

A Single Nucleotide Substitution Converts Potato Spindle Tuber Viroid (PSTVd) from a Noninfectious to an Infectious RNA for *Nicotiana tabacum*¹

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Mechanical inoculation of *Nicotiana tabacum* with the PSTVd isolate KF 440-2 from the host plant tomato resulted in the *de novo* emergence, replication, and accumulation of a new "tobacco variant," designated PSTVd NT. It produces no symptoms in tobacco but, like PSTVd KF 440-2, severe ones in tomato. The sequence analysis of PSTVd NT revealed a single nucleotide substitution from C → U at position 259. Autonomous viroid replication was also induced in tobacco by genomic integration of oligomeric cDNA copies of PSTVd KF 440-2. Although these cDNAs contained the original tomato-specific C₂₅₉, the circular PSTVd RNA subsequently accumulating in tobacco also exhibited the C₂₅₉ → U₂₅₉ substitution. In the secondary structure of PSTVd, nucleotide 259 is part of an internal loop analogous to loop E of eukaryotic 5S rRNA and presumed to be the only bulged extrahelical nucleotide of this loop. The C₂₅₉ in PSTVd KF 440-2 and in practically all other isolates and the U₂₅₉ in PSTVd NT of the loop E-like structure might be involved in protein binding and in viroid processing. The new variant PSTVd NT is genetically stable in both tobacco and tomato. © 1996 Academic Press, Inc.

INTRODUCTION

Potato spindle tuber viroid (PSTVd) is the first viroid that was discovered (Diener, 1971), structurally characterized (Sänger *et al.*, 1976), and sequenced (Gross *et al.*, 1978) and has thus become the prototype of this unusual class of small, single-stranded, and circular plant pathogenic RNA replicons. The correlation of its sequence with its virulence has been studied with various naturally occurring isolates (Schnölzer *et al.*, 1985; Puchta *et al.*, 1990; Owens *et al.*, 1992; Herold *et al.*, 1992; Lakshman and Tavantzis, 1993) and with *in vitro* generated PSTVd mutants (Hammond and Owens, 1987; Owens *et al.*, 1986, 1991; Loss *et al.*, 1991; Hammond, 1992; Lakshman and Tavantzis, 1992; Qu *et al.*, 1993; Hammond, 1994; Wassenegeger *et al.*, 1994b; Owens *et al.*, 1995; Hu *et al.*, 1996). From all these data the following conclusions can be drawn: (i) the chain length of the known PSTVd isolates varies between 341 and 361 nucleotides (nt); (ii) the number of nucleotide exchanges of the PSTVd variants is between 7 and 36 nt when compared with the canonical type strain sequence (Gross *et al.*, 1978) of PSTVd Di (Diener, 1971); (iii) although the primary structure of PSTVd RNA proved to be highly sensitive to experimentally introduced mutations in that a single point mutation that has no correlate in naturally

occurring variants can abolish viroid infectivity (Owens *et al.*, 1986, 1991), the emergence of a 341-nt-long infectious PSTVd mutant in transgenic tobacco (Wassenegeger *et al.*, 1994b) demonstrates that exceptions are possible; and (iv) despite the variations in primary structure, the overall PSTVd-specific native rod-like secondary structure is largely maintained.

At present the sequence of more than 20 infectious PSTVd variants is found in the nucleotide sequence databases GenBank and EMBL. Because some of these sequence variants originate from different plant species or different plant cultivars, selection processes are evidently taking place during the adaptation of the PSTVd RNA to the corresponding hosts.

Previous experiments (Wassenegeger *et al.*, 1994a) have shown that mechanical inoculation of tobacco does not result in a detectable replication and accumulation of the PSTVd strain KF 440-2 in such plants. PSTVd KF 440-2 had been originally isolated from field-grown potato plants and mechanically transmitted to the experimental host plant tomato (Fernow, 1967), in which it has been maintained ever since (KF relates to Karl Fernow, 440 was his sample number, and 2 denotes one of our subcultures from the original sample). PSTVd KF 440-2 has been sequenced, and it is highly infectious for tomato where it produces severe symptoms of disease (Schnölzer *et al.*, 1985). Here we report that transgenic expression of PSTVd KF 440-2 cDNA in tobacco plants can convert this isolate into a PSTVd variant that is able to infect tobacco plants. We further demonstrate that a

¹ The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession number X97387.

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single nucleotide substitution is sufficient to extend the host range of the new PSTVd variant in such a way that even after mechanical inoculation it replicates as efficiently in tobacco as in the experimental host plant tomato. This new PSTVd variant NT (NT for *Nicotiana tabacum*) can only have evolved *de novo* in tobacco plants, and it is genetically stable in both plant species.

MATERIALS AND METHODS

Plants and plant culture

Transgenic tobacco plants were produced as previously described (Wassenegger *et al.*, 1994a,b). *N. tabacum* plants (cv. "Petit Havana SRI") and *Lycopersicon esculentum* plants (cv. "Rentita") were maintained on MS medium (Murashige and Skoog, 1962) under sterile conditions in glass containers. Under nonsterile conditions both plant species were grown in soil.

Viroid infection

Viroid inoculation of tobacco and tomato plants was performed by rubbing the carborundum-dusted four primary leaves with 2.5 μg of total RNA per leaf corresponding to about 10 ng of viroid RNA per plant. The total RNA was isolated as described by Tabler and Sanger (1984) from the tomato cultivar (cv.) Rentita and from tobacco plants that had been infected with either the new tobacco variant PSTVd NT described in the present report or with PSTVd KF 440-2 (Schnolzer *et al.*, 1985), designated from now on as PSTVd KF. The accumulation of the newly synthesized PSTVd RNA was determined at weekly intervals postinoculation (p.i.) by Northern analysis.

RNA isolation and Northern blotting

For Northern blotting total RNA was extracted from leaf material in an 8 M guanidinium chloride buffer according to the procedure of Logemann *et al.* (1987). Glyoxalation of nucleic acids, PAGE, Northern blotting to Qiabrane Nylon membranes (Qiagen), and molecular hybridization with [α - ^{32}P]UTP-labeled PSTVd *in vitro* transcripts as probe were performed as previously described (Spiesmacher *et al.*, 1985).

PCR and sequencing

Performance of PCR, RT-PCR, the sequence of the primer pairs used (i.e., 325-H/326-R and 175-H/176-R), and cloning of the PCR products into the pTPCR vector were as previously described (Wassenegger *et al.*, 1994b). Cloned PCR products were sequenced according to Sanger *et al.* (1977) using Sequenase version 2.0 (Amersham).

RESULTS

Selection of a "tobacco-adapted PSTVd" in mechanically inoculated tobacco plants

Total RNA was isolated from PSTVd KF-infected tomato plants cv. Rentita bearing severe symptoms of growth retardation, stunting, and foliar epinasty. The presence of viroid RNA was verified by Northern analysis using PSTVd (+) RNA-specific, i.e., (–)-oriented, transcripts as probe (data not shown). The sequence of the isolated PSTVd KF was subsequently confirmed by sequencing of cloned PCR products that had been amplified with the two PSTVd-specific primer pairs 325-H/326-R and 175-H/176-R amplifying nt 326–359/1–325 or nt 176–359/1–175, respectively (Wassenegger *et al.*, 1994b). Ten micrograms of this viroid-containing tomato RNA per plant was then used for inoculation of four tobacco plants and of one tomato plant, all grown under sterile conditions. Inoculation of the tobacco plants was repeated every 2 weeks by rubbing again 10 μg of viroid-containing total RNA onto the four newly developing leaves of each plant.

After 7 weeks, Northern analysis revealed that, in contrast to the viroid-infected single tomato plant, none of the four tobacco plants had become infected with PSTVd (data not shown). Northern analysis was repeated at 2-week intervals until, after 5 months, in two of the tobacco plants an accumulation and hence an efficient replication of viroid RNA had taken place. To determine the sequence of the viroid RNA present in the tobacco plants, RT-PCR amplification of total RNA from both tobacco plants was performed with the two non-overlapping primer pairs 325-H/326-R and 175-H/176-R, respectively. Amplified PCR products were cloned into the pTPCR vector (Wassenegger *et al.*, 1994b). In each case, five independent clones were analyzed to guarantee (i) that mutations were not due to PCR errors, (ii) that no additional mutations were located within the sequences covered by the primers, and (iii) that there was no sequence variability among the RNA progeny. The corresponding viroid cDNA clones did not show any sequence heterogeneity, and the cDNA sequences from both infected plants were identical.

The sequence comparison between the "tobacco viroid" and the original PSTVd KF from tomato revealed a single point mutation at position 259 according to the canonical numbering of the PSTVd prototype (Gross *et al.*, 1978). In this new *N. tabacum* adapted variant PSTVd NT, nucleotide C₂₅₉ had changed to U (Fig. 1).

Selection of a "tobacco-adapted PSTVd" in transgenic tobacco plants

To determine whether the new PSTVd NT had evolved *de novo in planta* or whether a copy of this PSTVd isolate already existed in viroid-containing total RNA from to-

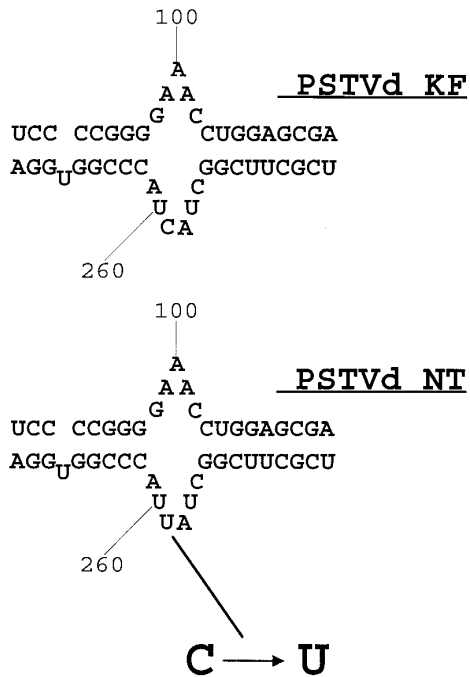


FIG. 1. Selected portions of the computed secondary structure of PSTVd sequence variants KF and NT. The single C \rightarrow U transition at position 259 is indicated in the PSTVd NT sequence. The numbering is according to Gross *et al.*, (1978).

mato, transgenic tobacco plants containing multimeric viroid cDNA copies were produced and also analyzed. The construction of the two transgenic tobacco plants, SRI-3(+) and SRI-4(-), and the sequence analysis of the transgenes have been described previously (Wassenegger *et al.*, 1994a). In the genome of the SRI-3(+) tobacco plant, 3.6 head-to-tail connected copies of a PSTVd KF cDNA unit were integrated, and in the SRI-4(-) tobacco plant, 4.4 copies of the PSTVd KF cDNA were inserted. In the SRI-3(+) plant, the PSTVd KF-specific cDNA was in (+) orientation downstream of the 35S cauliflower mosaic virus promoter (35S), whereas in the SRI-4(-) tobacco plant the orientation was reversed. In both plants viroid replication was induced by the 35S-driven *in vivo* transcription of the genome-integrated PSTVd cDNA (Wassenegger *et al.*, 1994a), which was unaltered as shown by PCR amplification and sequencing.

Total RNA was isolated from these plants and PSTVd cDNA was amplified by RT-PCR from circular RNAs which accumulated during replication in the SRI-3(+) and SRI-4(-) plants. Analysis of the cloned PCR products revealed that the sequences obtained from the SRI-3(+) and SRI-4(-) plants were identical, and again the only deviation from the original PSTVd KF sequence was the C \rightarrow U substitution at position 259. The fact that the C \rightarrow U substitution was found in all cloned PCR products also provides direct evidence that only the mutated viroid RNA had been amplified because the 35S-driven viroid-specific transcripts would show the original PSTVd KF sequence only.

Infectivity of PSTVd NT and PSTVd KF in tobacco

To compare the infectivity of the new PSTVd NT and the original PSTVd KF, each of 10 tobacco plants was mechanically inoculated with 10 μ g of total RNA that had been isolated from PSTVd NT-infected tobacco plants and from PSTVd KF-infected tomato plants, respectively. Eight weeks after inoculation, total RNA was isolated from these tobacco plants and subjected to Northern analysis using (+) PSTVd-specific RNA probes. At this time the RNA from 5 of the 10 PSTVd NT-inoculated plants gave strong viroid-specific hybridization signals typical for a systemic viroid infection (Fig. 2a, plant NT 1, 3, 4, 6, and 10). In three PSTVd NT-inoculated plants

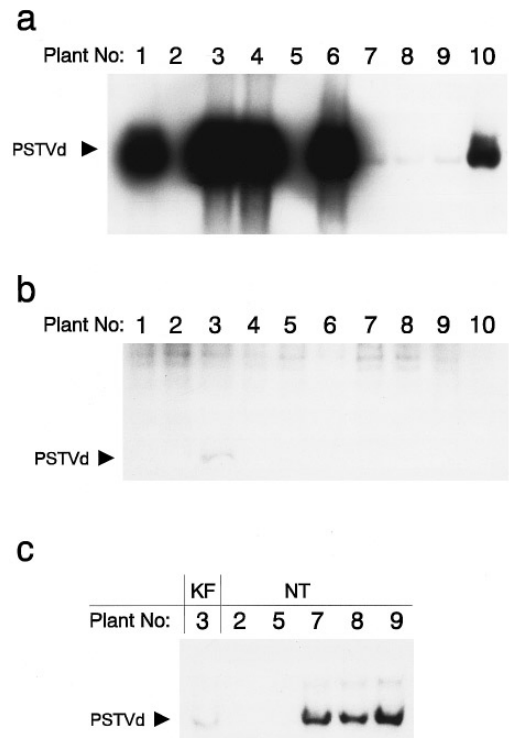


FIG. 2. Northern analysis of PSTVd replication and accumulation in tobacco plants 8 weeks after mechanical inoculation with the tobacco- and tomato-specific viroid isolate, respectively. To visualize the presence of mature circular PSTVd (+) RNA molecules that had accumulated during viroid replication, hybridization was performed with PSTVd-specific (-) RNA transcripts and autoradiographed for 3 hr (a), 16 hr (b), and 24 hr (c), respectively. (a) Lanes 1-10: total RNA from 10 tobacco plants inoculated with the tobacco isolate PSTVd NT. The signals show that extensive NT replication had taken place in plant NT 1, 3, 4, 6, and 10, respectively. (b) Lanes 1-10: total RNA from 10 tobacco plants inoculated with the tomato isolate PSTVd KF. Only lane 3 shows a faint hybridization signal indicative for a very early stage of a viroid infection. (c) Reevaluation of the uncertain samples from a and b. Upon longer exposure, the RNA from plant KF3 and from plants NT 7, 8, and 9 gave weak signals characteristic for a very early and a more advanced stage of a viroid infection, respectively, whereas plants NT 2 and 5 whose state was uncertain due to the overshadowing from the adjacent lanes had escaped infection and were viroid-free. The location of the monomeric circular PSTVd RNA is indicated by an arrowhead.

the infection was in an early stage which could be concluded from the weaker signals visible in Fig. 2a, plants 7–9.

To ensure that the infection of the tobacco with the tomato-specific PSTVd KF was not delayed compared to its infection with PSTVd NT, but indeed suppressed, Northern analysis was repeated 5 months p.i. In 9 of the 10 PSTVd KF-inoculated plants PSTVd RNA was still not detectable, whereas in a single tobacco plant the onset of a systemic viroid infection became visible (Fig. 2b, plant KF3).

To verify that the uncertain and weak signals in Figs. 2a and 2b were not due to the overshadowing or to a possible spill-over during the loading of the samples, an additional gel was run with aliquots of the corresponding total RNAs and analyzed. Figure 2c clearly demonstrates that upon longer exposure the uncertain sample from the PSTVd KF-inoculated tobacco plant KF3 and from the PSTVd NT-inoculated tobacco plants 7–9 gave weak signals characteristic for an early stage of viroid infection. However, for unknown reason, the PSTVd NT-inoculated tobacco plants 2 and 5 had escaped infection and were viroid-free.

A second Northern analysis was performed 7 months p.i. with RNA isolated from the newly developing young leaves and demonstrated that all those plants whose RNA had previously shown a weak signal had now developed a heavy systemic infection (data not shown).

Genetic stability of PSTVd NT in tobacco

RT-PCR was performed with total RNA from all viroid-infected tobacco plants including KF3. Analysis of the cloned PCR products revealed the PSTVd NT sequence in all clones, demonstrating its genetic stability in tobacco plants (data not shown). The fact that PSTVd NT accumulated also in the KF3 tobacco plant that had originally been inoculated with the PSTVd KF isolate from tomato again substantiates that PSTVd KF cannot replicate to a detectable level in tobacco unless the C → U substitution at position 259 has taken place.

Infectivity and genetic stability of PSTVd NT in tomato

The infectivity of PSTVd NT for tomato was analyzed after mechanical inoculation of 10 tomato plants each with 10 μ g of total RNA isolated from PSTVd NT-infected tobacco plants. Eight weeks p.i., accumulation of viroid progeny could be demonstrated in all test plants by Northern analysis. Cloning and sequencing of the RT-PCR products amplified from the total tomato RNA from the inoculated plants above revealed that the sequence of the PSTVd NT variant remained stable in tomato. No reversion of the nucleotide exchange has been observed as yet, i.e., after 4 months of culture (data not shown). Whereas all the viroid-infected tobacco plants remained free of symptoms, the tomato cultivar Rentita developed

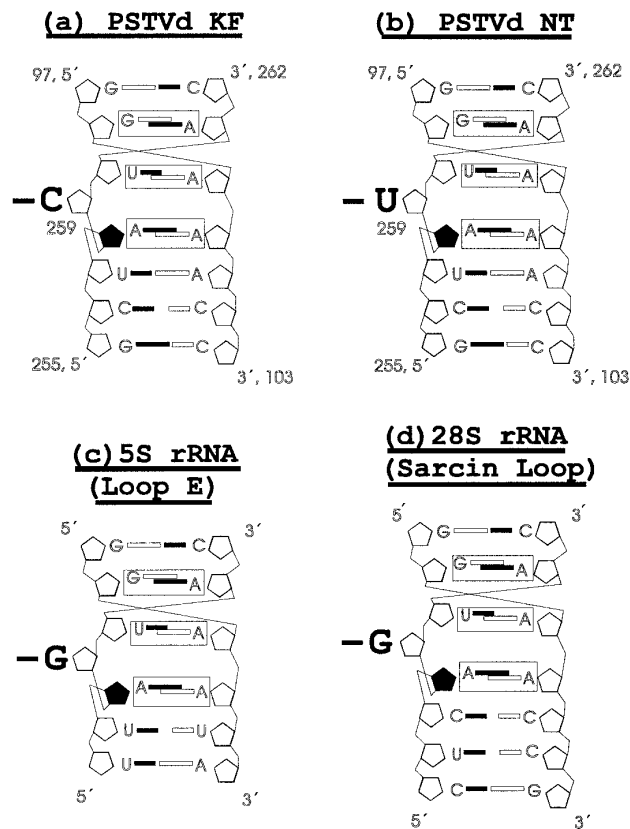


FIG. 3. Schematic representation of the proposed conformation of loop E-like structures in PSTVd KF, PSTVd NT, eukaryotic 5S rRNA, and 28S rRNA, respectively. (a) Loop E-like structure of PSTVd KF based on uv cross-linking data (Branch *et al.*, 1985) and on enzymatic as well as chemical probing (Gast *et al.*, 1996). (b) Analogous loop E-like structure of PSTVd NT. (c) Loop E structure of eukaryotic 5S rRNA as determined by NMR (Wimberly *et al.*, 1993). (d) A part of the loop E-like α -sarcin structure of 28S rRNA as determined by NMR (Szewczak *et al.*, 1995). Sugar residues of the RNA strands are represented by open pentagons and sugars of single reversed adenosine nucleotides that allow a local parallel-stranded orientation are filled in black. Boxed non-Watson-Crick G:A base pairs, reverse Hoogsteen A:U, and parallel A:A base pairs are conserved in all loop E-like structures. The single nucleotide exchange that distinguishes PSTVd NT from PSTVd KF occurs at the bulged position 259 in the loop E-like structure. The bulged extrahelical nucleotide in the major groove of the loop E region is assumed to be recognized by proteins (Shen *et al.*, 1995).

severe PSTVd-specific symptoms of growth retardations, stunting, and leaf epinasty irrespectively of whether PSTVd NT or PSTVd KF had been used for inoculation.

The C → U transition at position 259 in PSTVd affects the bulged nucleotide of an internal loop analogous to loop E of 5S rRNA

The nucleotide that is exchanged in PSTVd is located in an internal loop in the central region of the computed rod-like viroid structure (Fig. 1). This loop is homologous to loop E of *Xenopus laevis* 5S ribosomal RNA, whose three-dimensional structure has been solved by two-dimensional NMR (Wimberly *et al.*, 1993) (compare Figs. 3a and 3b with 3c). Loop E of 5S rRNA (Fig. 3c) contains

a number of noncanonical base pairs, namely a "sheared" G:A, a reverse-Hoogsteen A:U, a parallel-stranded A:A, and a U:U base pair, and an extrahelical G residue forming a nucleotide triple with the A:U pair in the major groove (Wimberly *et al.*, 1993). A second example has been found by NMR in the sarcin/ricin loop of 28S rRNA (Fig. 3d), which consists of a GAGA tetraloop and an adjacent loop E-like internal loop (Szewczak and Moore, 1995).

The sequence motif within the loop E structure of 5S and 28S rRNA, 5'-ANUA-3'/3'-AAG-5' (Figs. 3c and 3d), is present in most viroid RNAs. An E loop in PSTVd (Figs. 3a and 3b) is supported by its low nuclease accessibility in PSTVd-specific transcripts (Gast *et al.*, 1996) and by the uv crosslink that can be induced in this region both in PSTVd RNA and in 5S rRNA (Branch *et al.*, 1985). Photo-crosslinking experiments have also revealed the presence of a structure homologous to loop E which is essential for the catalytic function of the hairpin ribozyme (Butcher and Burke, 1994). It appears that all these loop E-containing molecules belong to a family of RNAs that has been selected for interactions with other RNAs or with proteins (Shen *et al.*, 1995).

In the case of PSTVd RNA, the bulged nucleotide of the loop E-like structure is located at position 259 in both the PSTVd KF and the PSTVd NT sequence, i.e., at the position where the C → U transition took place (Figs. 3a and 3b). Regarding the possible function of loop E one could argue that the nucleotide at position 259 might be important for viroid processing.

DISCUSSION

The influence of the host plant species and of specific nucleotide sequences during serial passages and the resulting selection process has been studied by Kurath and Palukaitis (1990) with cucumber mosaic virus satellite (sat) RNA clones. A rapid mutation rate appears to be the primary driving force for the emergence of distinct sequence variants in the various virus isolates. However, as demonstrated for the sat RNA of tobacco necrosis virus, the rapid host-dependent selection of a sat RNA strain from a given virus population consisting of different sequence variants ("quasispecies") seems to be more likely (Donis-Keller *et al.*, 1981). A similar situation has been reported by Semancik *et al.* (1993) for a heterogeneous citrus exocortis viroid (CEVd) population derived from a single source plant. They found that by sequential passage through alternative and genetically diverse hosts distinct sequence variants were selected. As a result, a common pattern of nucleotide exchanges emerged in all isolates derived from hybrid tomato tissue, of which five common exchanges were assumed to reflect a response to the selection pressure of the host. This observation prompted the designation "tomato signature" to these nucleotide exchanges.

The passage of the tomato-specific PSTVd KF through tobacco led to the emergence of PSTVd NT in which case only a single specific mutation occurred. In the transgenic plants PSTVd NT must have evolved *de novo*, and this term is used to illustrate that the source materials from which PSTVd NT arose were the 35S promoter-driven primary *in vivo* RNA transcripts from the transgenic viroid cDNA integrate that contained the original tomato-specific PSTVd KF sequence. With respect to their length and sequence, these primary PSTVd KF cDNA-derived RNA transcripts were found to be homogeneous (Wassenegger *et al.*, 1994a). This is most likely not the case when plants are mechanically inoculated with the quasi-species-containing population of viroid RNA as present in the total RNA from a viroid-infected tissue.

Because the PSTVd KF-specific cDNAs integrated into the genome of the SRI-3(+) and SRI-4(-) tobacco plants did not contain this mutation, we assume that the C → U substitution must have taken place during RNA synthesis. This could have happened during the primary RNA transcription from the plant genome-integrated PSTVd KF-specific cDNA template or during the subsequent viroid RNA/RNA transcription by the host plant-encoded DNA-dependent RNA polymerase II, which is known to mediate autonomous PSTVd replication (Schindler and Mühlbach, 1992).

The fact that PSTVd NT also emerged after inoculation of tobacco plants with the total RNA from PSTVd KF-infected tomato plants strengthens the assumption that the C → U substitution occurred during the viroid RNA/RNA replication process. As a consequence, PSTVd KF must have replicated autonomously in tobacco for several months at such a low rate that it could not be detected by Northern blotting as our analysis showed. Under this premise, tobacco could be considered a poor host plant for PSTVd KF because the replication and/or processing of its RNA is not functioning properly in its cells. Nevertheless, even a low rate of inappropriate replication and processing could generate over a period of several months a large number of mutated viroid RNA molecules. From this population of viroid-specific sequences, a new viroid variant could emerge that is capable of an efficient replication and processing in the tobacco plant without producing disease symptoms despite its accumulation and systemic invasion. A similar mechanism of a host plant-mediated selection of an RNA replicon has already been proposed for the occurrence of a 341-nt-long deletion mutant of PSTVd that also evolved in transgenic tobacco (Wassenegger *et al.*, 1994b). This mutant also exhibited the C → U substitution at the identical central position as PSTVd NT, suggesting that this exchange represents the "tobacco signature" of PSTVd.

It is noteworthy that the C → U substitution in PSTVd NT at position 259 has been previously described for a PSTVd variant from a wild *Solanum spp.*, and like PSTVd

NT this variant is also capable of infecting tomato (Owens *et al.*, 1992). However, when the entire PSTVd NT sequence is compared with that of the PSTVd variant from the wild *Solanum spp.*, 13 additional differences are found between both variants.

In the secondary structure of PSTVd KF the C₂₅₉ → U₂₅₉ substitution is located within an internal loop analogous to loop E of eukaryotic 5S rRNA (see Fig. 3). In fact, nucleotide 259 is presumed to be the only bulged extrahelical nucleotide of this internal viroid loop structure. Therefore, one can assume that the C → U substitution might convert the host specificity of PSTVd KF by extending the putative function of the loop E-like structure where this mutation occurred. In this context it is interesting to note that among the various exchanges found in distinct sequence variants of CEVd (Semancik *et al.*, 1993), a frequent U → G exchange at position 263 has been reported. In fact, if the secondary structure model of CEVd is analyzed, this substitution is also located within the loop E-like structure of this viroid.

Regarding the possible function and the effects of the mutational changes of the loop E-like structure in viroids, its influence on the processing of the multimeric viroid RNA precursor molecules can be invoked. It is generally accepted that during the final step of viroid replication these multimeric linear PSTVd (+) RNAs are cleaved to linear monomeric molecules which are then intramolecularly ligated to the mature circular PSTVd (reviewed by Sanger, 1987). It is also assumed that these viroid precursor molecules undergo a series of specific structural transitions (reviewed by Riesner, 1990). This has recently been demonstrated in *in vitro* processing experiments with precursor-like oligomeric linear PSTVd RNA transcripts incubated in the presence of extracts from potato cell nuclei (Baumstark and Riesner, 1995). From the analysis based on temperature gradient gel electrophoresis, "oligonucleotide mapping," and thermodynamic calculations it has been concluded that only one of the four possible secondary structures of this precursor-like RNA, namely the "extended middle" structure, is correctly processed in this system, i.e., cleaved to monomers which are then ligated to circular molecules (Baumstark and Riesner, 1995). In fact, most recent studies have shown that in this system cleavage takes place within a GAAA tetraloop and that the ligation of the monomers into the mature circular PSTVd RNA requires the refolding of this RNA into the viroid-specific rod-like structure and the exposure of the loop E-like domain with its bulged-out extrahelical nucleotide (Baumstark and Riesner, 1996).

Bulged nucleotides are known to be important for protein binding (Wu and Uhlenbeck, 1987; Gott *et al.*, 1991). Therefore, one can assume that in the case of PSTVd the bulged nucleotide at position 259 within its loop E-like structure might be involved in the processing of its RNA in that a putative protein required for intramolecular ligation binds to this domain. One could argue that the

corresponding tobacco protein recognizes and requires C₂₅₉ in this structure which results in an efficient replication, processing, and subsequent accumulation of mature circular PSTVd NT RNA molecules in this host. In tomato plants, however, U as well as C at position 259 must be accepted equally well by the tomato protein in question which would explain why both PSTVd KF and PSTVd NT replicate and accumulate in a similar fashion in this host. The isolation and characterization of these putative host-specific processing factors are the subject of our present studies.

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