Small satellite of arabis mosaic virus: autolytic processing of in vitro transcripts of (+) and (−) polarity and infectivity of (+) strand transcripts

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In vitro RNA transcripts of both (+) and (−) polarities were obtained from a circularly permuted dimer clone of the small satellite of arabis mosaic virus (sArMV). The transcripts show efficient self-cleavage at the two specific sites in the sequence where the monomers were joined. Autolytic processing of the full-length transcript in both orientations releases promoter-proximate fragments (+) or (−) P, promoter-distal fragments (+) or (−) D, and the monomer fragments (+) or (−) M. The presence of an OH group at their 5′ ends and a 2′,3′ cyclophosphate at their 3′ ends suggests that (+) and (−) M originated via two self-cleavage reactions within the full-length transcript of corresponding polarity. Infectivity assays showed that the (+) M fragment but not the (−) M fragment initiates replication as efficiently as the natural linear sArMV in Chenopodium quinoa. Two (−) fragments were identified which are the result of religation activity: a P-D fragment formed by religation of P and D, and c-M, which is the result of efficient self-ligation of (−) M. In contrast, linear (+) M self-ligates in vitro to a very limited extent but could be circularized enzymically in a wheat germ extract.

Introduction

Satellite RNAs of plant viruses can replicate in host cells only in the presence of a helper virus. Many of the presently known satellite RNAs associated with nepoviruses are of two different types: large, B-type satellites about 1.4 kb and small, D-type satellites 0.3–0.46 kb in size (Roossinck et al., 1992; Fritsch & Mayo, 1989). Arabis mosaic virus (ArMV) and chicory yellow mottle virus (CYMV) are the only known nepoviruses which can support both the large and the small type of satellite. CYMV supports both types of satellites simultaneously (Piazolla et al., 1989; Rubino et al., 1990), whereas the two types of satellite of ArMV are supported by different strains (Liu et al., 1990; Davies & Clark, 1983; Kaper et al., 1988). Another nepovirus supporting a small satellite is tobacco ringspot virus (TRSV) (Schneider, 1969). This satellite (sTRSV) was the first shown to exist in both a linear and a circular form (Linthorst & Kaper, 1984). Circular forms have also been found with the small satellites of ArMV (Kaper et al., 1988), CYMV (Rubino et al., 1990), the sobemoviral satellites (Jones et al., 1983; Gould & Hatta, 1981; Haseloff & Symons, 1982; Francki et al., 1983) and the satellite associated with the RPV strain of barley yellow dwarf virus (BYDV) (Miller et al., 1991). Many other small RNAs possessing linear and circular forms that are either of satellite-like or viroid-like nature may exhibit in vitro self-cleavage at specific sites (reviewed in Symons, 1992).

Arabis mosaic virus is widely found in different fruit and crop plants in Europe. The nettlehead disease of hop is one of the oldest known virus-related plant diseases and has been correlated with the presence of ArMV and its small satellite (Davies & Clark, 1983). In the indicator plant Chenopodium quinoa, the virus alone is asymptomatic but when co-inoculated with sArMV younger systemic leaves show yellow chlorotic spots. Like most other viral satellites the replication of sArMV significantly reduces the titre of ArMV RNA in the plant (unpublished data), suggesting competition of the two RNAs for the viral replicase. Although most larger nepovirus satellites do not influence viral symptoms, the large satellite of ArMV has been shown to exacerbate as well as attenuate disease in some host plants (Fritsch et al., 1993).

In this publication we report the construction of a circularly permuted dimeric cDNA clone of sArMV and show that in vitro RNA transcripts of (+) as well as (−)
polarity undergo efficient self-cleavage during transcription. We infer the chemical configuration of the 3' and 5' ends of the self-cleavage fragments with enzymic tests, and determine the exact position of the (−) strand self-cleavage site via sequence analysis. We demonstrate non-enzymic and enzymic mechanisms of circularization of linear (+) and (−) monomers. Furthermore we show that gel-eluted (+) transcripts are replicated in *Chenopodium quinoa* plants when co-inoculated with helper virus, whereas (−) transcripts are not.

**Methods**

*Virus propagation, purification and nucleic acid extraction.* The hop strain of ArMV (with and without satellite) was originally isolated from hop in Kent and Hereford, UK, and propagated in *Chenopodium quinoa* (Davies & Clark, 1983). Several subsequent passages in this laboratory ensured that one virus isolate remained satellite-free in this host. *C. quinoa* plants with four to six pairs of true leaves were dusted with carborundum and inoculated with a 50 μg/ml purified virion preparation or 20 μg/ml purified viral RNA in 50 mM-sodium phosphate buffer, pH 6.7. For infectivity studies of sArMV 2.5–5 μg/ml gel-eluted satellite RNA or RNA transcripts or 10 μg/ml total transcription mixture was added to the viral inoculum. The plants were placed in a growth chamber at 16–17 °C and a light intensity of 4000–6000 lux with an 18 h photoperiod. Tissue samples from systemically infected leaves were taken between 1 and 6 weeks post inoculation (p.i.) for further analysis.

Plants were harvested 4–5 weeks p.i. and virus was isolated according to the procedure of Davies & Clark (1983). Virions were purified by sucrose-gradient density centrifugation, fractionated on an IsCo fractionator, and concentrated by ultra centrifugation at 30,000 r.p.m. overnight and stored at a concentration of 0.5–2 mg/ml in 50 mM-sodium phosphate buffer, pH 6.7 at 4 °C. RNA was extracted from virions by SDS-phenol extraction (Kaper & West, 1972) followed by one ethanol precipitation and washing the precipitate with 70% ethanol.

*Isolation and characterization of nucleic acids from plants.* Total nucleic acid (TNA) was extracted from tissue as previously described (White & Kaper, 1989). TNA extracts or RNA extracted from virions were analysed by electrophoresis on a 6% polyacrylamide gel (PAGE) containing 7 M-urea and 1× TBE (89 mM-Tris, 89 mM-boric acid, 2.5 mM-EDTA). The same type of gel was used for fractionation and dephosphorylation pBSIIISK (+) DNA under conditions yielding preferentially multimeric inserts. Competent E. coli cells (INV5x F ’One Shot’; Invitrogen) were transformed with the ligation mixture, recombinant clones selected by blue-white screening, and plasmid preparations (He et al., 1990) analysed by restriction digestion. One clone, pBS7600, with the dimer cDNA in an orientation with the 5’ end of the (+) strand proximate to the T7 promoter of the plasmid, was grown in 50 ml LB culture and purified using a plasmid preparation kit (Qiagen) following the manufacturer’s instructions.

**Characterization of in vitro RNA transcripts**

Transcription of *BamHI* or *HindIII*-linearized pBS7600 to obtain (+) and (−) transcripts was performed according to Titus (1991) for 2 h at 37 °C with T7 or T3 RNA polymerase, respectively. After phenol-chloroform extraction and ethanol precipitation the transcripts were used for further analysis.

(i) 5’ End-labelling of RNA. To identify 5’ OH termini 1 μg transcription mixture was incubated for 30 min at 37 °C with 5 μl u polynucleotide kinase (US Biochemicals) in the presence of 50 μCi [γ-32P]ATP (Amersham, 300 Ci/mmol). To identify the 5’ pppG termini nonradioactive GTP in the transcription reaction mixture was partially replaced by 50 μCi [γ-32P]GTP.

(ii) 3’ End-labelling of RNA. To identify 3’ OH termini 4 μg transcription mixture was incubated for 16–24 h at 0 °C with T4 RNA ligase (New England Biolabs) in the presence of 50 μCi [γ-32P]PnP Cp (Amersham, 300 Ci/mmol) in a buffer consisting of 25 mM-HEPES, pH 7.5, 1.65 mM-dithiothreitol, 7.5 mM-MgCl₂, 1 mM-ATP, 5 mg/ml bovine serum albumin and 10% dimethyl sulphoxide. For general labelling of transcripts the UTP concentration in the transcription reaction was reduced to 20 μM and 50 μCi [α-32P]UTP was added.

**Sequence analysis.** The sArMV cDNA was sequenced by the dyeodeoxy chain termination procedure (Sanger et al., 1977) using end-labelled M13 forward and reverse primers. For the direct sequencing of the 5’ terminal portion of sArMV transcripts, they were 5’ end-labelled using [γ-32P]ATP, subjected to partial nuclease digestion, and the fragments

GTCGAGTACCGCCCAGA-
mononucleotides were separated by TLC in saturated ammonium sulphate 1 M-sodium acetate-propan-1-ol (80:18:2, by vol.) and phosphate buffer (Maniatis & Efstratiadis, 1980). The gel was digested to completeness with RNase T2, followed by nuclease P1. The (+) sArMV 5' end proximate to the T7 promoter was isolated and amplified. Nucleotide positions of the cDNA are numbered according to the (+) sArMV sequence. The locations of the junctions +J and −J, where the (+) and (−) transcripts of this cDNA clone self-cleave, are shown.

Comparison of its nucleotide sequence with that of the natural satellite (Kaper et al., 1988) showed an exchange of C for G at position 235. This difference persisted in all cDNA clones sequenced subsequently, whereas in a repeat of the dideoxy RNA sequence determination in natural sArMV at this position both a C and a G could be detected. This therefore raised the possibility that the cloned sequence represented one of two natural sArMV variants. However, as is shown later, the C to G change at position 235 did not affect the ability of sArMV clone transcripts to be replicated in Chenopodium quinoa.

Configuration of the 3' and 5' ends of self-cleavage and self-ligation products of (+) and (−) transcripts of pBS7600

Fig. 2 shows the 6% PAGE analysis of reaction mixtures resulting from in vitro transcription of BamHI-linearized pBS7600 with T7 polymerase, yielding transcripts of (+) polarity (lane 1), and HindIII-linearized pBS7600 with T3 polymerase, yielding transcripts of (−) polarity (lane 7). To facilitate identification of the gel-electrophoretic bands, the expected (+) and (−) transcription products, as well as the fragments resulting from their anticipated self-cleavage (and in some cases religation), are schematically depicted in Fig. 3, using designations previously adopted by Buzayan et al. (1986c) for analogous fragments observed after in vitro transcription of sTRSV permuted dimeric cDNA.

To confirm the designations given to the different-sized products resulting from pBS7600 transcription in the presence of [z-³²P]UTP (Fig. 2, lanes 1 and 7), and

Results

Cloning and nucleotide sequence of the cDNA of sArMV

A cDNA clone containing the complete sArMV sequence (Yang et al., 1993) was used for a PCR-based amplification of a permuted sArMV monomer cDNA (Fig. 1).
subsequent processing, their 3′ and 5′ ends were determined in a set of separate experiments. These involved end-labelling reactions performed with the transcription mixture designed to specifically identify the products’ termini, as can be deduced from Fig. 3 which shows schematically the products of self-cleavage and possible religation of the two junctions (J) in full-length (+) or (−) transcripts.

3′ Termini. Ligation of [5′-32P]pCp with the 3′-OH termini in the T7 transcription mixture containing (+) RNA strands generated labelled bands at positions P-M-D, M-D and D (Fig. 2, lane 2). With the T3 transcription mixture containing (−) RNA strands the [5′-32P]pCp reaction also resulted in labelling of bands at positions P-M-D, M-D and D (Fig. 2, lane 8), although the M-D and D positions were obviously different from similarly designated positions in the T7 mixture due to the different sizes of (+) and (−) stranded self-cleavage products (Fig. 3). The T3 mixture also contained another labelled band which might represent the heterologous religation of the 2′,3′ cyclophosphate terminus of P and the 5′-OH terminus of D (P-D; Fig. 2, lane 7). No attempt was made to identify the products with 2′,3′ cyclophosphate termini since these cannot be labelled directly (Buzayan et al., 1986b).

5′ Termini. The products with 5′-pppG ends in the transcription mixtures prepared with [γ-32P]GTP were identified by way of the labelled bands corresponding to positions P-M-D, P-M and P for both the T7 (Fig. 2, lane 3) and T3 mixtures (Fig. 2, lane 9); P-D, the product of heterologous religation could also be identified in the latter mixture. The products with 5′-OH termini in both the T7 and T3 mixtures showed as labelled bands the respective M-D, M and D positions after treatment with polynucleotide kinase and [α-32P]ATP (Fig. 2, lanes 4 and 10); however, in the T3 mixture additional bands were seen, the identities of which are unknown.

In Fig. 2, lane 1, the presence of bands corresponding in size to full-length transcript (+) P-M-D, and its partial self-cleavage (+) products P-M and M-D, in addition to the final products P, M and D (Fig. 3), suggests that self-cleavage does not occur in every transcript molecule and is not always complete. However, longer incubation of the transcription mixture, or reincubation of the products (after ethanol precipitation) in the presence of 10 mM-Mg2+ and 2 mM-spermidine did
not result in significant additional self-cleavage (data not shown).

**Self-ligated (−) sArMV monomeric transcription product**

General labelling of the T7 transcription mixture yielded six major products (Fig. 2, lane 1), representing the full-length transcript and the products of its self-cleavage (Fig. 3). Each product could furthermore be identified in the analysis of the 5' termini (Fig. 2, lanes 3 and 4). For the T3 transcription mixture eight major products were found upon general labelling (Fig. 2, lane 7), six of which represented the full-length transcript and its self-cleavage products (Fig. 3b). These six products were identified in the analysis of the 5' termini, as well as the seventh major band P-D (Fig. 2, lanes 8 and 9). However, the eighth labelled band in the mixture (Fig. 2, lane 7) did not show up in the analysis of either the 3' or 5' termini. This band co-migrated with the natural circular monomer of sArMV (Fig. 2, lane 6). After gel-elution and re-electrophoresis of this band an additional band appeared which co-migrated with linear sArMV (data not shown). Therefore this band was concluded to represent the circular monomer of sArMV (designated c-M), presumably generated from self-ligation of the linear monomer M (Figs 3 and 5a, lane 2). Religation between (−) self-cleavage products P and D, and self-ligation of (−) M has also been reported for (−) sTRSV in vitro transcription mixtures (Buzayan et al., 1986a, b).

**Sequence analysis of the 5' ends of (+) and (−) transcripts of pBS7600**

The direct RNA sequencing of about 60 nucleotides of the 5' termini of (+) M and D and (−) M and D fragments yielded a sequence identical to that determined from the natural satellite RNA (Kaper et al., 1988).
Fig. 4. Determination of the 5'-terminal sequence of (-) and (+) monomeric transcripts of sArMV. (a) Autoradiograph of enzymic sequencing reactions of 5'-end-labelled (-) M and (+) M separated on a 20% acrylamide-98% formamide gel. Large open regions represent bases which are resistant to nucleases. Bases are numbered according to the sArMV sequence. Bases which could not be identified are labelled according to the known sequence or derived from the sequence of the complementary transcript (in italics). Regions protected from nuclease digestion are marked off by solid lines (stem-loop, cf. Figs 7 and 8) or a bracket. Most cytidine residues are not identified because of overdigestion by the enzyme from B. cereus. L, Alkali ladder. (b) Autoradiograph of a cellulose thin-layer chromatogram of RNase T1- and nuclease P2-treated transcription fragments (-) and (+) M and (-) and (+) D. The position of the 5'-phosphorylated mononucleotides is labelled. 5'-pG and 5'-pU are not clearly separated under the chosen conditions.

and/or the cDNA. In Fig. 4(a) the results for (+) M and (-) M are shown. Nucleotides not identified in this gel were added according to the known sequence and from the sequence of the complementary transcript (in italics). Several regions in (+) and (-) M are obviously resistant to nuclease digestion: nucleotides 13–16 of (+) M (bracketed region) and the regions 26–31 and 36–41 of (+) M as well as regions 40–36 and 31–27 of (-) M (solid lines). These regions are obviously involved in strong basepairing in transcripts of both orientations. Region 13–16 of (+) M basepairs strongly with nucleotides 173–176 (see Fig. 7a); the region 27–40 in (+) and (-) M forms a stable tetraloop (see Figs 7 and 8) each with five GC basepairs and an additional U26G41.
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Fig. 5. Non-enzymic and enzymic ligation of monomeric sArMV transcripts. (a) Autoradiography of linear (+) and (−) M fragments gel-eluted from [γ-32P]UTP-labelled total T7 and T3 transcription mixtures. Denaturing 6% PAGE. Lane 1, linear (+) M fragment; lane 2, linear (−) M fragment. (b) Circularization of (+) M in wheat germ extract. Autoradiography of gel-eluted, 32P-labelled (+) M incubated for different times in wheat germ extract and analysed by denaturing 6% PAGE. Lane 1, gel-eluted (+) M, untreated; lanes 2–8, incubation of (+) M in wheat germ extract for t = 0, 5, 10, 15, 20, 25 and 30 min; lane 9, incubation of (+) M in heat-treated (2 min, 100 °C) wheat germ extract for 30 min. The positions of linear (l) and circular (c) sArMV are indicated.

Fig. 6. Infectivity of in vitro transcripts of pBS7600 in Chenopodium quinoa plants. Autoradiography of a Northern blot of total nucleic acid extracted 22 days p.i. from plants inoculated with helper virus and the following RNA: lane 1, total (−) transcription mixture; lane 2, total (+) transcription mixture; lane 3, gel-eluted (+) monomer; lane 4, gel-eluted full-length (+) transcript P-M-D; lane 5, linear sArMV from total nucleic acid. The positions of linear (l) and circular (c) monomeric and linear dimeric (d) sArMV are indicated.

Comparison of self-ligation of (−) M and (+) M

Examination of the in vitro self-ligation into circles of the linear monomers (−) M and (+) M eluted from gels with the transcription mixtures, and subsequently re-electrophoresing these RNAs showed more than 50% self-ligation propensity on the part of the (−) strands, but no self-ligation for the (+) strands (Fig. 5a) or only a very small amount (see Fig. 5b, lane 1). Almost identical results have previously been reported for (−) sTRSV transcripts where self-ligation appeared to be the
exact reversal of the self-cleavage reaction and the linear and circular forms are in equilibrium (Buzayan et al., 1986c). Self-ligation of (+) sTRSV was observed in a yield of about 1%, and more recently up to 5% when the RNA was stored under ethanol at −20 °C (Passmore & Bruening, 1993).

**In vitro circularization of (+) M in wheat germ extract**

To investigate the possibility that (+) M circularizes enzymically, gel-eluted linear (+) M was incubated in wheat germ extract for up to 30 min whereby it yielded increasing amounts of circular (+) M (Fig. 5b). The
Fig. 8. Secondary structures of (-) sArMV. (a) Optimal structure. (b) Suboptimal structure with the hairpin ribozyme structure (shaded area). Nucleotides 54 to 43 represent the substrate RNA, nucleotides 156 to 107 are the catalytic domain. The calculation was done with nucleotide 50 as the 5' end and nucleotide 51 as the 3' end of the (-) sArMV sequence.

Infectivity of (+) and (-) transcripts

RNA transcripts derived from clone pBS7600 were tested for infectivity in C. quinoa plants in the presence of
ArMV. Fig. 6 shows the results of Northern hybridization after 6% PAGE of TNA extracts isolated from systemically infected leaves 22 days p.i. Inoculation with total (+) transcription mixture resulted in successful replication of sArMV in systemically infected leaves (lane 2), whereas no accumulation of sArMV was observed when total (−) transcription mixture was used (lane 1). Inoculation of plants with gel-eluted full-length transcript (+) P-M-D also failed to initiate detectable replication (lane 4). Presumably, the concentration of (+) M, released from P-M-D by autolytic processing was too low to start replication. However, inoculation with the gel-eluted unit-length (+) M fragment resulted in accumulation in C. quinoa plants (lane 3) that was equally extensive as when natural linear sArMV was used (lane 5).

Discussion

The small satellite of ArMV has significant sequence homology with sTRSV and the small satellite RNA of CYMV. Among these satellites two sequence domains are highly conserved. One conserved sequence can form a hammerhead structure (Fig. 7b) in the (+) strand sequence (Kaper et al., 1988); the other forms a so-called hairpin or paperclip ribozyme structure (Fig. 8b) in the (−) strand (Rubino et al., 1990). Both structures apparently catalyse the self-cleavage of the corresponding RNA at a specific site within the conserved regions. These cleavage sites ...C_{300}-G_{1}... for (+) sArMV and ...A_{31}-G_{59}... for (−) sArMV have been confirmed by direct sequencing of the corresponding linear monomers. The full-length transcripts of both polarities showed extensive self-cleavage, essentially analogous to what has been reported for permuted dimer sTRSV transcripts (Gerlach et al., 1986). In addition, extensive self-ligation previously observed for (−) sTRSV transcripts (Buzayan et al., 1986c) was also noted for (−) sArMV transcripts. The combined evidence in this paper therefore suggests that the entire spectrum of autocyclic processing events known for sTRSV also takes place with sArMV, as was previously predicted on the basis of sequence homologies in the respective ribozyme domains of both the (+) and (−) strands of these two satellites (Kaper et al., 1988; Rubino et al., 1990). However, the self-cleavage occurring during transcription clearly is incomplete. This is evident from the presence of partial self-cleavage products as well as full-length transcripts in the transcription mixtures (Fig. 2). This could occur if a particular conformation of the transcript is needed for self-cleavage of the junctions, and not all transcript molecules assume this conformation. In addition, during the (−) transcription reaction very efficient religation of P, M and D must also have occurred, which is evident from the formation of new products such as P-D and c-M (Fig. 2).

The much greater tendency towards self-ligation of (−) versus (+) stranded monomers (Fig. 5a) may be explained in part when their computed secondary structures are considered (Figs 7 and 8). For (+) sArMV the most stable minimum free energy structure $S_{\text{opt}}$ (−105.8 kcal/mol) (Fig. 7a) is quite different from a suboptimal structure $S_{\text{subopt}}$ (−95.5 kcal/mol) (Fig. 7b) that contains the hammerhead. For (−) sArMV, on the other hand, there is little difference between the most stable secondary structure $S_{\text{opt}}$ (−99.9 kcal/mol) (Fig. 8a) and the suboptimal structure $S_{\text{subopt}}$ (−98.9 kcal/mol) containing the hairpin ribozyme motif (Fig. 8b). The likelihood of the $S_{\text{subopt}}$ structure actually existing in solution became evident during direct sequencing of the 5′ termini of (−) M and (−) D, which showed the presence of a double-stranded region (5 GC basepairs) resistant to nuclease cleavage at 55°C (Fig. 4a). These basepairs are part of a stem-loop (Fig. 8b, open arrow) which is not predicted by the FOLD program in the most stable structure $S_{\text{opt}}$ (Fig. 8a). Interestingly, if this stem-loop, which is localized outside the hairpin consensus sequence, is forced, the FOLD program automatically generates as the energetically most favourable the catalytically active structure $S_{\text{catal}}$ with the hairpin motif (Fig. 8b). This stem-loop also emerges in the (+) strand optimal structure, as well as in the hammerhead catalytic domain (hairpin II) (Fig. 7a, b) and was again identified during enzymic sequencing of the 5′ terminus of (−) M and (−) D by its nuclease resistance (Fig. 4a).

The (+) strand of sTRSV did not show a similar stem-loop in the most stable structure when the algorithm of Zuker & Stiegler (1981) was used (FOLD program of the GCG package). However, with the RNA folding program LINALL (Schmitz & Steger, 1992), and upon inclusion of thermodynamic parameters of unusually stable RNA hairpin tetraloops (Groeb & Uhlenbeck, 1988; Antao et al., 1991; Antao & Tinoco, 1991) a similar stem-loop was also found for the most stable structure of (+) sTRSV (data not shown). This stem-loop represents hairpin II of the hammerhead structure of (+) sTRSV (Kaper et al., 1988). In the most stable structure of the (+) strand of the D-type satellite of CYMV a stem-loop, identical to hairpin II of the hammerhead structure, could not be found with either algorithm (data not shown).

Interestingly, the optimal (+) strand secondary structure and the (−) strand ribozyme structure of sArMV are very similar in appearance (Figs 7a and 8b), which might be of importance for their recognition and replication by the viral replicase. Also for sTRSV the most stable (+) and the catalytically active (−) strand secondary structures are very similar (using the FOLD
program of the GCG package). In contrast to sArMV, however, the most stable structure of (−) sTRSV represents already the hairpin riboyme structure (data not shown).

The question of (+) circle formation for the nepovirus satellites sArMV and sTRSV (and also sCYMV) is central to an understanding of the mechanism of their replication. Assuming the fundamental correctness of a rolling circle replication mechanism, as proposed for sTRSV (Bruening et al., 1988), the first step must involve circularization of monomeric linear (+) strands, since these were shown to be the principal infectious entities (Fig. 6). Upon inoculation, circularization could occur via self-ligation (see Fig. 5b, lane 1), which would restore a conventional 3′−5′ phosphodiester bond. The main question is whether such self-ligation occurs to a degree sufficient to initiate the replicative process. Alternatively, circularization would have to occur enzymically. (+) sArMV with its 5′-OH and 2′,3′ cyclophosphate termini is an appropriate substrate for an enzyme such as the wheat germ ligase (Konarska et al., 1981). In Fig. 5(b) we show that (+) M will indeed circularize in a wheat germ extract. Prior heat treatment of the wheat germ extract completely abolishes this reaction, confirming that this circularization is an enzyme-dependent reaction. However, the junction generated would possess a 2′ phosphodiester bond in addition to the 3′−5′ phosphodiester bond. Such a junction has actually been found to exist in the encapsidated form of a small circular sobemovirus satellite and was reported to exhibit some unusual biochemical properties including resistance to alkali and most ribonucleases (Kiberstis et al., 1985). This type of phosphodiester linkage, if present in circular sArMV, might explain the blockage of reverse transcriptase we have repeatedly observed (unpublished results). It would be interesting to determine whether such a linkage could actually be part of a functional circular template for in vivo (−) strand transcription in nepovirus satellite replication.

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References


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