRNA (Fig. 3). These subcultures remained free of the satellite after more than 10 transfers in *N. glauca* over a period of 10 months. Inoculum that consisted of gel-purified full-length (genome-sized) ssRNA of TMV-U5/S+ yielded isolates of TMV-U5 that were free of the satellite dsRNA. After heating at 95°C, or storage at room temperature for up to 3 months sap of plants infected with TMV-U5/S+ remained infective for TMV-U5 and the satellite.
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Fig. 2. PAGE of dsRNAs from six N. glauca plants naturally infected with TMV-U5/S+ and CMV + CARNA 5 (lanes 1 to 6). DsRNAs from experimental single infections in N. tabacum (lane 7, TMV-U5/S+; lane 8, CMV + CARNA 5) are shown for comparison. △, RF of TMV-U5; ▼, satellite; ◇, CARNA 5.

Fig. 3. PAGE of dsRNAs from N. tabacum plants infected with six single local lesion isolates of TMV-U5/S+. △, RF of TMV-U5; ▼, satellite.

Host range and symptoms

No difference in host reaction was obtained following inoculation of plants with either TMV-U5/S+ or TMV-U5/S-. Plant species tested that reacted with similar systemic symptoms were: N. tabacum cv. Turkish, N. benthamiana Domin., N. clevelandii A. Gray, N. glauca, Vinca rosea L. and Physalis floridana Rydb. Plants that developed localized infection only were: Chenopodium
Fig. 4. (a) PAGE of dsRNAs from plants infected with TMV-U5/S⁺ (lane 1), TMV-U1/S⁺ (lane 2), TMV-U1 + TMV-U5/S⁺ (mixed infection) (lane 3) and TMV-U1/S⁻ (lane 4). Lane 5 contains a mixture of dsRNAs extracted from plants infected with TNV, CMV and CTV. (b) Autoradiograph of blotted gel. Probe used was cDNA transcribed from denatured satellite dsRNA. ▶️, RF of TMV; ◀️, satellite.

Analysis of dsRNAs extracted from inoculated plants indicated that the satellite dsRNA and TMV-U5 dsRNAs were always present in plants infected with TMV-U5/S⁺ (systemic or local infection), and TMV-U5 dsRNA, but not satellite dsRNA, was detected in plants infected with TMV-U5/S⁻. Satellite dsRNA was always the most prominent dsRNA when it was detected. No virus-specific dsRNA or symptoms were detected in plants that were not hosts of TMV-U5/S⁻ that were inoculated with TMV-U5/S⁺.

Electrophoresis of ssRNA

A single band corresponding to an RNA with a mol. wt. of 2.1 × 10⁶ was the only nucleic acid detected in agarose gels containing denatured ssRNAs of TMV-U1, ToMV, TMV-U5/S⁺ and TMV-U5/S⁻ extracted from purified nucleoproteins, after staining with ethidium bromide or toluidine blue O. A satellite ssRNA with a mol. wt. of 0.3 × 10⁶, corresponding to this single-stranded form of the 0.6 × 10⁶ satellite dsRNA, was not detected by this method, despite the fact that the satellite dsRNA was readily detected in plants inoculated with the purified virions of TMV-U5/S⁺ used to prepare ssRNA.

Nucleic acid hybridization

Denatured satellite dsRNA hybridized with cDNA made from satellite dsRNA (Fig. 4). In this experiment the probe did not hybridize to any readily detectable extent with dsRNAs of TMV-U5/S⁻, TMV-U1, CTV, TNV or CMV + CARNA 5. The probe did hybridize with a low molecular weight (approx. 0.3 × 10⁶) nucleic acid fraction (presumed to be the ssRNA form of...
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Fig. 5. (a) Agarose gel electrophoresis of the 2 M-LiCl-precipitable ssRNAs from *N. tabacum* plants infected with TMV-U1/S− (lane 3), TMV-U1/S+ (lane 4), TMV-U5/S+ (lane 5), TMV-U5/S− (lane 6) and from a non-inoculated plant (lane 2). The RNA of CMV was electrophoresed for comparison (lane 1). (b) Autoradiograph of blotted gel. Probe used was cDNA transcribed from denatured satellite dsRNA. △, CMV RNAs 1, 2; △, CMV RNA 3; ○, CMV RNA 4.

the satellite) in an agarose gel loaded with the 2 M-LiCl-precipitable nucleic acids extracted from plants infected with TMV-U5/S+ or TMV-U1/S+ (Fig. 5). No hybridization was obtained with the 2 M-LiCl-precipitable nucleic acid extracted from healthy *N. tabacum* nor with that extracted from plants infected with TMV-U5/S− or TMV-U1. cDNA made from TMV-U5/S− ssRNA hybridized strongly with the RF and other dsRNAs of TMV-U5, but not with the satellite dsRNA, nor with the dsRNAs of TMV-U1, CTV, TNV or CMV + CARNA 5. It did hybridize strongly with TMV-U5/S+ and TMV-U5/S− genomic RNA, but only weakly with TMV-U1. No detectable signal could be obtained when satellite cDNA was used to probe a blotted gel that contained ssRNA from TMV-U1, TMV-U5/S− and TMV-U5/S+.

**Infectivity of the satellite RNA**

Purified preparations of satellite dsRNA were not infectious when inoculated alone or together with TMV-U5/S− ssRNA. Melted satellite dsRNA was infectious when inoculated together with a TMV-U5/S− ssRNA. Nevertheless, it had low levels of infectivity (infectious in only two out of eight trials). When satellite ssRNA (obtained from gels used to electrophorese 2 m-LiCl-precipitable ssRNAs from infected plants) was mechanically inoculated to *N. tabacum* cv. Turkish no symptoms or virus-specific dsRNAs were detected. When inoculated together with ssRNA of TMV-U5/S−, or TMV-U1, satellite dsRNA was recovered from infected plants. *Nicotiana silvestris* plants inoculated with crude sap from a mixed infection in *N. tabacum* (TMV-U5/S+ and TMV-U1/S−) yielded a TMV isolate with characteristics of TMV-U1 that had the satellite dsRNA associated with it (TMV-U1/S+). No satellite dsRNA was recovered from plants infected with PVX or CMV which were initially co-inoculated with satellite ssRNA and ssRNA of PVX or CMV.

**DISCUSSION**

From these results we conclude that our TMV-U5 field isolate is similar to that described by Bald & Goodchild (1960) and Siegel & Wildman (1954). This TMV-U5 isolate showed little nucleotide sequence homology with TMV-U1 and ToMV. This is not surprising since Randles *et al.* (1981) suggested that there are isolates of TMV from *N. glauca* with different degrees of relationship to TMV-U1.

The use of dsRNA analysis to detect infections of RNA viruses and satellites in plants has been well established (Morris & Dodds, 1979; Morris *et al.*, 1983; Rosner *et al.*, 1983; Dodds *et al.* 1983; Dodds *et al.* 1984).
After analysing the dsRNAs of plants infected with a field isolate of TMV-U5 we have detected a previously unreported dsRNA with mol. wt. 0.6 × 10^6 and we presumed it to be the RF of a satellite RNA. Plant virus satellites are low mol. wt. RNAs that depend completely on a specific helper virus (or related group of viruses) for their replication, share little nucleotide sequence homology with their helpers, may or may not code for their own capsid protein and are not host DNA-coded (Murant & Mayo, 1982; Francki, 1985). The dsRNAs of some satellite genomes accumulate in relatively large quantities in infected tissue (Schneider & Thompson, 1977; Habili & Kaper, 1981). Several properties of the dsRNA (mol. wt. 0.6 × 10^6) found in plants infected with some isolates of TMV-U5 support our conclusions that it is the RF of a satellite RNA. These include: the relative abundance and size of the dsRNA, ability to isolate the dsRNA from all hosts tested which were susceptible to the helper (same host range as the helper) and failure to isolate it from hosts that are not susceptible to the helper, ability to obtain subcultures of the helper that were unable to induce the satellite dsRNA in single infections, isolation of an RNA of approximately 0.3 × 10^6 which is presumably the single-stranded genomic form of the satellite dsRNA, inability of this ssRNA form to replicate by itself, and its ability to do so (by inducing the accumulation of satellite dsRNA) when associated with a specific helper (TMV-U5/S^- or TMV-U1), lack of appreciable nucleotide sequence homology with its helper or host nucleic acids, and inability of the satellite dsRNA to accumulate when attempts were made to associate it with two other unrelated viruses.

The fact that TMV-U5 and TMV-U1 but not two other viruses support satellite replication indicates that TMV-U5 and TMV-U1 have some common features which are involved in their role as helper, despite the apparent low sequence homology between these two viruses detected by Northern blot hybridization analysis in this study. It is possible that the satellite and both helpers compete for a similar replicase as suggested by Kaper (1982) for CMV and its satellite RNA. Satellite dsRNA accumulated in large amounts when associated with TMV-U5, and consistently in lesser amounts when associated with TMV-U1. This suggests that replication may be more efficient when the satellite is associated with its original helper as reported by Mossop & Francki (1979) with CMV, tomato aspermy virus and CMV satellite RNA.

The host range of the satellite was the same as that of the helper virus and one consequence of this was a change in the host range of the satellite when the helper was changed. TMV-U5/S^- was not able to infect N. silvestris systemically, but TMV-U1/S^- was. Satellite dsRNA (and ssRNA) was obtained from tissue of this host only when it was systemically infected with TMV-U1/S^- . This indicates that satellite replication is more helper-specific than host-dependent. We have been unable to identify a host which will support only the helper and not the satellite and vice versa. These results make it very unlikely that a second undetected virus and neither TMV-U5 nor TMV-U1 was the true helper for the satellite.

Satellites of several viruses can increase or decrease the severity of symptoms induced by the helper (Takanami, 1981; Altenbach & Howell, 1981; Gallitelli & Hull, 1985). There was no evidence of visible change in symptoms or host range of TMV-U5 associated with the presence of the satellite dsRNA, a result similar to that obtained with satellite RNA of tomato black ring virus (Murant & Mayo, 1982).

An interesting observation is the failure to detect a satellite ssRNA in RNA purified from virions of TMV-U5/S^- by gel electrophoresis and nucleic acid hybridization. Since the same preparations could induce satellite dsRNA accumulation, it is likely that satellite ssRNA was present in these nucleoprotein preparations but could not be detected.

Some properties of this satellite RNA are similar to the satellite virus of TNV (Kassanis, 1981), and include the high thermal inactivation point, high stability, RNA size and lack of additional dsRNAs other than the RF in extracts from infected plants. Work is underway which should elucidate how the RNA is encapsidated, its frequency among TMV-U5 field isolates, and whether it has any relationship with other satellites.

This is the first report to indicate that satellites may be associated with tobamoviruses. It will not be surprising if similar associations are found with other elongated viruses. Our results have shown that analysis of double-stranded RNA may well prove to be a useful technique initially to detect and analyse such satellites.
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REFERENCES


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