VIROLOGY 238, 478–485 (1997)
ARTICLE NO. VY978853

The Coat Protein of Turnip Crinkle Virus Is Involved in Subviral RNA-Mediated Symptom Modulation and Accumulation

Qingzhong Kong,* Jong-Won Oh,† Clifford D. Carpenter,* and Anne E. Simon*,[1]

*Department of Biochemistry and Molecular Biology and Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, Massachusetts 01003; and †Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003

Received August 7, 1997; returned to author for revision September 4, 1997; accepted September 17, 1997

Some satellite (sat-) and defective interfering (DI) RNAs associated with plant viruses intensify or ameliorate the symptoms of the virus. We recently demonstrated that the TCV coat protein (CP) is involved in symptom modulation by sat-RNA C. Two additional subviral RNAs have now been tested for effect of the CP on symptom modulation. DI RNA G, which normally intensifies the symptoms of TCV, is able to attenuate symptoms if the TCV CP is replaced with the CP of cardamine chlorotic fleck virus. DI RNA G had no effect on the symptoms of TCV with a single base alteration in the CP open reading frame, unlike sat-RNA C, which was able to ameliorate the symptoms of the mutant TCV. Using a hybrid sat-RNA constructed from sat-RNA C and TCV (which shares a similar 3′-end region with DI RNA G), the 3′-terminal 53 bases of sat-RNA C were found to be involved in symptom attenuation, which was directly correlated with the lack of detectable viral genomic RNA in whole plants. Sat-RNA D had no effect on the symptoms of mutant or wild-type TCV. The accumulation of TCV subviral RNAs in plants and protoplasts was also found to be strongly influenced by the presence or absence of the wild-type TCV CP.

INTRODUCTION

Most plants are naturally resistant to most viruses. In many instances, resistance involves an interaction between a product encoded by a resistance gene of the host and an elicitor specified by the virus. This recognition event leads to induction of the host defense response, resulting in the limitation of virus replication and/or spread (White and Antoniw, 1990; Dawson and Hilf, 1992; Hammond-Kosack and Jones, 1996). Resistance to viruses can also be mediated by small virus-associated satellite (sat-) RNAs, which share partial or limited sequence similarity with their helper virus (Roossinck *et al.,* 1992), or defective interfering (DI) RNAs, which are completely or nearly completely derived from the helper virus (Roux *et al.,* 1991). There are also sat- and DI RNAs that intensify the symptoms of their helper viruses (Li *et al.,* 1989; Collmer and Howell, 1992; Romero *et al.,* 1993).

Specific nucleotide residues of sat-RNAs associated with cucumber mosaic virus (CMV; Masuta *et al.,* 1989; Devic *et al.,* 1990; Jaegle *et al.,* 1990; Sleat and Palukaitis, 1990) and peanut stunt virus (Naidu *et al.,* 1991a,b) have been implicated in sat-RNA-mediated resistance or symptom intensification. Resistance mediated by sat-RNAs is generally (Kaper and Collmer, 1988; Naidu *et al.,* 1991a,b; Roossinck *et al.,* 1992), but not always (Harrison *et al.,* 1987), accompanied by a substantial decrease in virus titer, leading to the suggestion that more than one mechanism may be responsible for sat-RNA-mediated symptom attenuation (Roossinck *et al.,* 1992). One suggested mechanism is a competition between the sat-RNA and helper virus for limited factors required for replication (Wu and Kaper, 1995), which leads to a reduction in the level of virus with a concomitant decrease in virus symptoms. Alternatively, a sat-RNA may induce a host factor(s) that leads to plant resistance or tolerance, although no experimental evidence is available for this possibility (Roossinck *et al.,* 1992). The finding that symptom modulation by a CMV sat-RNA is dependent on specific strains of CMV (Sleat and Palukaitis, 1990; Sleat *et al.,* 1994) and that in *Nicotiana* spp. sat-RNA symptom modulation is regulated by a single, partially dominant host gene (Masuta *et al.,* 1993) suggests that determinants from the host, helper virus, and sat-RNA interact to produce resistance plants.

Turnip crinkle carmovirus (TCV) has a broad host range that includes *Arabidopsis thaliana.* As shown in Fig. 1A, TCV has a single-stranded RNA genome of 4054 bases encoding five proteins that are required for virus replication (p28 and p88), movement (p8, p9), and encapsidation and movement [coat protein (CP)] (Hacker *et al.,* 1992; White *et al.,* 1995). TCV systemically infected all tested ecotypes of Arabidopsis except ecotype Dijon (Di-0; Li and Simon, 1990; Simon *et al.,* 1992). Resistance of

[1] To whom correspondence should be addressed at Department of Biochemistry and Molecular Biology, Lederle Graduate Research Center, University of Massachusetts, Amherst, MA 01003-4505. Fax: 413-545-4529. E-mail: simon@biochem.umass.edu.

0042-6822/97 $25.00

Di-0 to TCV involves inhibition of virus cell-to-cell movement and is associated with induction of a host defense response in inoculated and uninoculated leaves (Simon *et al.,* 1992; Uknes *et al.,* 1993; Kong *et al.,* in press).

The related carmovirus, cardamine chlorotic fleck (CCFV), is able to systemically infect Di-0 and other ecotypes of Arabidopsis (Oh *et al.,* 1995). TCV containing the CP ORF of CCFV in place of its own CP ORF (TCV-CP_{CCFV}) and TCV-CPm, a TCV derivative in which the initiating AUG of the CP ORF was altered to ACG (Fig. 1A), were able to overcome the resistance of Di-0 to TCV, indicating that the CP is the viral determinant involved in the elicitation of resistance (Kong *et al.,* 1995, in press). Plants and protoplasts infected with TCV-CPm accumulated about 20% of the wild-type level of a protein that migrated to a similar position as wild-type CP in polyacrylamide gels and cross-reacted with CP-specific antibodies. No virions could be isolated in TCV-CPm-inoculated protoplasts, suggesting that the CP produced by TCV-CPm was not wild-type and was probably synthesized from an undetermined, in-frame, non-AUG initiation codon (Kong *et al.,* in press). TCV-CP_{CCFV} induced moderate stunting symptoms and TCV-CPm produced more severely stunted plants that commonly had bushy stems and curled leaves (Kong *et al.,* in press).

TCV is associated with several satellite (sat-), defective interfering (DI), and chimeric sat/DI RNAs (Simon and Howell, 1986; Li *et al.,* 1989), some of which can modify the symptoms of TCV. Sat-RNA C (356 bases) is a chimeric RNA composed of a nearly full-length second sat-RNA (sat-RNA D; 194 bases) at the 5′ end and two regions of TCV at the 3′ end (Fig. 1B). Sat-RNA C intensifies the symptoms of wild-type TCV on all hosts where the virus produces visible symptoms (Li *et al.,* 1989; Li and Simon, 1990). Using susceptible Arabidopsis ecotypes, inclusion of sat-RNA C in the inoculum resulted in the death of the plant by 3 weeks postinoculation (Simon *et al.,* 1992). Sat-RNA D, which shares little consecutive sequence similarity with TCV beyond the seven 3′-terminal nucleotides, had no effect on TCV symptoms (Li and Simon, 1990). Unexpectedly, sat-RNA C eliminated the moderate symptoms produced by TCV-CP_{CCFV} on Di-0 and the susceptible ecotype Col-0 and inhibited the accumulation of TCV-CP_{CCFV} genomic RNA in plants and protoplasts (Kong *et al.,* 1995). Sat-RNA C also attenuated symptoms of about 70% of plants inoculated with TCV-CPm (Kong *et al.,* in press). Attenuation of symptoms by sat-RNA C was directly correlated with undetectable levels of viral genomic RNA in whole plant extracts (Kong and Simon, in press). However, the presence of sat-RNA C in TCV-CPm-inoculated protoplasts did not substantially affect the level of viral genomic RNA, suggesting that sat-RNA C may not be greatly influencing TCV-CPm replication or stability, but rather may be involved in inhibiting virus movement (Kong *et al.,* in press).

DI RNA G (346 bases) is a DI RNA associated with TCV isolate TCV-B (Li *et al.,* 1989). DI RNA G is composed (from 5′ to 3′) of 10 bases from the 5′ end of sat-RNA D, 12 bases of unknown origin, 99 bases from near the 5′ end of TCV, and then 94% sequence similarity with 225 bases of the TCV untranslated 3′-terminal sequence including an imperfect repeat of 36 bases (Fig. 1B). DI RNA G intensified the symptoms of TCV on all hosts tested (Li *et al.,* 1989). In this paper, we report the testing of DI RNA G, sat-RNA D, and a sat-RNA C/TCV hybrid RNA (sat-RNA C*) for effects on the symptoms of TCV-CP_{CCFV} and TCV-CPm.

MATERIALS AND METHODS

Origin of viruses, subviral RNAs, and plant inoculations

Constructs containing full-length cDNAs for TCV, CCFV, TCV-CP_{CCFV}, TCV-CPm, sat-RNA C, DI RNA G, and sat-RNA D, downstream from T7 RNA polymerase promoters, have been described (Li and Simon, 1991; Song and Simon, 1994; Carpenter *et al.,* 1995; Oh *et al.,* 1995; Kong *et al.,* in press). For the construction of sat-RNA C*, in which the 3′-terminal 100 bases of sat-RNA C are replaced with the corresponding sequence from TCV, plasmid pT7C+, containing full-length sat-RNA C cDNA downstream of a T7 RNA polymerase promoter (Song and Simon, 1994), was digested with *Spe*I (position 257 in sat-RNA C) and *Sma*I (located just downstream from the cDNA). The larger fragment was ligated to the small *Spe*I–*Sma*I fragment of pT7TCVms, which contains a full-length cDNA of TCV isolate TCV-M (Oh *et al.,* 1995). For each virus/subviral RNA combination, at least 10 Arabidopsis seedlings at the six- to eight-leaf stage were mechanically inoculated with full-length transcripts synthesized *in vitro* using T7 RNA polymerase as previously described (Kong *et al.,* 1995).

RNA gel blot analysis

RNA samples were subjected to electrophoresis on agarose gels, transferred to nitrocellulose (Nitoplus 2000, MSI), and probed with ^{32}P-labeled oligonucleotides to detect viral and subviral RNAs. After autoradiography, the blots were briefly submersed in water at 100° to remove the probe before the next hybridization. The probes for genomic RNA and sat-RNA C were complementary to positions 3892–3912 and 175–199, respectively. The sat-RNA C probe also weakly hybridized to DI RNA G. The ribosomal RNA probe was described elsewhere (Kong *et al.,* 1995). The probes for DI RNA G and sat-RNA D were complementary to positions 140–159 and 44–59, respectively. Autoradiograms were scanned with a laser densitometer and values normalized to the levels of ribosomal RNAs.

Protoplast inoculations and RNA extraction

Protoplasts were prepared from cultured callus made from seedlings of Arabidopsis ecotype Col-0 as previously described (Kong *et al.,* in press). Protoplasts (2×10^7) were inoculated with 80 μg of genomic RNA and 8 μg of subviral RNA transcripts synthesized *in vitro* as previously described (Kong *et al.,* 1995). Total RNA was extracted from protoplasts collected at various times postinoculation as has been described (Kong *et al.,* 1995).

RESULTS AND DISCUSSION

Effect of sat-RNA D and DI RNA G on virus-induced symptoms in Col-0 and Di-0 plants

Sat-RNA C intensified the symptoms of TCV and attenuated the symptoms of TCV-CP_{CCFV} and TCV-CPm (Kong *et al.,* 1995, in press). Since sat-RNA C shares 90% sequence similarity with sat-RNA D in its 5′ region and 90% sequence similarity with DI RNA G in its 3′ region (Fig. 1B), examining the effect of the latter two subviral RNAs on the symptoms of TCV-CP_{CCFV} and TCV-CPm should provide clues concerning the region(s) of sat-RNA C that are involved in symptom attenuation.

Col-0 and Di-0 seedlings were inoculated with transcripts of TCV, TCV-CP_{CCFV}, or TCV-CPm genomic RNAs, with and without transcripts of the subviral RNAs, and plants photographed at 16 days postinoculation (DPI; Fig. 2). TCV-inoculated Di-0 plants were nearly or completely symptomless, regardless of the presence of any subviral RNA (Fig. 2D). In TCV-inoculated Col-0 plants, DI RNA G intensified symptoms of all plants (i.e., increased stunting), although usually to a lesser extent than sat-RNA C (Fig. 2A). In TCV-CP_{CCFV}-inoculated Col-0 and Di-0 plants, DI RNA G eliminated or almost eliminated symptoms on all plants (Figs. 2B and 2E). DI RNA G had little or no effect on the symptoms of TCV-CPm, unlike sat-RNA C, which attenuated symptoms on about 70% of inoculated plants (Figs. 2C and 2F). Sat-RNA D had no reproducible effect on the symptoms of TCV, TCV-CP_{CCFV}, or TCV-CPm, in Di-0 or Col-0.

In addition to sat-RNA D, sat-RNA C, and DI RNA G, we tested a chimeric sat-RNA composed of the 5′ 256 bases of sat-RNA C and the 3′ 103 bases of TCV (sat-RNA C*). While the 3′-end regions of TCV, sat-RNA C, and DI RNA G are similar, the 3′-end sequences of TCV and DI RNA G exhibit greater similarity (Fig. 1C). As shown in Fig. 2, sat-RNA C*, like DI RNA G, had little effect on symptoms produced by TCV-CPm and attenuated the symptoms of TCV-CP_{CCFV} on Col-0 or Di-0 plants, while intensifying the symptoms of TCV on Col-0. These results indicate that the 3′ 53 nucleotides of sat-RNA C*, which contains eight positional differences with sat-RNA C (adjacent alterations are counted as a single positional difference), is the region responsible for attenuation of TCV-CPm symptoms.

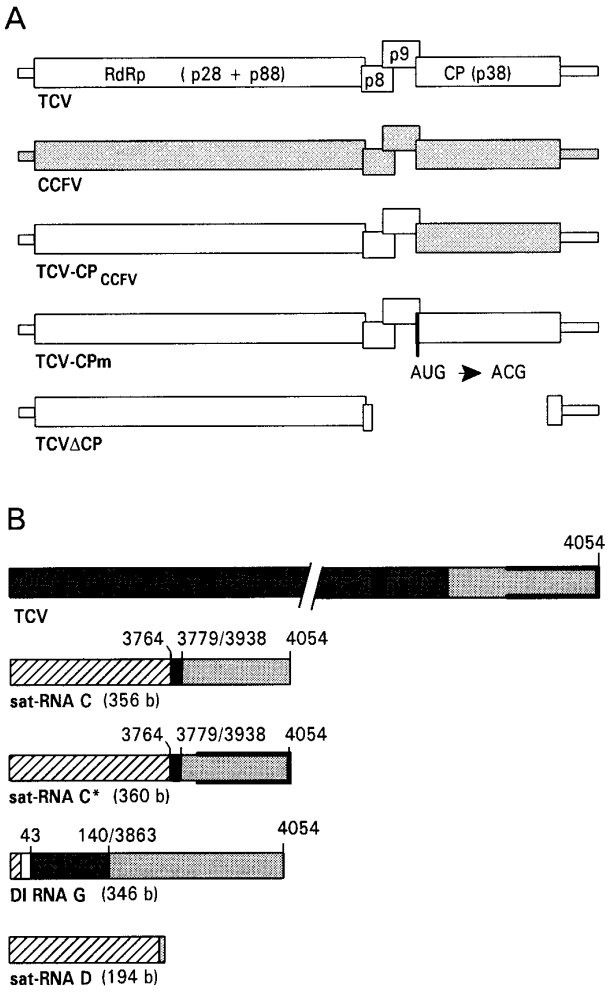

FIG. 1. Genomic and subviral RNAs used in this study. Similar sequences are shaded alike. (A) Genomic RNAs. ORFs and untranslated regions are represented by thick and thin boxes, respectively. TCV-CPm has a point mutation in the CP initiation codon. (B) Subviral RNAs. Numbers above the bars indicate the positions of the TCV-related sequence. Sat-RNA C* has the 3′-terminal 100 bases (thick line) replaced with the corresponding sequence of TCV genomic RNA. (C) Alignment of the 3′-end sequences of sat-RNA C (satC), DI RNA G (DI-G), and the TCV genomic RNA, which is the same sequence as sat-RNA C* (C*). Only differences among the RNAs are indicated. "." indicates absence of the base in the sat-RNA C sequence.

Effect of TCV-associated subviral RNAs on virus accumulation in Col-0 and Di-0 plants

To study the mechanism of symptom modulation, the effect of sat-RNA D, sat-RNA C, DI RNA G, and sat-RNA

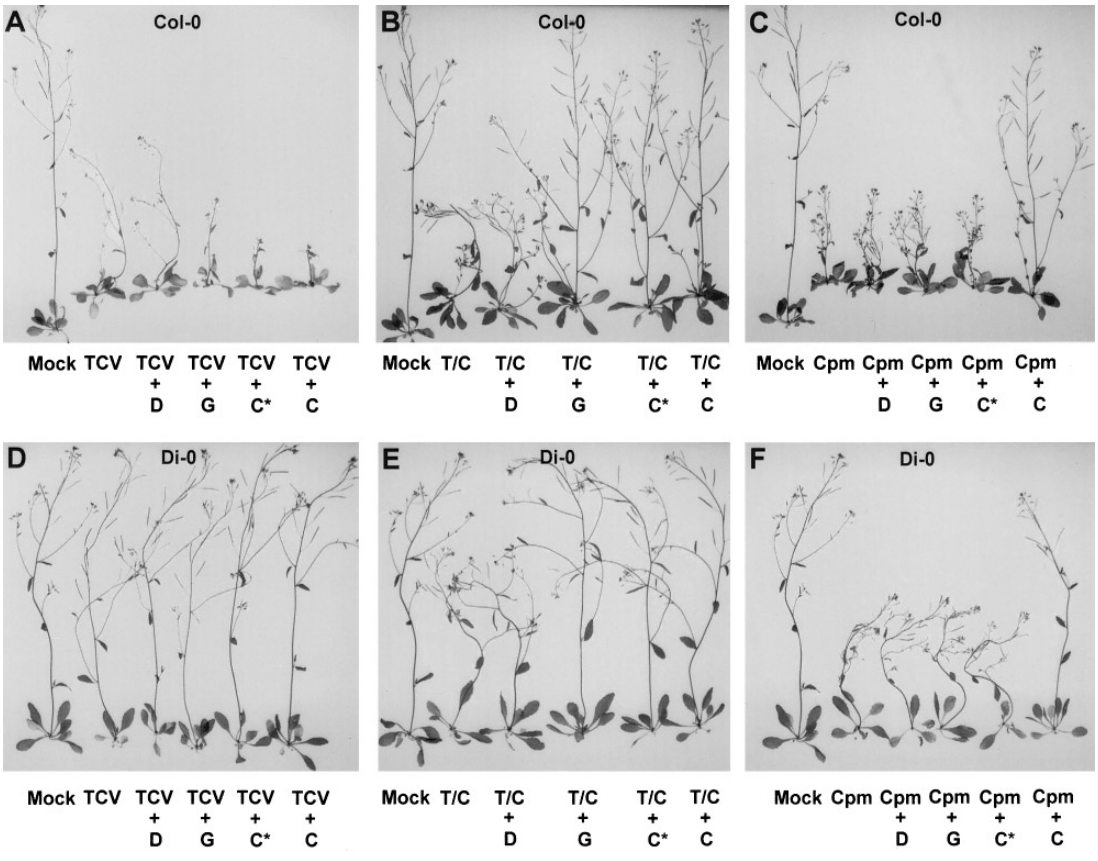

FIG. 2. Comparison of the effects of subviral RNAs on symptom expression. Col-0 (A, B, C) and Di-0 (D, E, F) seedlings at the six- to eight-leaf stage were inoculated on the oldest leaf pair with transcripts of helper virus in the presence or absence of subviral RNAs. Plants were treated with buffer only (mock), helper virus alone; helper virus plus sat-RNA D (+D), helper virus plus DI RNA G (+G), helper virus plus sat-RNA C* (+C*), and helper virus plus sat-RNA C (+C). Representative plants were photographed at 16 DPI. (A and D) TCV helper virus. (B and E) TCV-CP_{CCFV} helper virus (T/C). (C and F) TCV-CPm helper virus (Cpm).

C* on viral genomic RNA accumulation was examined in Col-0 and Di-0 plants. Seedlings were coinoculated on the oldest leaf pair with transcripts of TCV, TCV-CP_{CCFV}, or TCV-CPm genomic RNAs, with and without a subviral RNA, and leaves from three typical plants for each infection were collected and pooled at 16 DPI. Total RNA isolated from these plants was subjected to RNA gel blot analysis (Fig. 3). In TCV-inoculated Di-0 plants, there was no detectable virus accumulation, regardless of the presence of subviral RNAs. In TCV-inoculated Col-0 plants, sat-RNA D and sat-RNA C were associated with reductions in TCV genomic RNA levels of 28 and 42%, respectively, while DI RNA G and sat-RNA C* were associated with increases in TCV genomic RNA levels of 69 and 25%, respectively. This result shows that sat-RNA C, sat-RNA C*, or DI RNA G, all of which intensify the symptoms of TCV in Col-0, are not necessarily associated with a reduction or elevation in levels of TCV genomic RNA and that a particular amount of the genomic RNA does not appear to correlate with symptom intensification.

In Col-0 plants inoculated with TCV-CP_{CCFV}, sat-RNA D had little effect on TCV-CP_{CCFV} genomic RNA levels, while DI RNA G, sat-RNA C*, or sat-RNA C, all of which attenuated symptoms, were associated with a reduction in TCV-CP_{CCFV} genomic RNA to undetectable levels. In Di-0 plants inoculated with TCV-CP_{CCFV}, the presence of sat-RNA D led to a 67% decrease in the level of TCV-CP_{CCFV} genomic RNA with no discernible effect on symptoms. Sat-RNA C, sat-RNA C*, and DI RNA G, all of which attenuated symptoms, were associated with undetectable levels of TCV-CP_{CCFV} genomic RNA in Di-0. In Col-0 or Di-0 plants inoculated with TCV-CPm and sat-RNA C, symptoms were attenuated and TCV-CPm genomic RNA levels were reduced to below the level of detection. Sat-RNA D, DI RNA G, and sat-RNA C*, none of which had an effect on TCV-CPm symptoms, did not change the levels of TCV-CPm genomic RNA. Taken together, these data indicate that inoculation of plants with combinations of viral and subviral RNAs that result in symptom attenuation is correlated with a reduction in viral genomic RNA to undetectable levels. In addition, viral and subviral RNA combinations that had no effect on symptoms or intensified symptoms nearly always resulted in less than

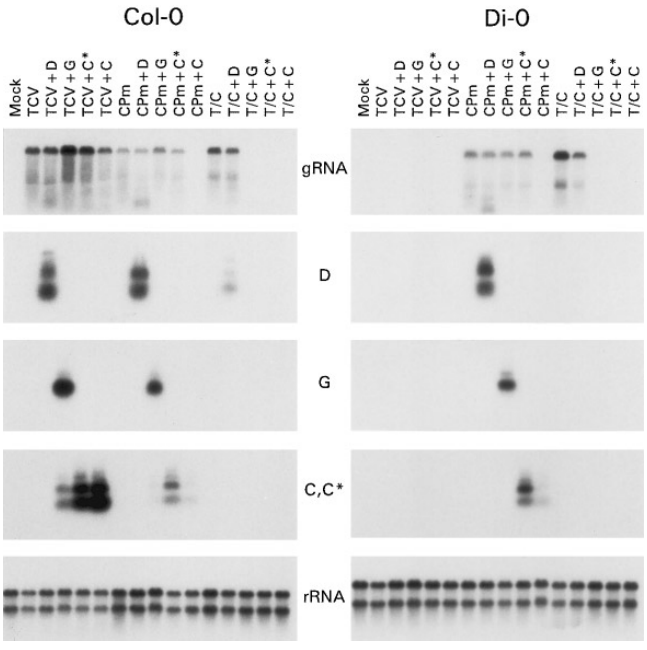

FIG. 3. Effect of subviral RNAs on accumulation of TCV, TCV-CP_{CCFV}, and TCV-CPm in Col-0 and Di-0 plants. Two micrograms of total RNA isolated from three pooled Col-0 (left panel) or Di-0 (right panel) plants inoculated 16 days previously with TCV, TCV-CPm (CPm), or TCV-CP_{CCFV} (T/C) and sat-RNA D (D), DI RNA G (G), sat-RNA C* (C*), or sat-RNA C (C) was subjected to RNA gel blot analysis. Blots were sequentially hybridized with probes for the RNAs indicated between the panels. The probe for sat-RNAs C and C* also hybridized weakly to DI RNA G. gRNA, viral genomic RNA; rRNA, ribosomal RNA.

a 50% decrease, little change, or an increase in viral genomic RNA levels.

As described above, sat-RNA C*, which has no effect on symptoms of TCV-CPm, and sat-RNA C, which attenuates the symptoms of most TCV-CPm-infected plants, differ at only eight positions all within the 3′-terminal 53 bases. Since sat-RNA C symptom attenuation occurs as a consequence of altering the CP of TCV (by replacing it with the CP of CCFV or altering the N-terminus as with TCV-CPm), this suggests that the 3′-terminal 53-base region interacts directly or indirectly with the CP. Since sat-RNA C has only a limited effect on the levels of TCV-CPm genomic RNA in protoplasts (Kong *et al.,* in press), the direct or indirect interaction of the TCV wild-type CP with the sat-RNA C 3′-terminal region likely obviates the ability of the sat-RNA to attenuate symptoms.

Effect of subviral RNAs on viral RNA accumulation in protoplasts

TCV-CP_{CCFV} supported sat-RNA D to much lower levels in both Col-0 and Di-0 plants when compared with TCV and TCV-CPm (Fig. 3), suggesting that a wild-type or near wild-type TCV CP might be important for efficient sat-RNA D accumulation. We previously showed that the opposite was true for sat-RNA C in Col-0 protoplasts; three- to fourfold more sat-RNA C accumulated when TCV-CP_{CCFV} or TCVΔCP (TCV with a deletion of the CP ORF; Fig. 1A) were the helper viruses than when TCV was the helper virus (Kong *et al.,* 1995). To determine the effect of TCV CP on the accumulation of sat-RNA D or DI RNA G in protoplasts, Col-0 protoplasts were coinoculated with TCV, TCV-CP_{CCFV}, or TCVΔCP, in the absence or presence of sat-RNA D or DI RNA G, and the level of the genomic and subviral RNAs at 16 and 24 h postinoculation (PI) was quantified by RNA gel blot analysis.

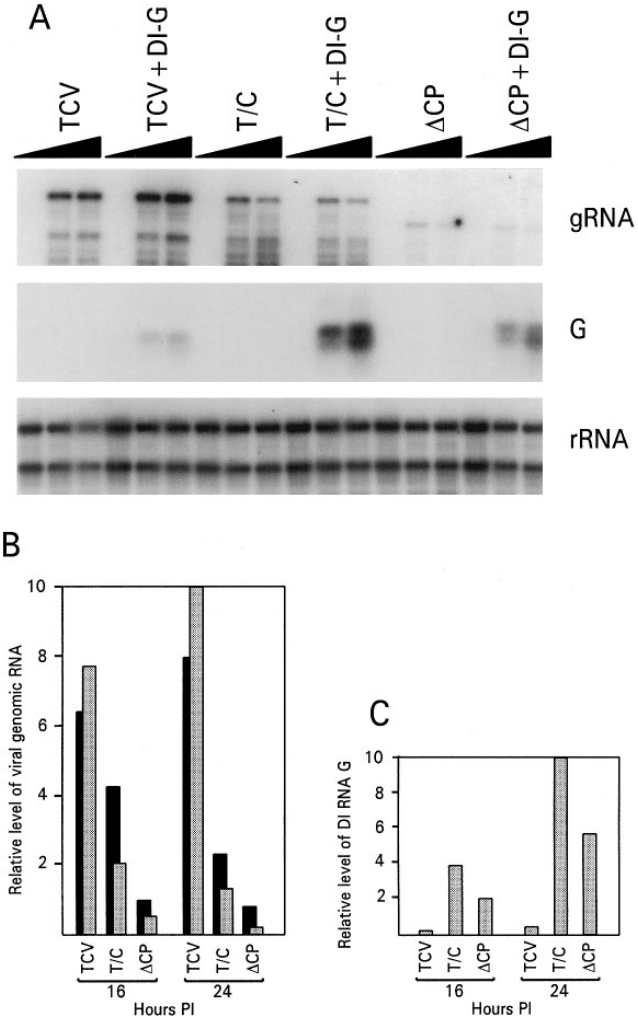

FIG. 4. Effect of DI RNA G on accumulation of TCV, TCV-CP_{CCFV}, and TCVΔCP in protoplasts. Col-0 protoplasts were inoculated in the absence or presence of DI RNA G and the helper viruses TCV, TCV-CP_{CCFV} (T/C), or TCVΔCP (ΔCP). (A) RNA was extracted from samples collected at 0, 16, and 24 h postinoculation (increasing triangle height) and subjected to RNA gel blot analysis. Blots were sequentially hybridized with probes specific for TCV (gRNA), DI RNA G (G), and ribosomal RNA (rRNA). (B) Autoradiograms were scanned with a densitometer and the data normalized to ribosomal RNA levels. Genomic RNA levels in the absence and presence of DI RNA G are denoted by black and gray bars, respectively. (C) Normalized levels of DI RNA G. PI, postinoculation.

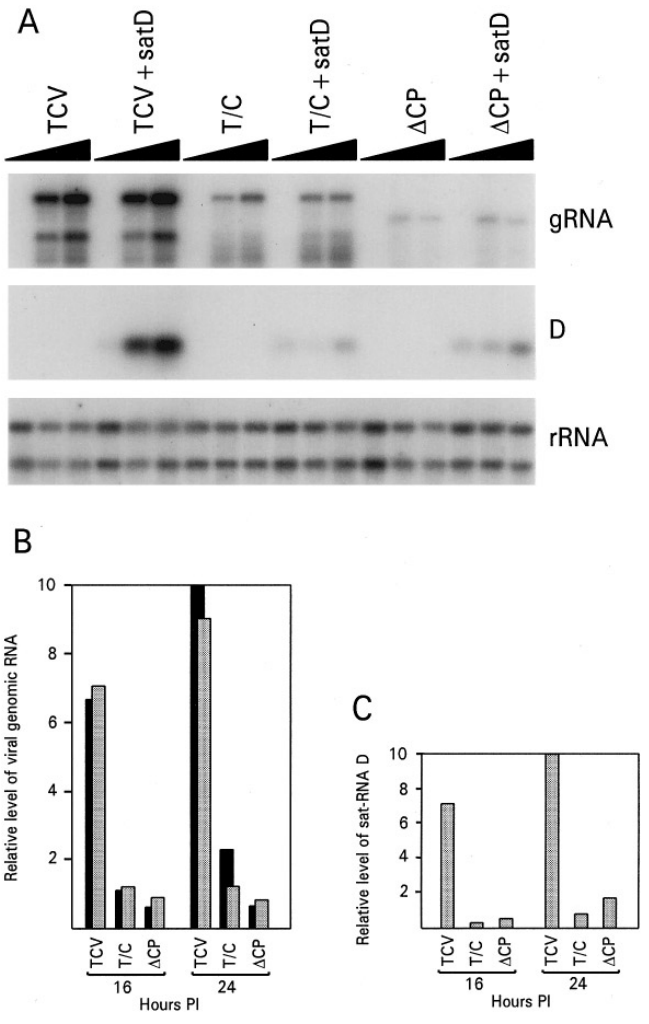

FIG. 5. Effect of sat-RNA D on accumulation of TCV, TCV-CP_{CCFV}, and TCVΔCP in protoplasts. Col-0 protoplasts were inoculated in the absence or presence of sat-RNA D and the helper viruses TCV, TCV-CP_{CCFV} (T/C), or TCVΔCP (ΔCP). (A) RNA was extracted from samples collected at 0, 16, and 24 h postinoculation (increasing triangle height) and subjected to RNA gel blot analysis. Blots were sequentially hybridized with probes specific for TCV (gRNA), sat-RNA D (D), and ribosomal RNA (rRNA). (B) Autoradiograms were scanned with a densitometer and the data normalized to ribosomal RNA levels. Genomic RNA levels in the absence and presence of sat-RNA D are denoted by black and gray bars, respectively. (C) Normalized levels of sat-RNA D. PI, postinoculation.

DI RNA G, while not substantially affecting the level of TCV genomic RNA, reduced the accumulation of TCV-CP_{CCFV} and TCVΔCP genomic RNAs by an average of 40 and 65%, respectively, over the course of the experiment (Figs. 4A and 4B). These reductions in genomic RNA levels due to the presence of DI RNA G are similar to reductions previously found for sat-RNA C (Kong *et al.,* 1995). The level of DI RNA G was an average of 22-fold higher when inoculated with TCV-CP_{CCFV} and 11-fold higher when inoculated with TCVΔCP than when inoculated with TCV (Figs. 4A and 4C). The helper virus association led to opposite effects on the accumulation of sat-RNA D. Levels of sat-RNA D were an average of 16-fold higher when associated with TCV than with TCV-CP_{CCFV} and 5-fold higher with TCV than with TCVΔCP (Figs. 5A and 5C). The decrease in sat-RNA D levels when TCV-CP_{CCFV} or TCV-CPm were used as helper viruses in protoplasts was similar to the decrease found when sat-RNA D was coinoculated with TCV-CP_{CCFV} in plants (see Fig. 3). In addition, sat-RNA D did not consistently reduce the accumulation of any of the genomic RNAs.

CP is known to have an important role in the replication of alfalfa mosaic virus (van der Kuyl *et al.,* 1991; Neeleman *et al.,* 1993) in addition to roles in other viruses such as elicitation of resistance (Culver *et al.,* 1994; Kavanagh *et al.,* 1992; Köhm *et al.,* 1993; Oh *et al.,* 1995; Taraporewala and Culver, 1996), symptom expression (Heaton *et al.,* 1991, 1995; Neeleman *et al.,* 1991; Shintaku *et al.,* 1992; Suzuki *et al.,* 1995; Rao and Grantham, 1995), and movement (Allison *et al.,* 1990; Chapman *et al.,* 1992; Hacker *et al.,* 1992; Hilf and Dawson, 1993; Dolja *et al.,* 1994; Rao and Grantham, 1995; Oparka *et al.,* 1996). Our results suggest that the TCV CP may be involved in the replication of the TCV subviral RNAs possibly by competing for binding to promoter sequences with the virus RNA-dependent RNA polymerase (RdRp). Sat-RNA C and DI RNA G, both of which do not accumulate efficiently in the presence of the wild-type TCV CP, share similar 3′-end sequences and probably promoters for minus-strand synthesis (Song and Simon, 1995). Sat-RNA D, however, shares little sequence and no structural similarity with sat-RNA C and DI RNA G at the 3′ end (Carpenter and Simon, 1996). While a competition between the RdRp and CP for binding to the 3′ ends of sat-RNA C and DI RNA G could account for the depressed accumulation of these subviral RNAs in the presence of wild-type TCV CP, the reduced level of sat-RNA D when associated with TCV-CP_{CCFV} and TCVΔCP may be a function of the less efficient accumulation of their genomic RNAs in protoplasts compared with wild-type TCV.

ACKNOWLEDGMENTS

We thank H. Guan for providing sat-RNA C*. This work was supported by NSF Grants MCB-9315948 and MCB-9419303 awarded to A.E.S.

REFERENCES

Allison, R., Thompson, C., and Ahlquist, P. (1990). Regeneration of a functional RNA virus genome by recombination between deletion mutants and requirement for cowpea chlorotic mottle virus 3a and coat genes for system infection. *Proc. Natl. Acad. Sci. USA* 87, 1820–1824.

Carpenter, C. D., and Simon, A. E. (1996). *In vivo* repair of 3′-end deletions in a TCV satellite RNA may involve two abortive synthesis and priming events. *Virology* 226, 153–160.

Carpenter, C. D., Oh, J.-W., Zhang, C., and Simon, A. E. (1995). Involvement of a stem-loop structure in the location of junction sites in viral RNA recombination. *J. Mol. Biol.* 245, 608–622.

Chapman, S., Hills, G., Watts, J., and Baulcombe, D. (1992). Mutational analysis of the coat protein gene of potato virus X: Effects on virion morphology and viral pathogenicity. *Virology* 191, 223–230.

Collmer, C. W., and Howell, S. H. (1992). Role of satellite RNA in the expression of symptoms caused by plant viruses. *Annu. Rev. Phytopathol.* 30, 419–442.

Culver, J. N., Stubbs, G., and Dawson, W. (1994). Structure–function relationship between tobacco mosaic virus coat protein and hypersensitivity in *Nicotiana sylvestris*. *J. Mol. Biol.* 242, 130–138.

Dawson, W. O., and Hilf, M. E. (1992). Host-range determinants of plant viruses. *Annu. Rev. Plant Physiol.* 43, 527–555.

Devic, M., Jaegle, M., and Baulcombe, D. (1990). Cucumber mosaic virus satellite RNA (strain Y): Analysis of sequences which affect systemic necrosis on tomato. *J. Gen. Virol.* 71, 1443–1449.

Dolja, V. V., Haldeman, R., Robertson, N. L., Dougherty, W. G., and Carrington, J. C. (1994). Distinct functions of capsid protein in assembly and movement of tobacco etch potyvirus. *EMBO J.* 13, 1482–1491.

Hacker, D. L., Petty, I. T. D., Wei, N., and Morris, T. J. (1992). Turnip crinkle virus genes required for RNA replication and virus movement. *Virology* 186, 1–8.

Hammond-Kosack, K. E., and Jones, J. D. G. (1996). Resistance gene-dependent plant defense responses. *Plant Cell* 8, 1773–1791.

Harrison, B. D., Mayo, M. A., and Baulcombe, D. C. (1987). Virus resistance in transgenic plants that express cucumber mosaic virus satellite RNA. *Nature* 328, 799–802.

Heaton, L. A., Lee, T. C., Wei, N., and Morris, T. J. (1991). Point mutations in the turnip crinkle virus capsid protein affect the symptoms expressed by *Nicotiana benthamiana*. *Virology* 183, 143–150.

Heaton, L. A., and Laakso, M. M. (1995). Several symptom-modulating mutations in the coat protein of turnip crinkle carmovirus result in particles with aberrant conformational properties. *J. Gen. Virol.* 76, 225–230.

Hilf, M. E., and Dawson, W. O. (1993). The tobamovirus capsid protein functions as a host-specific determinant of long-distance movement. *Virology* 193, 106–114.

Jaegle, M., Devic, M., Longstaff, M., and Baulcombe, D. (1990). Cucumber mosaic virus satellite RNA (Y strain): Analysis of sequences which affect yellow mosaic symptoms on tobacco. *J. Gen. Virol.* 71, 1905–1912.

Kaper, J. M., and Collmer, C. W. (1988). Modulation of viral plant diseases by secondary RNA agents. *In* "RNA Genetics" (E. Domingo, J. J. Holland, and P. Ahlquist, Eds.), Vol. III, pp. 171–194. CRC Press, Boca Raton, FL.

Kavanagh, T., Goulden, M. G., Santa Cruz, S., Chapman, S., Barker, I., and Baulcombe, D. (1992). Molecular analysis of a resistance-breaking strain of potato virus X. *Virology* 189, 609–617.

Kohm, B. A., Goulden, M. G., Gilbert, J. E., Kavanagh, T. A., and Baulcombe, D. C. (1993). A potato virus X resistance gene mediates an induced nonspecific resistance in protoplasts. *Plant Cell* 5, 913–920.

Kong, Q., Oh, J.-W., and Simon, A. E. (1995). Symptom attenuation by a normally virulent satellite RNA of turnip crinkle virus is associated with the coat protein open reading frame. *Plant Cell* 7, 1625–1634.

Kong, Q., Wang, J., and Simon, A. E. (1997). Satellite RNA-mediated resistance to turnip crinkle virus in Arabidopsis involves a reduction in virus movement. *Plant Cell,* in press.

Li, X. H., and Simon, A. E. (1990). Symptom intensification of cruciferous hosts by the virulent satellite RNA of turnip crinkle virus. *Phytopathology* 80, 238–242.

Li, X. H., and Simon, A. E. (1991). *In vivo* accumulation of a turnip crinkle virus DI RNA is affected by alterations in size and sequence. *J. Virol.* 65, 4582–4590.

Li, X. H., Heaton, L., Morris, T. J., and Simon, A. E. (1989). Defective interfering RNAs of turnip crinkle virus intensify viral symptoms and are generated de novo. *Proc. Natl. Acad. Sci. USA* 86, 9173–9177.

Masuta, S., and Takanami, Y. (1989). Determination of sequence and structural requirements of pathogenicity of a cucumber mosaic virus satellite RNA (Y-sat RNA). *Plant Cell* 1, 1165–1173.

Masuta, C., Suzuki, M., Kuwata, S., Takanami, Y., and Koiwai, A. (1993). Yellow mosaic symptoms induced by Y satellite RNA of cucumber mosaic virus is regulated by a single incompletely dominant gene in wild Nicotiana species. *Phytopathology* 83, 411–413.

Naidu, R. A., Collins, G. B., and Ghabrial, S. A. (1991a). Symptom-modulating properties of peanut stunt virus satellite RNA sequence variants. *Mol. Plant–Microbe Interact.* 4, 268–275.

Naidu, R. A., Collins, G. B., and Ghabrial, S. A. (1991b). Peanut stunt virus satellite RNA: Analysis of sequences that affect symptom attenuation in tobacco. *Virology* 189, 668–677.

Neeleman, L., van der Kuyl, A. C., and Bol, J. F. (1991). Role of alfalfa mosaic virus coat protein gene in symptom formation. *Virology* 181, 687–693.

Neeleman, L., Van Der Vossen, E. A. G., and Bol, J. F. (1993). Infection of tobacco with alfalfa mosaic virus cDNAs sheds light on the early function of the coat protein. *Virology* 196, 883–887.

Oh, J.-W., Kong, Q., Song, C., Carpenter, C. D., and Simon, A. E. (1995). Open reading frames of turnip crinkle virus involved in satellite symptom expression and incompatibility with *Arabidopsis thaliana* ecotype Dijon. *Mol. Plant–Microbe Interact.* 8, 979–987.

Oparka, K. J., Roberts, A. G., Roberts, I. M., Prior, D. A. M., and Santa Cruz, S. (1996). Virol coat protein is targeted to, but does not gate, plasmodesmata during cell-to-cell movement of potato virus X. *Plant J.* 10, 805–813.

Rao, A. L. N., and Grantham, G. (1995). Biological significance of the seven amino-terminal basic residues of brome mosaic virus coat protein. *Virology* 211, 42–52.

Romero, J., Huang, Q., Pogany, J., and Bujarski, J. J. (1993). Characterization of defective interfering RNA components that increase symptom severity of broad bean mottle virus infections. *Virology* 194, 576–584.

Roossinck, M. J., Sleat, D., and Palukaitis, P. (1992). Satellite RNAs of plant viruses: Structures and biological effects. *Microbiol. Rev.* 56, 265–279.

Roux, L., Simon, A. E., and Holland, J. J. (1991). Effects of defective interfering viruses on virus replication and pathogenesis in vitro and in vivo. *Adv. Virus Res.* 40, 181–211.

Shintaku, M. H., Zhang, L., and Palukaitis, P. (1992). A single amino acid substitution in the coat protein of cucumber mosaic virus induces chlorosis in tobacco. *Plant Cell* 4, 751–757.

Simon, A. E., and Howell, S. H. (1986). The virulent satellite RNA of turnip crinkle virus has a major domain homologous to the 3′-end of the helper virus genome. *EMBO J.* 5, 3423–3428.

Simon, A. E., Li, X. H., Lew, J. E., Stange, R., Zhang, C., Polacco, M., and Carpenter, C. D. (1992). Susceptibility and resistance of *Arabidopsis thaliana* to turnip crinkle virus. *Mol. Plant–Microbe Interact.* 5, 496–500.

Sleat, D. E., and Palukaitis, P. (1990). Site-directed mutagenesis of a plant viral satellite RNA changes its phenotype from ameliorative to necrogenic. *Proc. Natl. Acad. Sci. USA* 87, 2946–2950.

Sleat, D. E., Zhang, L., and Palukaitis, P. (1994). Mapping determinants with cucumber mosaic virus and its satellite RNA for the induction of necrosis in tomato plants. *Mol. Plant–Microbe Interact.* 7, 189–195.

Song, C., and Simon, A. E. (1994). RNA-dependent RNA polymerase from plants infected with turnip crinkle virus can transcribe (+)- and (−)-strands of virus-associated RNAs. *Proc. Natl. Acad. Sci. USA* 91, 8792–8796.

Song, C., and Simon, A. E. (1995). Synthesis of novel products in vitro by an RNA-dependent RNA polymerase. *J. Virol.* 69, 4020–4028.

Suzuki, M., Kuwata, S., Masuta, C., and Takanami, Y. (1995). Point mutations in the coat protein of cucumber mosaic virus affect symptom expression and virion accumulation in tobacco. *J. Gen. Virol.* 76, 1791–1799.

Taraporewala, Z. F., and Culver, J. N. (1996). Identification of an elicitor active site within the three dimensional structure of tobacco mosaic tobamovirus coat protein. *Plant Cell* 8, 169–178.

Uknes, S., Winter, A. M., Delaney, T., Vernooij, B., Morse, A., Friedrich, L., Nye, G., Potter, S., Ward, E., and Ryals, J. (1993). Biological induction of systemic acquired resistance in Arabidopsis. *Mol. Plant–Microbe Interact.* 6, 692–698.

van der Kuyl, A. C., Neeleman, L., and Bol, J. F. (1991). Role of alfalfa mosaic virus coat protein in regulation of the balance between viral plus and minus strand synthesis. *Virology* 185, 496–499.

White, R. F., and Antoniw, J. F. (1991). Virus-induced resistance responses in plants. *Crit. Rev. Plant Sci.* 9, 443–455.

White, K. A., Skuzeski, J. M., Li, W., Wei, N., and Morris, T. J. (1995). Immunodetection, expression strategy and complementation of turnip crinkle virus p28 and p88 replication components. *Virology* 211, 525–534.

Wu, G., and Kaper, J. M. (1995). Competition of viral and satellite RNAs of cucumber mosaic virus for replication in vitro by viral RNA-dependent RNA polymerase. *Res. Virol.* 146, 61–67.