specifically cited studies of nitric oxide sticking probabilities, energy transfer, and desorption rates on close-packed metal surfaces where specific and general conclusions about the molecular dynamics and interaction potential have been drawn. In order to attain this level of understanding, high-resolution experiments and detailed theories are combined. We anticipate a comparable level of understanding to unfold for elementary reactive processes at surfaces. Key to progress in this direction is the evolution of sensitive time-resolved measurements of the populations of adsorbed molecules and their intermediate configurations.

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A Replication Cycle for Viroids and Other Small Infectious RNA's

Andrea D. Branch and Hugh D. Robertson

Viroids are infectious circular RNA molecules consisting of 250 to 400 nucleotides; these viroids replicate and cause disease in various economically important plants (*1–3*). We have analyzed the viroid-specific nucleic acids in tomato plants infected by potato spindle tuber viroid (PSTV) and have used these data to construct a model for PSTV replication. Our experiments presented in this article suggest that the pathway for PSTV replication proposed here may also apply to other viroids and to two nonviroid classes of small pathogenic RNA molecules found in plants. These additional species include (i) virus satellite RNA's that are packaged as linear molecules such as the satellite of tobacco ringspot virus, TobRV (*4–6*) and (ii) the small circular viral RNA's ("virusoids") that have recently been discovered (*7–10*). In light of experiments suggesting that viroids do not encode proteins (*11–14*), we propose possible host functions of the machinery used for viroid replication.

Summary. Experimental data concerning viroid-specific nucleic acids accumulating in tomato plants establish, together with earlier studies, the major features of a replication cycle for viroid RNA in plant cells. Many features of this pathway, which involves multimeric strands of both polarities, may be shared by other small infectious RNA's including certain satellite RNA's and "virusoid" RNA's which replicate in conjunction with conventional plant viruses. The presence, in host plants, of an elaborate machinery for replicating these disease agents suggests a role for endogenous small RNA's in cellular development.

The early studies of viroid replication were directed toward detecting viroid-specific nucleic acids (minus strands) that could serve as the template for synthesis of mature, infectious RNA (plus strands). Minus strands composed of RNA were first discovered in 1978 by Grill and Semancik during their studies of citrus exocortis viroid (CEV) (*15*) and now have also been detected in plants infected by PSTV (*16–18*), avocado sunblotch viroid (ASBV) (*19*), and chrysanthemum stunt viroid (CSV) (*20*). In addition, double-stranded RNA has been found in association with the satellite of TobRV (*4*) and the virusoid of velvet tobacco mottle virus (VTMoV) (*21*). The minus strand RNA of PSTV appears to contain regions complementary to the entire plus strand and therefore could act as its template (*22*). Experiments by several groups of investigators have established that host plants do not contain detectable amounts of viroid-specific DNA (*23–25*). Thus, key experimental questions about viroid replication concern the size and structure of the RNA minus strands, the form of plus strand intermediates, and the composition of the replication complex.

Multimeric Minus Strands

Analysis of RNA filter hybridization (Northern blot) experiments (*26*) using two different denaturing gel electrophoretic systems established that multimeric minus strands accumulate during PSTV replication (*17*). To determine whether multimeric minus strands could be detected in an additional viroid system, hybridization experiments were carried out with nucleic acids extracted from tomato plants infected by CEV. The nucleic acids were denatured by treatment with glyoxal and dimethyl sulfoxide (*27*) and then separated by gel electrophoresis. The pattern of complementary nucleic acids detected after hybridization to ^{125}I-labeled CEV (Fig. 1B) was similar to the pattern of multimeric minus strands of PSTV (Fig. 1, A and C).

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The most prominent minus strand RNA's have the appropriate sizes to contain between two and five repeats of the viroid monomer (unit length).

Minus strands greater than unit length have also been reported in a variety of systems including PSTV (*18, 28*), ASBV (*19*), and the virusoid of VTMoV (*21*). A definitive demonstration of multimeric minus strands has been provided by studies of the satellite of TobRV (*6*).

The existence of multimeric minus strands in cells infected by PSTV, CEV, and other agents raises the question of their involvement in replication. To investigate the possibility that the multimeric minus strands of PSTV serve as templates for plus strand synthesis, we examined the profiles of PSTV-specific RNA's present in tomato plants at intervals after infection. As shown in Fig. 2, A and B, as soon as plus strands could be detected (day 14), multimeric minus strands were also present. Thus, the time course of multimeric minus strand synthesis indicates that minus strands are present at the appropriate times to play a direct role in replication.

Despite the widespread occurrence of multimeric minus strands and their presence relatively soon after inoculation, it is possible that they are intermediates in minus strand synthesis (or by-products) and that the true template for plus strand synthesis is a unit length minus strand species, which was not detected in previous studies for technical reasons. As illustrated in Fig. 1A, lane a, unit length minus strands are not evident in Northern hybridization experiments. In fact, a slight gap is present at this region of the blot.

The reconstruction experiment shown in Fig. 3 was performed in order to ascertain whether any unit length minus strands of PSTV that might have been present in extracts of infected plants could be detected and at the same time to assess the general usefulness of Northern blot analysis for picking up both strands of an RNA · RNA duplex in the presence of an excess of one of them. The unit length minus strands required for this experiment were prepared by treating nucleic acids of infected plants with pancreatic ribonuclease under conditions leading to the digestion of single- but not double-stranded RNA's (*17*); this treatment is accompanied by the appearance of duplexes ("cores") containing unit length minus strands (along with unit length plus strands) (*16, 17, 29*). The enzymatically generated (unit length) minus strands could be readily detected in samples containing no other plant nucleic acids (as in Fig. 3, lane c) or when mixed with nucleic acids of uninfected plants (lane b). However, detection of unit length minus strands was made impossible by the addition of only 10 nanograms of unlabeled PSTV plus strands (lane e)—presumably because the added plus strands took all the available minus strands and hybridized with them, outcompeting the ^{125}I-labeled PSTV used as a radioactive probe. This same result (no detection of minus strands at the unit length position) was obtained when the cores were mixed with nucleic acids of infected plants (lane g).

To eliminate the free progeny plus strands (whose presence might interfere with detection of minus strands), we used cellulose CF11 chromatography (*30*). This procedure can effectively separate mature PSTV (which chromatographs as single-stranded RNA) from duplexes containing viroid plus and minus strands, which chromatograph as typical double-stranded RNA (*23*). Fractions enriched for double-stranded RNA's were studied by RNA · RNA hybridization, and predominantly multimeric minus strands were detected. Unit length minus strands, while clearly visible, did not form a prominent band (figure 5 in *31*). The presence of multimeric minus strands in the double-stranded RNA fraction supports the hypothesis that they are involved in replication. Furthermore, if the multimeric minus strands shown in Figs. 1 and 2 were copied, they would give rise to plus strands ranging in size up to at least 2000 bases. Thus, the existence of multimeric minus strands led to the prediction that plus strand synthesis might also involve a multimeric species (*17, 29*).

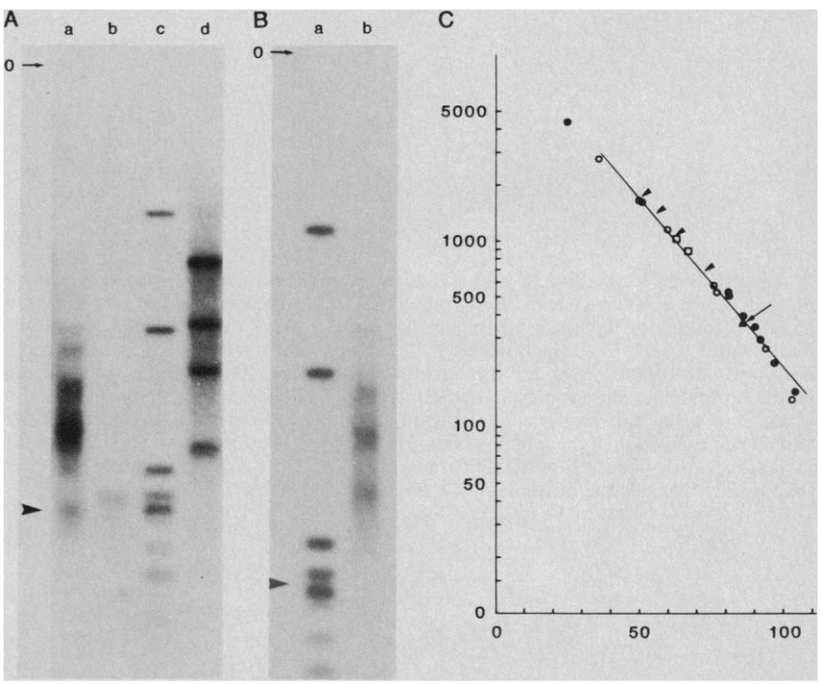

Fig. 1. (A) Detection of multimeric PSTV minus strands by RNA · RNA hybridization. Samples were treated with glyoxal and dimethyl sulfoxide (*27*) and then fractionated in 1.6 percent agarose gels (10 m*M* phosphate buffer, *p*H 7.1) (*26*). Nucleic acids were transferred to nitrocellulose. Filters were incubated with double strength (2×) TESS (*17*) containing ^{125}I-labeled PSTV (*60*) at 200,000 dpm/ml, and RNA (2 μg/ml) from uninfected tomato plants, washed, and prepared for autoradiography. (Lane a) 5 μg of nucleic acids from PSTV-infected tomato plants; (lane b) 4000 dpm of ^{125}I-labeled PSTV size marker (359 bases) (*14*); (lane c) 3000 dpm of ^{32}P-labeled pBR322 DNA restriction fragments with sizes of 4362, 1631, 515, 506, 396, 344, 298, 221, 220, 154, and 75 bases (*61*); (lane d) 30,000 dpm of ^{32}P-labeled bacteriophage T7 early messenger RNA (mRNA) size markers, including four prominent bands ranging from 2740 to 560 bases (*62*); "0," origin of electrophoresis; arrowhead, the bromphenol blue dye marker. (B) Detection of multimeric CEV minus strands by hybridization. Nucleic acids were denatured with glyoxal and dimethyl sulfoxide; they were then fractionated by electrophoresis, transferred to nitrocellulose, and hybridized to ^{125}I-labeled CEV in 2× TESS at 73°C. (Lane a) pBR322 restriction fragments; (lane b) 5 μg of nucleic acids of CEV-infected tomato plants. (C) Molecular size determination of PSTV minus strand RNA. The logs of the molecular sizes of a series of fully denatured RNA and DNA size markers were plotted against the distance migrated during electrophoresis and used to estimate the sizes of PSTV minus strand RNA's fractionated in the same gel. The data represent the average figures obtained from three separate experiments. Marker species included ^{32}P-labeled restriction fragments of pBR322 (*61*) and bacteriophage T7 early mRNA's (*62*). The ^{125}I-labeled PSTV RNA is indicated by an arrow, and the four most prominent PSTV multimeric minus strand components are denoted by arrowheads.

Multimeric Plus Strands

Low levels of PSTV plus strands which migrate more slowly than mature viroid RNA can be detected in Northern blots of nucleic acids of infected plants (Fig. 2A). We have studied these longer than unit length plus strands in several ways and have examined their potential for serving as precursors to mature circular progeny. For example, nucleic acids of infected plants were fractionated in nondenaturing gels and then blot analysis was done on RNA's eluted from gel regions which contained RNA's migrating more slowly than a PSTV marker. Molecular size analysis revealed that monomer, dimer, and trimer length PSTV-specific RNA's were all present (*31*). In addition, samples were analyzed before and after cellulose CF11 chromatography. The fraction containing double-stranded RNA was enriched for plus strands (compare lanes a and b in Fig. 4), suggesting that long plus strands, like their multimeric minus strand counterparts, may be intermediates of viroid replication. Recently, a set of multimeric plus strands of sizes appropriate for two to five repeats of the ASBV monomer have been reported (*19*). Dimer length RNA's of coconut cadang-cadang viroid (CCCV) also accumulate to high levels as well (*32*), while multimeric plus strands of TobRV satellite RNA (*6*) and VTMoV virusoid RNA (*21, 33*) have also been detected. Finally, plus strand RNA's of a size commensurate with dimers and trimers of the peanut stunt virus (PSV) satellite RNA have been identified (*34*).

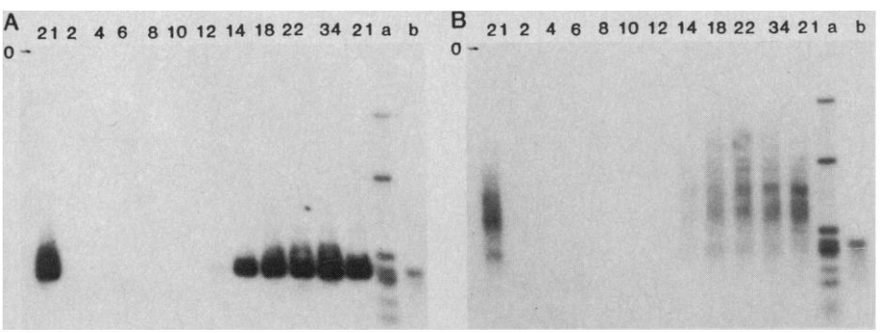

Fig. 2. Analysis of PSTV multimeric plus and minus strands present at various times following infection. Nucleic acids were extracted from PSTV-infected plants at various times after inoculation. Portions of the LiCl supernatant fraction of these extracts were denatured by treatment with glyoxal (*27*), fractionated by electrophoresis in 1.6 percent agarose gels, transferred to nitrocellulose (*26*) and then hybridized to either (A) ^{32}P-labeled PSTV complementary DNA to detect plus strands (*29*) or (B) ^{125}I-labeled PSTV to detect minus strands (*17*). Numerals across the top of the filter indicate the number of days that elapsed between inoculation and extraction of the tomato plants; "0," origin of electrophoresis of the gel. Lane a on the right-hand side of each panel demonstrates pBR322 DNA size markers (see legend to Fig. 1), while lane b indicates the position of ^{125}I-labeled PSTV RNA.

RNA Processing and the Generation of Viroid Progeny RNA Molecules

For multimeric plus strands to play a direct role in viroid replication, cleavage and ligation are required. If cleavage and circularization are not tightly coupled processes in vivo, unit length linear plus strands might accumulate in infected plants. In fact, unit length linear plus strands have been widely detected and studied (*35, 36*). Although their biological role and biochemical composition remain obscure, their infectivity has now been established (*36*). The apparent formal similarity between viroid circularization and RNA splicing suggests that at least some of these molecules might have characteristics of RNA's which are intermediates in the splicing reaction.

In another system, yeast transfer RNA (tRNA) (*37, 38*), the RNA splicing intermediates have highly characteristic cyclic phosphate (2′,3′) termini which are also required by the RNA ligase of wheat germ, as first shown by Konarska, Filipowicz, and Gross (*39*). Through a series of studies with colleagues (*40*), it was found that infected plants contain a population of linear PSTV molecules which are ready to be ligated by the RNA ligase of wheat germ and appear to have the 2′,3′-phosphate moiety charac-

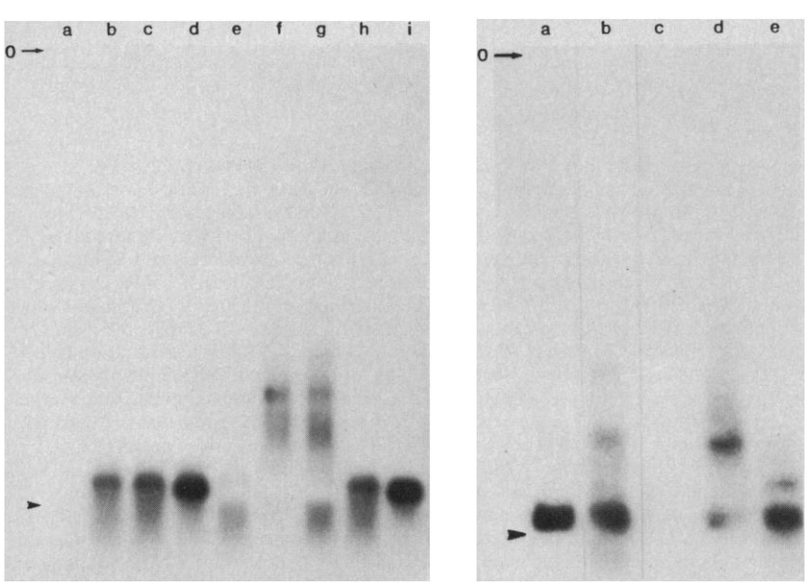

Fig. 3 (left). Large accumulation of plus strands can prevent detection of PSTV minus strands by Northern hybridization. Nucleic acids were denatured by treatment with glyoxal and dimethyl sulfoxide, fractionated by electrophoresis, transferred to nitrocellulose, and hybridized to ^{125}I-labeled PSTV RNA in 2× TESS at 73°C. (Lane a) Nucleic acids of uninfected tomato plants (NA/T); (lane b) NA/T mixed with pancreatic ribonuclease-resistant cores of nucleic acids of PSTV-infected plants; (lane c) ribonuclease-resistant cores; (lane d) ^{125}I-labeled PSTV RNA (size marker); (lane e) NA/T cores and 10 ng of unlabeled PSTV RNA; (lane f) nucleic acids of PSTV infected plants (NA/PSTV); (lane g) NA/PSTV and cores; (lane h) cores alone; (lane i) ^{125}I-labeled PSTV RNA; "0" denotes the origin of electrophoresis of the gel. Fig. 4 (right). Enrichment of multimeric viroid plus strands in nucleic acid fractions from PSTV-infected plants. RNA extracted from viroid-infected plants (*2*) was enriched for double-stranded RNA (dsRNA) by two successive purifications on cellulose CF11 columns (*30*). A sample of the dsRNA was glyoxalated and then fractionated by sucrose density gradient centrifugation (*63*). Three fractions of different *S* value were obtained. Top (0 to 7*S*); middle (7 to 20*S*); and bottom (20 to 40*S*). RNA fractions were again treated with glyoxal, fractionated by electrophoresis in 1.6 percent agarose gels, blotted onto nitrocellulose and hybridized to ^{32}P-labeled PSTV complementary DNA (*17, 29*). Control RNA ran in lane a and dsRNA–enriched RNA in lane b. Bottom, middle, and top sucrose density gradient fractions were run in lanes c, d, and e, respectively; "0" marks the origin of electrophoresis; the arrowhead indicates the position of a bromphenol blue dye marker.

teristic of molecules that have been cleaved from precursors of larger molecular size (*40*). To determine whether the cell-free circularization of the ^{125}I-labeled viroid RNA studied previously followed the mechanism described by Filipowicz and co-workers (*39, 41*), we incubated unlabeled linear PSTV RNA with the wheat germ RNA ligase in a reaction mixture containing γ-^{32}P-labeled adenosine triphosphate (ATP). ^{32}P-Labeled viroid RNA's were produced with the electrophoretic mobilities of circular and linear viroid size markers (Fig. 5B); the products of this reaction can be compared to those of ligations carried out with ^{125}I-labeled PSTV (Fig. 5A). Studies of both Konarska *et al.* (*41*) and Greer *et al.* (*42*) demonstrated that the phosphate incorporated into the newly formed 3′,5′-phosphodiester bond by the wheat germ ligase is provided by ATP, while the phosphate originally present as a 2′,3′ (cyclic) moiety is shifted onto the 2′ position. Thus, when the ligation of tRNA half-molecules was carried out in the presence of [γ-^{32}P]-ATP, label was incorporated exclusively into the junction site. This observation suggests a procedure that might be used to determine the point in the viroid molecule at which ligation takes place in vivo. Therefore, ^{32}P-labeled circular viroid RNA's (Fig. 5B) are being analyzed to determine the terminal sequences present on the linear RNA molecules that act as substrates for the wheat germ ligase. Preliminary data suggest that only one or a few junction sites are recovered with the appropriate properties (data not shown). A similar approach was applied by Kikuchi and co-workers (*43*) to study that subset of PSTV RNA's which could be labeled at their 5′ ends by the action of bacteriophage T4 polynucleotide kinase prior to ligation by the wheat germ enzyme.

The presence of longer than unit length plus strands in double-stranded RNA fractions of cells replicating either TobRV satellite (*6*) or the VTMoV virusoid (*21*) suggests that synthesis of these agents may also involve a precursor which is cleaved to produce the unit length species. In the case of TobRV satellite, further evidence consistent with a cleavage mechanism has been obtained, namely: 5′ hydroxyl (*6*) and 3′ phosphate (*44, 45*) termini on the mature satellite RNA's, rather than the 5′ triphosphate and 3′ hydroxyl termini expected of primary transcripts. If multimeric plus strands are involved in satellite and virusoid RNA replication, an RNA ligation step is also required. In the case of TobRV satellite—where the encapsidated form is primarily linear (*4, 6*)—Bruening and co-workers have presented a model for replication in which ligation to produce a circular form is the first step (*6, 45*). Circularization could also initiate PSV satellite replication. In the case of VTMoV and other virusoids, conversion to circular form occurs on most molecules prior to packaging (*7*).

Multimeric PSTV plus strands would be ideal substrates with which to study the final steps of viroid replication. However, since the processing of PSTV plus strand multimers appears to be highly efficient under our growth conditions, multimeric forms are rare. To obtain fractions with a high proportion of dimer length PSTV plus strands, we chromatographed nucleic acids of infected plants on cellulose CF11, denatured the RNA's eluting in the double-stranded RNA fraction, and separated them by centrifugation in sucrose density gradients (Fig. 4, lanes c to e). In other systems (such as CCCV), plus strand dimers accumulate to high levels and can be purified from stained gel bands (*32*).

The cleavage and ligation reactions needed to process a multimeric precursor into a mature circular RNA could be carried out by enzymes normally concerned with host cell splicing, as suggested by the capacity of the wheat germ RNA ligase to circularize linear PSTV monomers in vitro. Alternatively, a concerted self-splicing mechanism, similar to the self-cleavage and ligation reactions shown by Cech and colleagues to occur during *Tetrahymena* ribosomal RNA processing (*46*), could account for these steps.

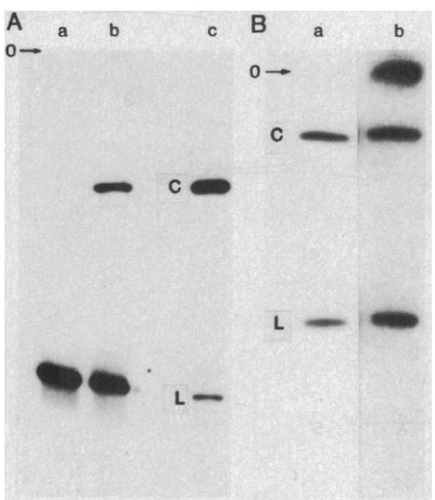

Fig. 5. (A) Viroid circularization by the RNA ligase from wheat germ. ^{125}I-Labeled PSTV natural linear RNA molecules and ^{32}P-labeled tRNA half-molecules (*64*) were incubated without (lane a) and with (lane b) wheat germ RNA ligase and then fractionated by electrophoresis in 5 percent polyacrylamide gels containing 7*M* urea (*40*). (Lane c) The position of ^{125}I-labeled circular (*C*) and linear (*L*) PSTV RNA's; "0" marks the origin of electrophoresis. (B) Polyacrylamide gel analysis of viroid RNA labeled in vitro with [γ-^{32}P]ATP during an RNA ligase reaction with an enzyme activity from wheat germ. In a preparation scale reaction (10 μl), 5.0 ng of highly purified linear PSTV RNA was incubated in the presence of 40 μCi of [γ-^{32}P]ATP and a fraction containing an RNA ligase activity from wheat germ for 20 minutes at 30°C. A portion of this reaction (0.5 μl) was treated with proteinase K and fractionated by electrophoresis (lane b) in a gel containing 7*M* urea, as described previously (*40*). Lane a depicts ^{125}I-labeled circular and linear PSTV size markers; "0" marks the origin of electrophoresis of the gel.

Discussion

The studies reported here and elsewhere establish that PSTV-infected cells accumulate partially double-stranded RNA structures that contain multimeric strands of both polarities. Similar complexes have been detected in plants replicating other viroids as well as a number of viroid-like RNA's. We must now consider how such structures could be generated and evaluate the potential of their various components for serving as intermediates in replication.

Historically, a number of mechanisms have been proposed to describe RNA replication (*47–50*), including one introduced in 1965 by Brown and Martin in which circular RNA is used as a template (Fig. 6A) (*50*). This progenitor of the "rolling circle" model was later applied to replication of circular single-stranded DNA molecules of bacteriophage ϕX174 (*51*). In the Brown and Martin model, a circular RNA is copied to give greater than unit length complementary strands which are then cut down to progeny-size RNA's.

On the basis of the evidence of multimeric PSTV minus strands, we suggested that PSTV replication involves copying of the input circular plus strand by way of a rolling circle mechanism (*17*). Combining this idea with additional data, including that reported in this article, we have developed the hypothetical replication cycle shown in Fig. 6B. It begins with the entrance of an infecting circular plus strand (marked "+") into the cell and the initiation of minus strand synthesis (step 1). The plus strand is copied into a multimeric complementary strand (marked "−"; step 2). The multimeric minus strands, with each viroid repeat delineated by short dividers, then serve as the template for the production of multimeric plus strands (step 3), which must be cleaved to give unit length mole-

cules with characteristic end groups (as in step 4) and circularized (step 5) to yield progeny circles.

At present, we favor this hypothetical scheme because it relies exclusively on viroid-specific nucleic acid species that can be readily detected in PSTV-infected plants and incorporates all of the viroid-related RNA's that have been well-characterized. A similar rolling circle model based on studies of TobRV has been developed (*6*).

There is widespread agreement from studies on PSTV, other viroids, virusoids, and satellites that multimeric plus strands exist and play an important role in replication. Debate continues regarding the pathway of their synthesis and, in particular, regarding the form of their minus strand template. Another way to generate multimeric plus strands and still account for the presence of multimeric minus strands is shown in Fig. 6C. In this scheme, circular plus strands are copied into multimeric minus strands (steps 1 and 2). These give rise to monomeric minus intermediates (steps 2 and 3a; shown in brackets to represent uncertainty in the reaction mechanism), which are then ligated (step 3b) and copied in a second rolling circle step to yield multimeric plus strands (steps 4 and 5). The partially double-stranded RNA structures we have studied, which contain plus and minus strand multimers, could constitute a mixture of the two types of rolling circle structures (Fig. 6C, steps 1 and 4). Choosing between the pathways shown in Fig. 6, B and C, depends on an experimental search for complexes containing circular minus strands and multimeric plus strands. Additional experiments are also needed to explain the observation that both plus and minus strands accumulate preferentially as RNA's with sizes that are multiples of unit length, perhaps due to pauses in synthesis, specific cleavage, or a combination of the two.

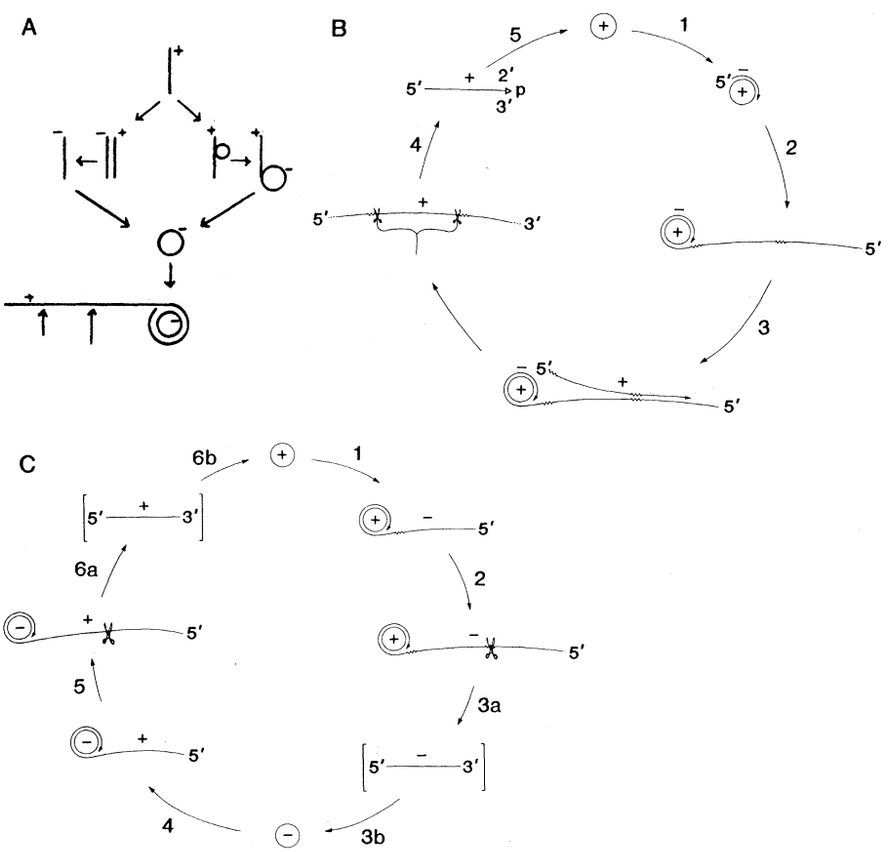

Fig. 6. (A) A replication scheme introducing a circular RNA template. In this scheme, a linear input plus strand (+, at the top) is copied to give a circular minus strand (−). Two alternative pathways are presented for this process. (i) Unit length linear minus strands are synthesized and then converted to circular form (shown on the left); or (ii) minus strands arise as minute circular molecules which then increase in circumference (shown on the right). In whatever way the mature circular minus strand is synthesized, it is copied through multiple rounds of synthesis into a plus strand containing tandem repeats of the input RNA. Specific cleavages (at positions indicated by arrows) produce unit length plus strands [(*50*) courtesy of *Nature (London)*]. (B) A rolling circle model for PSTV replication. This cycle, showing a circular infecting plus strand which is copied into a multimeric minus strand (steps 1 and 2), incorporates the various PSTV-specific nucleic acids identified in infected tomato plants (see text). After being synthesized on its multimeric minus strand template (step 3), the multimeric plus strand is shown as an isolated species indicating that rearrangements may be required to facilitate processing. It is also possible that cleavage and ligation take place while the plus strand remains within the replication complex. A 2′,3′ phosphate moiety (arising after cleavage—step 4) is indicated by an arrowhead. (C) A replication cycle for PSTV involving two rolling circles. This model, a variant of the cycle outlined in (B), illustrates how multimeric minus strands (which arise by copying of circular plus strands; step 1) could be processed (step 2), converted into circular RNA's (step 3), and copied via a rolling circle mechanism to give multimeric plus strands. The linear monomeric − and + strands (see steps 3a and 6a) are shown in brackets to indicate that these may be unstable reaction intermediates.

Certain studies suggest that it may be worthwhile to consider models for viroid replication that do not involve multimeric minus strands. In the case of ASBV, it has been proposed that only unit length minus strands are relevant to replication (*19*). In support of this proposal is the observation that multimeric minus strands were not detected in studies of ASBV. However, since multimeric ASBV plus strands accumulate to high levels in infected tissues, it is possible that multimeric ASBV minus strands were present, but not readily detectable. As noted above (see Fig. 3), for any given size class, whenever one member of a pair of complementary strands is present in large excess over the other, the minority strand is difficult, or impossible, to detect by Northern hybridization analysis. Furthermore, the ASBV minus strands evident in the blots were primarily longer than unit length species and ranged in size up to about 1000 bases (figure 2 in *19*). The additional experimental data implicating multimeric minus strands in viroid replication can be summarized as follows: (i) longer than unit length minus strands have been detected in every system in which they have been carefully sought, (ii) multimeric minus strands can be detected as soon after infection as plus strands can be identified by hybridization analysis (Fig. 2), and (iii) multimeric minus strands are isolated principally as components of complexes in which they are hydrogen-bonded to plus strands—a property characteristic of molecules undergoing replication.

As models of the RNA intermediates of viroid replication become more exact, questions emerge regarding the host components required to make viroid replication a successful process. Since viroids do not appear to code for proteins (*11–14*), it may be that PSTV and other viroids subvert a preexisting host cell pathway for their own ends; virusoid and satellite RNA's may do the same. The components of the pathway could come

either from (i) macromolecules not normally involved in RNA-dependent RNA synthesis which are recruited for this purpose by the pathogen, or (ii) species that are active in the host cell, copying endogenous RNA templates. Reports of enzymatic activities that copy viroid RNA in vitro (*52–55*) have not clearly identified the host cell polymerase that copies viroid RNA in vivo. Furthermore, except for the wheat germ RNA ligase, candidates for the RNA processing enzymes to carry out the cleavage and ligation of viroid RNA have not been detected in host plants. It is likely that synchronized growth of viroid and other RNA pathogens in cultured cells will be necessary before all the steps of their replication pathway can be worked out and then duplicated in carefully designed cell-free systems.

So far, only plant cells have been shown to be capable of copying viroid-like RNA molecules; however, other organisms may also have the necessary machinery. Thus, PSTV RNA has been introduced into cultured *Drosophila* cells by means of liposome fusion (*56*). The PSTV is stable after introduction, and preliminary results (not shown) indicate that the viroid may stimulate synthesis of RNA's related to those in the replication cycle described in this article.

If cells have the inherent capacity to copy certain RNA molecules faithfully, it is interesting to consider the possible host cell function of the enzymatic machinery which replicates viroids. Whether there is a normal endogenous RNA template, and if so, what its role in gene regulation might be—and whether viroid symptoms result from interference with the function of such a host RNA—remain to be determined. Circular RNA's have been detected in a number of eukaryotic cells (*57–59*), and several cases would seem to lend themselves well to a search for complementary strand synthesis. A campaign to reveal endogenous replicating RNA circles would benefit from an increasing knowledge of the intermediates in the replication of viroids and other infectious circular RNA molecules.

Conclusions

We think the following statements will help to summarize present knowledge concerning the role of viroids and viroid-like RNA molecules.

1) PSTV and other viroids replicate by direct RNA to RNA copying.

2) Analysis of complexes containing plus and minus strands has revealed many features of the viroid replication cycle.

3) Both multimeric plus and minus strands arise during viroid infection, suggesting the use of a rolling circle mechanism for the synthesis of at least one (and possibly both) strands.

4) RNA processing—in fact, specific cleavage and ligation reminiscent of RNA splicing—is required to generate progeny monomeric circular RNA molecules from the multimeric plus strands present in viroid replication complexes.

5) If PSTV and other viroids encode no polypeptides, all components required for efficient viroid replication by the pathway described here must preexist in host cells.

6) Two other classes of small pathogenic RNA—the virusoids and certain viral satellites—form structures in infected cells which suggest that their replication cycles resemble that of PSTV and other viroids in most respects.

7) Although no host cell RNA species have yet been found which could fill the role of endogenous template for the set of host components required for viroid replication, it seems highly probable that the normal function of this host pathway—and its disruption by the proliferation of viroid or viroid-like RNA—could provide the key for explaining the diseases caused by these agents.

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