MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 1, No. 4, pp. 175-181, 1988

Pathogenicity Regulation by Satellite RNAs of Cucumber Mosaic Virus: Minor Nucleotide Sequence Changes Alter Host Responses

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Received 29 March 1988. Accepted 12 May 1988.

Six satellite RNAs of cucumber mosaic virus (CMV) could be differentiated on the basis of symptom expression they elicit in tomato and tobacco, and all but two could be differentiated by gel electrophoretic migration. Three of the satellite RNAs (B_2-sat, G-sat, and WL_1-sat RNA) ameliorated the symptoms induced by CMV on tomato, whereas three others (B_1-sat, B_3-sat, and WL_2-sat RNA) induced chlorosis on tomato, the extent and nature of which was CMV-strain dependent. By contrast, B_2-sat RNA induced chlorosis in tobacco, whereas WL_1-sat and G-sat RNAs did not. Thus, the symptoms observed were dependent on the host species, the particular satellite RNA, and also the strain of helper virus, suggesting that a complex association of at least three factors is involved in symptom elicitation. Comparisons of the nucleotide sequences of pairs of satellite RNAs inducing the various chlorotic responses suggest that only a few nucleotide changes in specific domains are required for the elicitation of different host responses.

Additional keywords: RNA replication, RNA fingerprinting.

Cucumber mosaic virus (CMV) is a tripartite RNA plant virus with a broad host range (Francki *et al.* 1979). CMV particles also contain a subgenomic RNA, designated RNA 4, which encodes the viral coat protein (Schwinghamer and Symons 1975). In addition, several isolates of CMV also contain a fifth RNA species that is a satellite RNA. This is an RNA species that contains sequences unrelated to the nucleic acid sequence of the CMV RNAs and is nonessential to the replication of CMV, but that is itself dependent on CMV for both replication and encapsidation (Francki 1985). The presence of a satellite RNA usually has an effect on the symptoms induced by the helper virus. In most cases the symptom response is attenuated, but in some cases the symptom response is either modified or exacerbated. Four satellite-mediated symptom responses have been reported: (1) the satellite RNA ameliorated the symptoms induced by CMV to varying extents on all host species tested (Mossop and Francki 1979); (2) as in (1), except on tomato in which the satellite RNA induced a systemic necrosis independent of the strain of helper virus (Waterworth *et al.* 1979); (3) as in (1), except on tomato in which the satellite RNA induced a white-leaf chlorosis (Gonsalves *et al.* 1982); and (4) as in (1), except on tobacco in which the satellite RNA induced a bright yellow chlorosis (Takanami 1981; Kaper *et al.* 1986).

We have isolated six satellite RNAs (from three strains of CMV), and their nucleotide sequences recently have been reported (Garcia-Arenal *et al.* 1987). This paper describes some of the pathological properties of these six satellite RNAs, which fit into the above four symptom-response groups. Whereas no correlation between specific nucleotide sequence changes and the various pathological responses described for 11 satellite RNAs could be established (Garcia-Arenal *et al.* 1987), comparisons between four satellite RNAs that differ by only 3–4% of their nucleotide sequence suggests that only a few specific nucleotide changes result in the elicitation of certain symptom responses.

MATERIALS AND METHODS

Propagation and purification of CMV strains and satellite RNAs. CMV strains B-, L_2-, and WL- were obtained from D. Gonsalves; strains Fny- and Pf- were obtained from T. Zitter; strains D- and S- were obtained from J. Kaper; and strain G- was obtained from R. Francki. All strains were propagated in either squash (*Cucurbita pepo* cv. Elite), tobacco (*Nicotiana tabacum* 'Xanthi nc'), tomato (*Lycopersicon esculentum* 'Rutgers'), or *Nicotiana clevelandii*. The virus was purified and RNA extracted as previously described (Palukaitis and Zaitlin 1984). RNA (100 μg/ml of CMV and 5 μg/ml of satellite) was inoculated in 0.1 M Na_2HPO_4, pH 9.2, and sap or virus (10–50 μg/ml) was inoculated in 0.05 M sodium phosphate, pH 7.0. Tomato and squash were inoculated at the cotyledon stage, tobacco was inoculated at the two–four leaf stage, and *N. clevelandii* was inoculated at various stages, preferably before flowering. Plants were maintained in either a greenhouse or in environmentally controlled chambers, with a 14-hr photoperiod and day and night temperatures of 20–26° C and 18–24° C, respectively.

Origin, isolation, and purification of specific satellite RNAs. All satellite RNAs were purified from stocks of viral RNA by electrophoresis on nondenaturing 5% polyacrylamide gels, electroelution from the gels, and concentration by ethanol precipitation (Palukaitis and Zaitlin 1984).

A preparation of B-sat RNA, purified from B-CMV RNA, was combined with a satellite-free strain of CMV (L_2-CMV) in two separate experiments (Fig. 1). In one experiment, tomatoes were inoculated with L_2-CMV and B-sat RNA. Sap from infected tomato was inoculated to tobacco, and the progeny virus contained a B-sat RNA variant later designated B_1-sat RNA, after repeated passages

through several hosts (tomato, tobacco, and squash) to detect additional variants. In a second experiment, the same inoculum was applied to tobacco; the progeny virus contained a B-sat RNA variant later designated B_2-sat RNA, after multiple passage through tomato, tobacco, and squash.

B-CMV was also propagated in tobacco and squash and purified from squash. The satellite RNA purified from this preparation was designated B_3-sat RNA (Fig. 1). B_3-sat RNA was combined with L_2-CMV RNA and inoculated to tobacco, in which they induced a mild mosaic. After multiple passage through various hosts, L_2-CMV containing B_3-sat RNA was purified from *N. clevelandii*.

WL-CMV RNA, originally propagated in squash, was passaged further through squash by sap inoculation. The virus was transferred to *N. clevelandii*, the progeny virus isolated, and the satellite RNA purified. This satellite RNA was designated WL_1-sat RNA (Fig. 1).

Sap from a tomato leaf expressing the "white-leaf" disease (Gonsalves *et al.* 1982) was inoculated to tomato, where it reproduced the white-leaf phenotype. Sap from such tomatoes was used to inoculate *N. clevelandii* plants. Virus and satellite RNA were purified from such plants (as described), and the satellite RNA was designated WL_2-sat RNA (Fig. 1).

Transfer of WL-CMV from tomato to squash and back to tomato by sap inoculation produced WL-CMV that was free of any detectable satellite RNA. G-sat RNA was obtained in an inoculum of a pseudorecombinant CMV between M-CMV and G-CMV, designated $M_1M_2G_3$-CMV, but referred to here as G-CMV. G-CMV was passaged through tobacco, squash, or *N. clevelandii*. The isolation and purification of G-sat RNA was previously reported (Palukaitis and Zaitlin 1984). D-sat and S-sat RNA were purified from preparations of D-CMV and S-CMV propagated in *N. clevelandii*.

RNA fingerprint analysis. Digestion of satellite RNAs with RNase T1, labeling of the RNA fragments with polynucleotide kinase and ^{32}P-γ-ATP, two-dimensional gel electrophoretic separation of the labeled fragments, and autoradiography were carried out as previously described (Palukaitis and Zaitlin 1984).

Semi-denaturing polyacrylamide gel electrophoresis. Fractionation of RNAs was done either on 6% polyacrylamide gels containing 7 M urea in a Tris-borate/EDTA buffer, prepared and subjected to electrophoresis as previously described (Palukaitis and Symons 1980), or on 9% polyacrylamide gels containing 8 M urea in a Tris-acetate/EDTA buffer, as described by Kaper *et al.* (1981). Gels were stained for 15 min in 1 μg/ml of ethidium bromide and rinsed twice with water before photography.

RESULTS AND DISCUSSION

Physical and pathological differentiation of six satellite RNAs. Kaper and his colleagues (Kaper *et al.* 1981; Garcia-Luque *et al.* 1984; Collmer and Kaper 1986) were able to differentiate three satellite RNAs of CMV by their relative electrophoretic mobilities under partially denaturing conditions. Although the six satellite RNAs described below could be differentiated on the basis of either their biological or their pathological properties, as well as by their nucleic acid sequence (Garcia-Arenal *et al.* 1987), all but two of the satellite RNAs could also be differentiated from each other and from two other satellite RNAs by electrophoresis on a semi-denaturing, polyacrylamide gel (Fig. 2). That is, only B_1-sat RNA and B_2-sat RNA showed identical electrophoretic mobilities on such gels containing either 9% (Fig. 2) or 8% polyacrylamide (results not shown). Although B_3-sat RNA showed a mobility very similar to the other B-sat RNAs, it could be differentiated more readily by increasing the time of the electrophoresis. For these eight satellite RNAs for which the nucleotide sequences are known, there

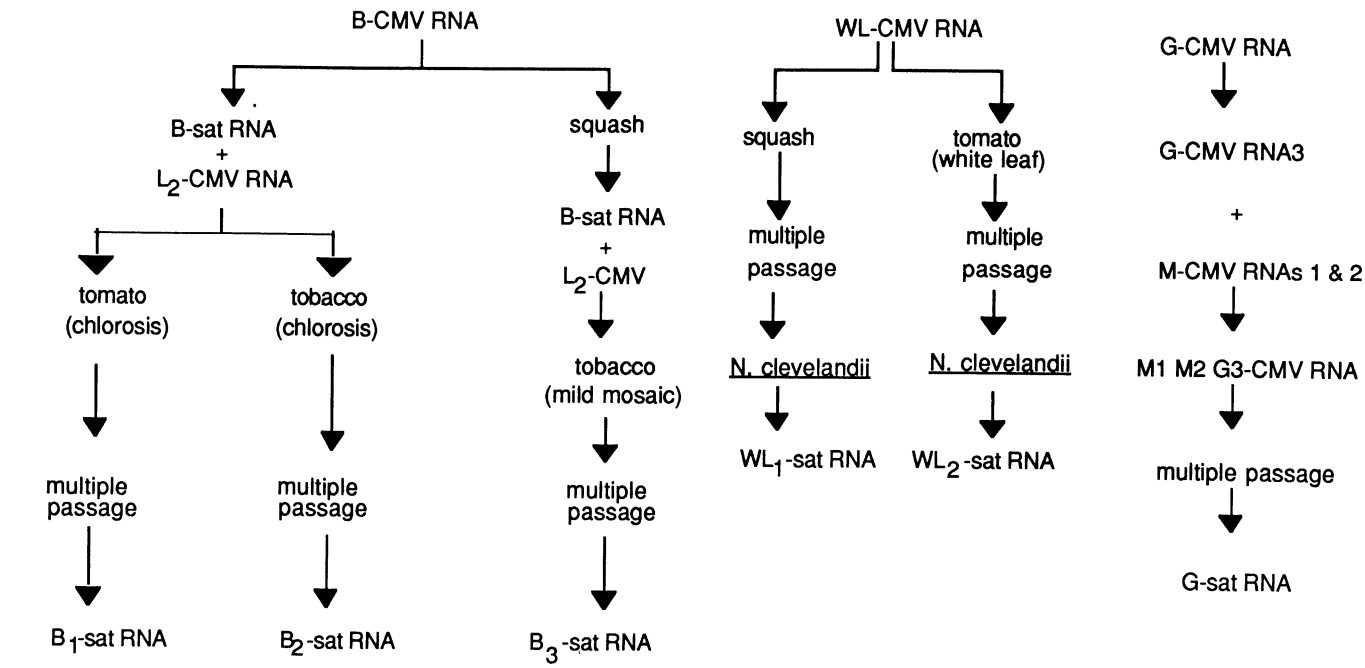

Fig. 1. Diagram showing the origins of the six satellite RNAs: B_1-sat, B_2-sat, and B_3-sat RNAs all originated from B-CMV; WL_1-sat and WL_2-sat RNAs originated from two separate samples of WL-CMV; G-sat RNA originated from G-CMV, but was maintained in the pseudorecombinant M1M2G3-CMV (probably originating as a contaminant of G-CMV RNA 3). "Multiple passage" indicates repeated propagation and transfer in a variety of host species (tomato, tobacco, and/or squash), and not necessarily the host species initially inoculated, using sap as an inoculum. Virus containing that satellite RNA indicated was purified from either tobacco, squash, tomato, or *Nicotiana clevelandii*.

was no correlation between their molecular weight and their electrophoretic mobility in this semi-denaturing gel.

The analysis of the range of pathological responses induced by the six satellite RNAs of CMV on tomato in the presence of one of four strains of CMV (Table 1) indicated that these satellite RNAs either ameliorated the virus-induced symptoms or exacerbated the symptom response by inducing chlorosis: B_2-sat RNA, G-sat RNA, and WL_1-sat RNA ameliorated the symptoms induced by L_2-CMV and WL-CMV; B_1-sat RNA induced leaf yellowing with both strains of helper virus; B_3-sat RNA induced leaf yellowing with L_2-CMV, but amelioration with WL-CMV; and WL_2-sat RNA induced leaf whitening with WL-CMV and leaf yellowing with L_2-CMV. B_1-sat RNA, B_2-sat RNA, and B_3-sat RNA induced similar symptoms with Q-CMV and L_2-CMV (Table 1). Most of the satellite RNAs, however, only slightly attenuated the symptoms induced by two other strains of CMV: Fny-CMV and Pf-CMV (Table 1). Thus, the nature of the symptoms induced by a particular satellite RNA of CMV is dependent on both the satellite RNA and the strain of helper virus.

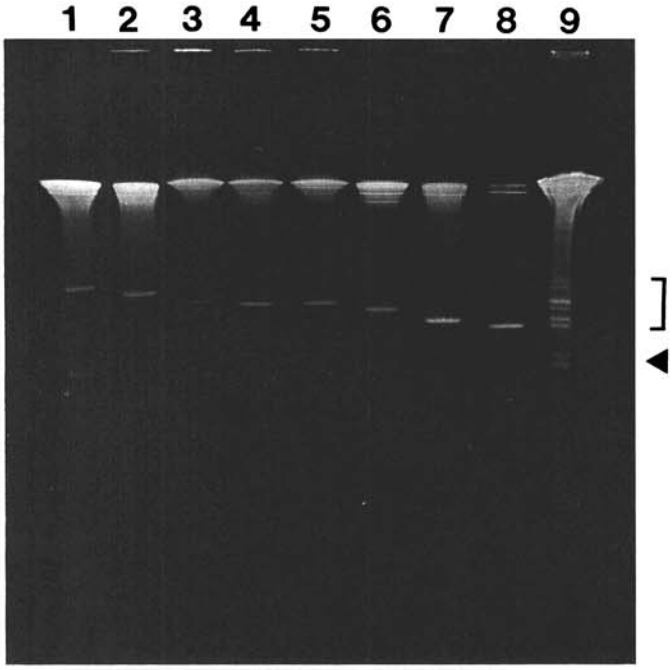

Fig. 2. Electrophoresis under semi-denaturing conditions of eight satellite RNAs on a 9% polyacrylamide gel containing 8 M urea. Viral RNAs samples were S-CMV containing the S-sat RNA (lane 1); G-CMV containing the G-sat RNA (lane 2); L_2-CMV containing either the B_3-sat RNA (lane 3), the B_1-sat RNA (lane 4), or the B_2-sat RNA (lane 5); WL-CMV containing the WL_2-sat RNA (lane 6); D-CMV containing D-sat (lane 7); WL-CMV containing WL_1-sat RNA (lane 8); and a mixture of each of the above (lane 9). The gel was stained with ethidium bromide before photography under UV transillumination. The arrowhead indicates the position of CMV RNA 5 (a subgenomic RNA present in some strains of CMV) and the bracket indicates the zone of migration of the eight satellite RNAs.

Most of the strains of CMV used in this study induced few or no symptoms on tobacco. In one case, however, the presence of a satellite RNA had a marked effect on the pathological response in tobacco (Fig. 3). Although neither L_2-CMV nor L_2-CMV containing B_1-sat RNA induced any systemic symptoms on tobacco, L_2-CMV containing B_3-sat RNA induced a mild mosaic, and L_2-CMV containing B_2-sat RNA induced a bright yellow chlorosis. Thus, there was a reversal of the pathological responses associated with B_1-sat, B_2-sat, and B_3-sat RNA in the presence of L_2-CMV on tobacco compared with tomato. Early preparations of B_2-sat RNA also contained some B_1-sat RNA (B_1'-sat); i.e., in some experiments there was segregation of the symptoms induced by two satellites in both tomato and tobacco. This enabled us to establish a culture of B_2-sat RNA that on repeated passage through several hosts remained stable. The B_1'-sat RNA was not characterized further.

After about 1 yr in passage, the symptom responses described were always observed, whether after serial passage of a particular satellite RNA:CMV strain combination, in either tobacco, squash, or tomato, or after altering the host species used for the propagation of the virus (i.e., squash versus *N. clevelandii*). The possibility could not be ruled out, however, that minor contamination plus host-specific amplification of a given satellite RNA was the explanation of the reversal of symptom response between tobacco and

Table 1. Symptoms induced on tomato by five strains of cucumber mosaic virus with or without various satellite RNAs

	Satellite RNAs						
CMV strains	**None**	**B_1-sat RNA**	**B_2-sat RNA**	**B_3-sat RNA**	**G-sat RNA**	**WL_1-sat RNA**	**WL_2-sat RNA**
L_2-CMV	Stunting; fern-leaf	Severe stunting; leaf-distortion; yellow leaves; white fruit	Mild fern-leaf	Stunting leaf distortion; yellow leaves;[a] white fruit	Mild fern-leaf	Mild fern-leaf to symptomless	Severe stunting; leaf distortion; yellow leaves; white fruit
WL-CMV	Stunting; severe fern-leaf	Severe stunting; leaf-distortion; yellow veins; white fruit	Mild fern-leaf	Stunting; fern-leaf	Fern-leaf	Symptomless	Stunting; leaf distortion; white leaves;[a] white fruit
Q-CMV	Symptomless	Yellow-green mosaic	Symptomless	Yellow-green mosaic	NT[b]	NT	NT
Fny-CMV	Severe stunting; severe fern-leaf; mosaic	Stunting; fern-leaf; scattered yellow patches	Stunting; mild fern-leaf; mild mosaic	NT	Stunting; mild fern-leaf; mosaic	Stunting; fern-leaf; mosaic	Stunting; fern-leaf; mosaic
Pf-CMV	Severe stunting; fern-leaf; severe leaf distortion	Stunting; mild fern-leaf	Stunting; mild fern-leaf;	NT	NT	Severe stunting; fern-leaf	Stunting; fern-leaf

[a] There was variation in the extent of chlorosis in different experiments, sometimes limited to veinal chlorosis.
[b] NT = Not tested.

tomato. Therefore, CMV was isolated from both tobacco and tomato plants infected with either L_2-CMV and B_1-sat RNA or L_2-CMV and B_2-sat RNA, and the satellite RNAs were purified and subjected to RNA fingerprint analysis (Fig. 4), which is capable of detecting less than 5% contamination.

The pattern of RNase T1-resistant oligonucleotides was distinct for each of the satellite RNAs (B_1-sat or B_2-sat). However, the pattern was the same for a given satellite RNA, regardless of whether that satellite RNA was propagated in tobacco or tomato (Fig. 4, A cf. B and C cf. D). The same results were observed with the B_3-sat RNA (not shown). Thus, at least in this case, there has not been a detectable host-specific selection and amplification of a contaminating satellite RNA, and the various pathological responses observed are due to interactions between a defined satellite RNA, a given helper virus, and a specific host.

Satellite sequence variations associated with differential replication of satellite RNAs in squash. For unknown reasons, most satellite RNAs of CMV are replicated to a much lower level in cucurbit than in solanaceous hosts (Kaper and Tousignant 1977; Jacquemond and Leroux 1982). However, the WL_1-sat RNA replicates efficiently in both kinds of hosts. In fact, WL_1-sat RNA was first amplified by WL-CMV serially passaged through squash by sap inoculation, a procedure that can cause the loss of the white-leaf phenotype associated with WL_2-sat RNA and WL-CMV (Gonsalves *et al.* 1982).

To examine the effect of serial passage in squash on satellite RNA levels, WL-CMV containing either WL_1-sat RNA or WL_2-sat RNA was propagated first in *N. clevelandii* in which both satellite RNAs were propagated efficiently (Fig. 5, lanes 2 and 6). WL-CMV RNA purified from *N. clevelandii* was inoculated to squash, and at 2 wk postinoculation, CMV was purified and the encapsidated RNAs analyzed by PAGE (Fig. 5, lanes 3 and 7). Similar samples were analyzed after two and three passages through squash. In Figure 5, it can be seen that the level of encapsidated WL_1-sat RNA remained high during serial passage in squash, but the level of encapsidated WL_2-sat dropped in the first passage and remained low during subsequent passages. The effect of other helper virus strains on the replication and/or encapsidation of WL_1-sat RNA in squash was not examined. By contrast with WL_1-sat RNA, G-sat RNA propagated in squash was also present at much lower levels than G-sat RNA propagated in tobacco (Fig. 5, lanes 10 and 11, respectively).

Northern blots of total RNA extracted from squash inoculated with CMV containing satellite RNAs other than WL_1-sat RNA always showed lower levels of both satellite RNAs and their various replicative forms than did RNA extracted from either infected tobacco or tomato plants (results not shown). Thus, a reduced efficiency of replication of the other satellite RNAs of CMV in squash is considered a more likely explanation of the this phenomenon, rather than some host specific effect on the encapsidation of satellite RNAs by CMV coat protein.

A comparison of the nucleotide sequence of WL_1-sat RNA with those of other satellite RNAs showed a limited number of sequence alterations specific to WL_1-sat RNA scattered through the molecule (Garcia-Arenal *et al.* 1987). A more restricted comparison between WL_1-sat RNA and WL_2-sat RNA showed no variation in sequence in the 3′ end 43 residues. However, there are seven substitutions in WL_1-sat RNA (cf. WL_2-sat RNA) at about 50 residues from the 3′ end (Garcia-Arenal *et al.* 1987). Furthermore, residues 11 and 13 are also different, although the 5′ end 23 residues of WL_1-sat are identical to the corresponding sequences of a number of other satellite RNAs, some of which have been shown to be propagated poorly in squash (Kaper and Tousignant 1977; Kaper *et al.* 1988). Thus, the sequence domain(s) controlling the level of sat RNA in squash appears to be located elsewhere than either the 5′ or 3′ terminal 50 residues.

Localization of nucleotide sequence domains eliciting chlorosis in tomato. A comparison of nucleotide sequences of the three B-sat RNAs and the WL_2-sat RNA in pairwise combinations (Table 2) reveals several interesting patterns to the limited (3–6%) variation observed between these satellite RNAs.

B_1-sat and B_2-sat RNAs differ in only 10 nucleotide positions, one of which is a duplication of an A residue (No. 230) at the boundary of a hypervariable region (residues

Fig. 3. Tobacco plants inoculated with L_2-CMV containing either the B_1-sat RNA (**A**), the B_2-sat RNA (**B**), or the B_3-sat RNA (**C**). Both L_2-CMV (not shown) and L_2-CMV containing the B_1-sat RNA showed no systemic symptoms in greenhouse-grown plants.

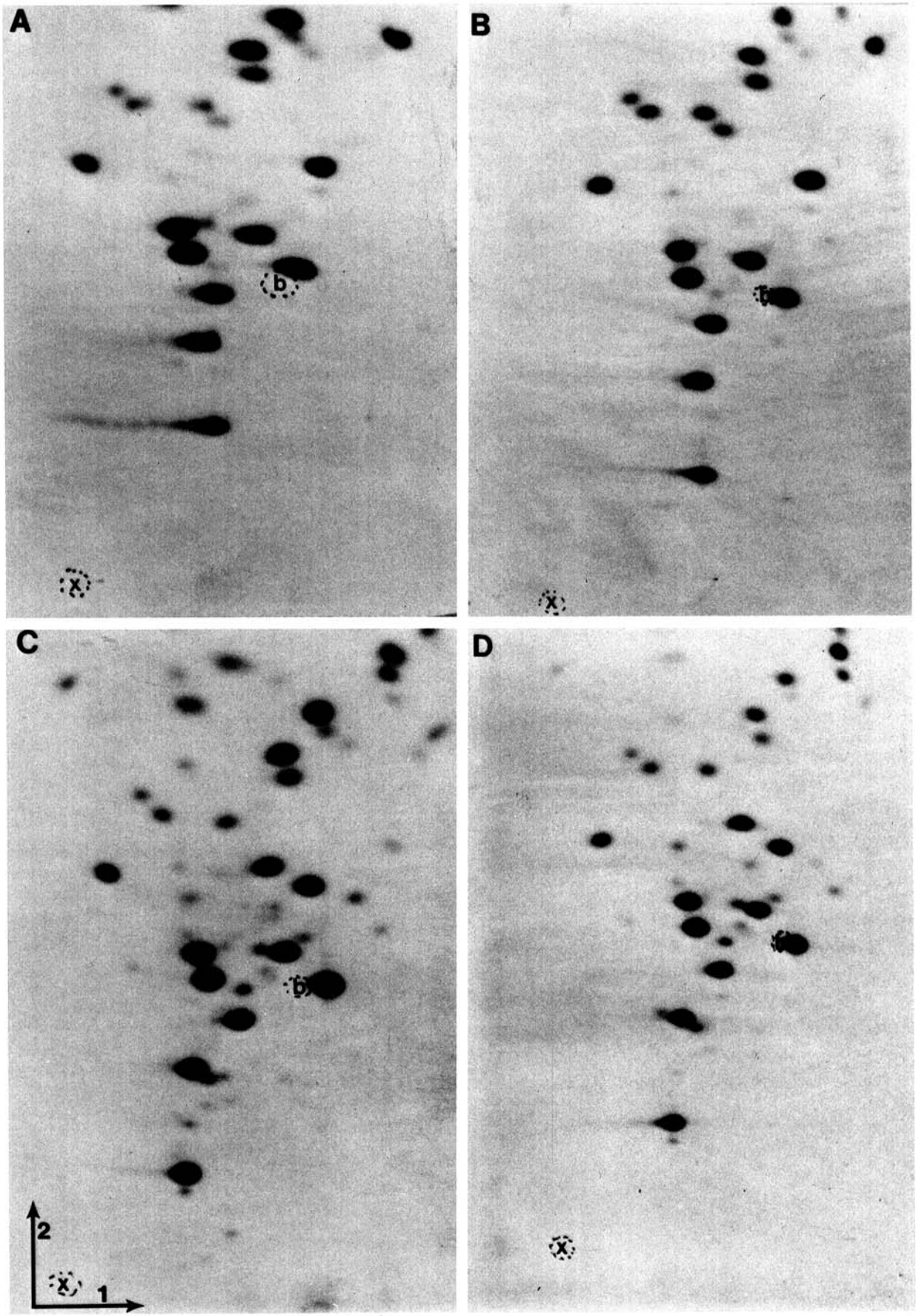

Fig. 4. Autoradiograms of 5′-^{32}P-labeled oligonucleotides separated by two-dimensional gel electrophoresis. RNA samples were digested with RNase T_1 before 5′-end labeling. The samples were B_1-sat RNA propagated in either tobacco (**A**) or tomato (**B**) and B_2-sat RNA propagated in either tobacco (**C**) or tomato (**D**). The positions of the marker dyes bromophenol blue and xylene cyanol blue are marked by b and x, respectively. The horizontal and vertical arrows (**C**) indicates the directions of electrophoresis of the first dimension gel and the second dimension gel, respectively.

231–236 by our numbering, but residues 224–229 of Richards *et al.* 1978) in B_2-sat RNA. However, nucleotide sequence alterations in this region do not appear to influence the pathogenicity of satellite RNAs (Richards *et al.* 1978; Kurath and Palukaitis 1987; Kaper *et al.* 1988).

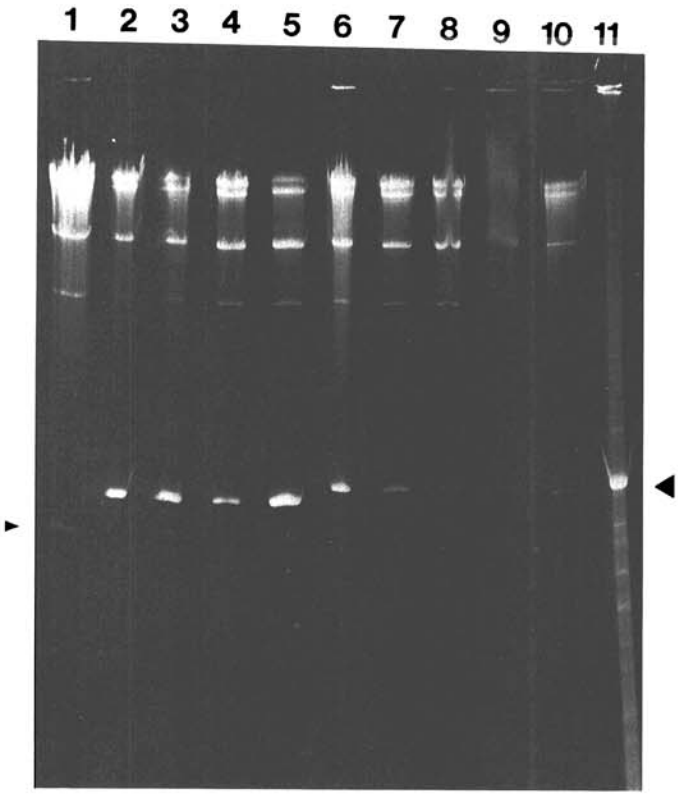

Fig. 5. PAGE of CMV and satellite RNAs. WL-CMV RNA (lane 1) containing either WL_1-sat RNA (lane 2) or WL_2-sat RNA (lane 6) was propagated in and extracted from *N. clevelandii*. These viral RNAs were inoculated onto and passaged through squash once (lanes 3 and 7), twice (lanes 4 and 8), and three times (lanes 5 and 9). Lanes 2–5 contain WL/WL_1-CMV RNA and lanes 6–9 contain WL/WL_2-CMV RNA. Lanes 10 and 11 contain G-CMV (with G-sat RNA), propagated in either squash or tobacco, respectively. Lanes 2–10 contain 2.5 g of viral RNA and lanes 1 and 11 contain 8 g of RNA. The genomic G-CMV RNAs extracted from tobacco (lane 11) had degraded partially. The position of satellite RNA is indicated by the large arrowhead (right) and the CMV RNA 5 is indicated by the small arrowhead (left).

Thus, all remaining nine sequence differences between B_1-sat and B_2-sat RNAs occur in the 5′ half of the sequence, between residues 41 and 171.

Although B_1-sat and B_3-sat RNAs both can induce chlorosis in tomato, they differ in nucleotide sequence by 18 residues, nine of which occur in the 3′ half of the satellite RNA. Seven of the remaining nine nucleotide differences between B_1-sat and B_3-sat RNA also occur in the ameliorative B_2-sat RNA.

B_2-sat and B_3-sat RNAs differ by 14 nucleotides. However, nine of these changes are in the 3′ half of the sat RNA molecule at positions that differentiate B_1-sat from B_3-sat RNA, one insertion is at the hypervariable region, and two of the four remaining substitutions also occur between B_1-sat and B_3-sat RNA. Thus, sequence alterations at residues 91 and 149 are the only remaining substitutions that correlate with a change in pathogenicity. For the following reasons, however, these sequence changes in themselves are unlikely to be solely responsible for the induction of chlorosis in tomato: (1) Although the chlorosis-inducing B_1-sat and B_3-sat RNAs both contain U residues at positions 91 and 149, and B_2-sat RNA contains an A (91) and a C (149) residue, the ameliorative WL_1-sat RNA also contains U residues at both of these positions. (2) The chlorosis-inducing WL_2-sat RNA contains U and C residues at positions 91 and 149, respectively, as do the ameliorative satellite RNAs, G-sat RNA (Garcia-Arenal *et al.* 1987), and (1)CARNA 5 (Kaper *et al.* 1988). (3) Neither alterations in the secondary structure nor the formation of new sequence elements (Garcia-Arenal *et al.* 1987) resulting from the above specific sequence substitutions could be correlated with the changes in pathogenicity. (4) Alterations in the amino acid sequence of the open reading frame (ORF) beginning at residue 138 also could not be correlated with the changes in pathogenicity, because the C → U substitution at residue 149 of B_2-sat RNA cf. B_3-sat RNA resulted in a conservative replacement of alanine for valine in an otherwise identical polypeptide. Furthermore, the corresponding ORFs for B_1-sat and WL_2-sat RNA contain entirely different amino acid sequences.

The localization of the chlorosis-inducing domain to the 5′ half of the molecule also extends to a comparison of the WL_2-sat RNA with the B_2-sat RNA (Table 2), which differ by only 10 sequence substitutions located between residues

Table 2. Nucleotide sequence variation betweeen pairs of satellite RNAs

Paired satellite RNAs	Nucleotide positions showing variation[a]																									Number of changes
	41	84	91[b]	97	102	144	149[b]	153	160	170	171	185	190	193	214	230	270	301	321	326	329	330	337	342	343	
B_1/B_2	C	A	U			∇[c]	U	∇	U	A	∇					∇										10
	U	C	A			C	C	U	C	∇	U					A										
B_1/B_3	C	A				∇		∇	U	A	∇	C		U			C	A	A	C	C	A	U	∇	A	18
	U	C				C		U	C	∇	U	U		C			U	U	G	U	U	∇	∇	C	∇	
B_2/B_3			A				C					C		U		A	C	A	A	C	C	A	U	∇	A	14
			U				U					U		C		∇	U	U	G	U	U	∇	∇	C	∇	
B_1/WL_2				∇	∇	∇	U	∇			∇		C		U											8
				U	G	C	C	U			U		U		A											
B_2/WL_2	U	C	A	∇	∇				C	∇			C		U	A										10
	C	A	U	U	G				U	A			U		A	∇										
B_3/WL_2	U	C		∇	∇		U		C	∇		U	C	C	U		U	U	G	A	U	∇	∇	C	∇	20
	C	A		U	G		C		U	A		C	U	U	A		C	A	A	C	C	A	U	∇	A	

[a] The nucleotide positions are numbered according to Garcia-Arenal *et al.* (1987).
[b] Nucleotide variation between B_1-sat RNA and B_2-sat RNA also observed between B_2-sat RNA and B_3-sat RNA, but not between B_1-sat RNA and B_3-sat RNA.
[c] ∇ indicates a deletion at that position with respect to the satellite to which it is compared.

41 and 230. Eight of these substitutions also occur in comparisons of WL_2-sat RNA and either B_1-sat RNA and/or B_3-sat RNA. Thus, once again a few sequence alterations correlate with major changes in pathogenicity. The additional sequence differences between the three chlorosis-inducing satellite RNAs (B_1-sat, B_3-sat, and WL_2-sat RNA) presumably relate to the variation in the extent of chlorosis observed with these three satellite RNAs (Table 1).

The results presented here concerning the localization of a pathogenesis domain, specific to chlorosis in tomato, between residues 85 and 195 (Table 2), are in contrast to our previous results on the localization of the sequence domain specifying necrosis in tomato, which was exclusive of the sequences between residues 70 and 160 (Kurath and Palukaitis 1987). In that report we had cloned and characterized a variant (pDsat1) of a necrosis-inducing satellite that contained 10 substitutions between residues 70 and 170 (using the numbering of Garcia-Arenal *et al.* 1987), such that this domain was only one residue different from the ameliorative satellite RNA, (1)CARNA 5 (Kaper *et al.* 1988). The single alteration was an insertion of a U after residue 96. However, this insertion is also seen in the ameliorative WL_1-sat (Garcia-Arenal *et al.* 1987). Although each of the 10 sequence substitutions also was observed in WL_1-sat RNA, the latter satellite RNA was not identical to pDsat1 throughout this domain, contrary to our previous assertion (Kurath and Palukaitis 1987). WL_1-sat RNA contained 10 additional substitutions between residues 114 and 154 with respect to pDsat1. At each of the latter 10 positions, however, the nucleotide sequence of pDsat1 was identical to the corresponding sequence of the ameliorative G-sat RNA. Thus, the nucleotide sequence domain specifying necrosis on tomato appears to be located outside a sequence domain that specifies the induction of chlorosis in tomato. This would imply that satellite RNAs of CMV may not have a single pathogenicity domain as has been observed for viroids (Schnölzer *et al.* 1985; Visvader and Symons 1986), but rather suggests that certain sequence substitutions within a particular sequence background in a given domain will result in a change in the interactions between that satellite RNA, the host, and the helper virus, leading to an altered pathogenic response. Furthermore, these pathogenicity domains are specific to tomato, and presumably other sequence domains specify the interactions leading to induction of chlorosis in tobacco by the B_2-sat RNA (Fig. 3). The synthesis of full-length clones and infectious transcripts of the B_1-sat RNA and the WL_1-sat RNA and recombination between the cloned DNAs of different pathogenic satellite RNAs opens the possibility of further delimiting either the specific nucleotide sequences or the sequence domains controlling the various pathogenic responses.

ACKNOWLEDGMENTS

This work was supported in part by Grant 84-09851 from the National Science Foundation and Grant DE-FG02-86BR13505 from the Department of Energy.

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