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Plant pathogenic RNAs and RNA catalysis

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ABSTRACT

The rolling circle replication of small circular plant pathogenic RNAs requires a processing step to convert multimeric intermediates to monomers which are then circularized. Eleven such RNAs are known so far, two are viroids, one is viroid-like and the remainder are satellite RNAs dependent on a helper virus for replication. The processing step is RNA-catalysed in all cases, at least *in vitro*. All plus forms of these RNAs self-cleave via the hammerhead structure whereas only eight of the minus RNAs self-cleave, five via the hammerhead structure and three via the hairpin structure. There are about 20 other viroids where the processing mechanim has yet to be determined but they are likely candidates for a new type of self-cleavage reaction which is predicted to be conserved in all these viroids. Hepatitis delta RNA is the only circular pathogenic RNA known to self-cleave in the animal kingdom. It is feasible that more single-stranded circular pathogenic RNAs are waiting to be discovered and these could be prospective for new types of self-cleavage reactions.

Small single-stranded circular plant pathogenic RNAs have been a rich source of self-cleaving RNAs and there is considerable promise of more to be found. All such self-cleaving RNAs identified so far range in size from 246 to >400 nucleotides (nt) and are replicated by a host RNA polymerase via a rolling circle mechanism (1). Two of these are viroids which are able to replicate in the plant independently of a helper virus. The remainder are satellite RNAs, so called because they require specific input from a helper RNA virus for their replication.

The aim of this article is to place as much emphasis on where the future lies as on a concise review of a number of aspects of RNA catalysis in small plant pathogenic RNAs. I refer the reader to review type articles which cover both broad and specific aspects relevant to RNA catalysis as considered here (2–11).

Self-cleavage and rolling circle replication

Self-cleavage is a term used for the reaction whereby single-stranded RNAs are cleaved at a specific site in the absence of a cofactor apart from a divalent cation. In the reactions identified so far, cleavage occurs by nucleophilic attack by the 2′-hydroxyl at the cleavage site on the internucleotide phosphate such that the cleavage fragments contain a 2′, 3′-cyclic phosphate and a 5′-hydroxyl (Fig. 1). The reaction is therefore distinguished from the self-splicing of Group I introns which requires a guanosine cofactor which is covalently incorporated at the 5′-end of the intron (Fig. 2A). The 3′-hydroxyl of the 5′-exon then attacks the phosphate at the 5′-end of the 3′-exon to give the spliced exons and the release of the excised intron. In the case of the self-splicing of Group II introns, the initial step is the attack by the 2′-hydroxyl of an A residue internal to the intron on the phosphate at the exon–intron boundary (Fig. 2B) followed by a similar second step to that of Group I splicing.

All evidence indicates that the circular pathogenic RNAs are replicated by a rolling circle mechanism (1,8) *in vivo* (Fig. 3). The replication cycle involves the copying of the dominant circular plus (+) strand by a host or viral-coded RNA polymerase to give a longer than unit length minus (–) strand. For most of the self-cleaving circular RNA pathogens so far identified, this long (–) strand can self-cleave *in vitro* to give monomeric products (Fig. 3A). *In vivo*, these monomers are circularised and then copied to produce a linear (+) strand which self-cleaves to monomers which circularize to produce the dominant circular (+) progeny found *in vivo*. For those multimeric (–) RNAs which are not processed (Fig. 3B), the RNA is copied to give a multimeric (+) strand which then undergoes cleavage to monomers which are then circularised.

Circular, self-cleaving plant pathogenic RNAs

The *in vitro* self-cleaving plant pathogenic RNAs identified so far all fall into three groups (Table 1); those where both the (+) and (–) RNAs self cleave via the hammerhead structure (Fig. 4A), those where only the (+) RNA is cleaved via the hammerhead structure, and the remainder where the (+) RNA is cleaved by the hammerhead structure and the (–) RNA is cleaved via the hairpin structure (Fig. 4B).

Of the 21 viroids identified so far (Fig. 5), only two have shown specific self-cleavage *in vitro*. Avocado sunblotch viroid (ASBV), a nuisance pathogen in the avocado industry, was the first viroid to show hammerhead self-cleavage in 1986 (12). This viroid at 246–251 nt, together with coconut cadang cadang viroid (CCCV) at 246 nt, are the two smallest viroids isolated so far; however the processing mechanism during replication of CCCV has yet to be determined. A most interesting difference between these two viroids as well as the 368–463 nt citrus exocortis viroid (CEV) and the 341–361 nt potato spindle tuber viroid (PSTV) is their intracellular localisation; ASBV is found on the chloroplast thylakoid membranes while CCCV, CEV and PSTV are localised in the nucleus of host plants (13–15). The only other viroid isolated so far which has been shown to self-cleave is the 337–338 nt peach latent mosaic viroid (PLMV) and, like ASBV, both (+) and (–) RNAs self-cleave via the hammerhead structure (16; Fig. 4A).

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Table 1. Plant pathogenic RNAs that self-cleave *in vitro*

	Size (nt)	RNA self-cleavage structure	
		(+) RNA	(–) RNA
Viroids			
Avocado sunblotch viroid (ASBV)	246–251	hammerhead	hammerhead
Peach latent mosaic vioid (PLMV)	337–338	hammerhead	hammerhead
Viroid-like RNA			
Carnation small viroid-like RNA (CarSV RNA)	275	hammerhead	hammerhead
Satellite RNAs			
Sobemoviruses:			
Lucerne transient streak virusoid (vLTSV)	322–324	hammerhead	hammerhead
Solanum nodiflorum mottle virusoid (vSNMV)	377	hammerhead	—
Subterraneum clover mottle virusoid (vSCMoV)	322 and 328	hammerhead	—
Velvet tobacco mottle virusoid (vVTMoV)	365–366	hammerhead	—
Nepoviruses:			
Arabis mosaic virus satellite RNA (sARMV)	300	hammerhead	hairpin
Chicory yellow mottle virus satellite RNA (sCYMV)	457	hammerhead	hairpin
Tobacco ringspot virus satellite RNA (sTRSV)	359–360	hammerhead	hairpin
Luteovirus:			
Barley yellow dwarf virus satellite RNA (sBYDV)	322	hammerhead	hammerhead

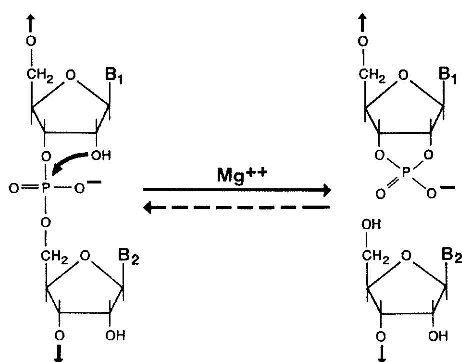

Figure 1. The self-cleavage reaction. The reaction is a non-hydrolytic, phosphoryl transfer reaction and is reversible in the hairpin ribozyme but not in the hammerhead ribozyme.

Carnation small viroid-like RNA (CarSV RNA) is something of an enigma. Its 275 nt sequence was reported in 1992 together with the demonstration that both (+) and (–) strands self-cleave via the hammerhead structure (17). However, purified RNA inoculated on carnations is not infectious (18), in contrast to ASBV which is infectious when appropriately inoculated on healthy avocado seedlings (19). The plot thickened when CarSV RNA sequences were isolated from infected carnation as DNA tandem repeats (18), which can perhaps be compared with a tandemly repeated 330 bp satellite II DNA of the newt, the transcript of which self-cleaves via the hammerhead structure (20). A further complication is that CarSV DNA can be found directly fused to DNA sequences of carnation etched ring caulimovirus, a pararetrovirus (18). Obviously, this is a complex system yet to be fully resolved.

Most of the self-cleaving RNAs in Table 1 fall under the heading of satellite RNAs, i.e., RNAs which are completely dependent on a helper virus for replication. The helper viruses involved come from three different families. The Sobemoviruses (southern bean mosaic virus family) specifically encapsidate the four circular viroid-like satellite RNAs or virusoids. Members of the other two virus families encapsidate the linear form of the satellite RNA but the circular forms which are essential for rolling circle replication can be isolated from total plant RNA.

The hammerhead ribozyme structure is becoming well characterized

The small size of the hammerhead structure (Fig. 4A), where fewer than 40–50 nt can form an active self-cleaving molecule, has led to an explosion of effort into its characterisation and manipulation both *in vitro* and *in vivo*. It was the discovery and characterization of the self-cleavage of ASBV and of the virusoid of lucerne transient streak virus (vLTSV) (12,21–23), together with the predicted self-cleavage sites of two other virusoids (21) and the data from the self-cleavage of the (+) form of the satellite RNA of tobacco ringspot virus (sTRSV) (10,24), which defined the hammerhead structure and the naming of it (21).

The first *in trans* reactions with the hammerhead structure involved two short sequences derived from the sequence of the top and bottom strands of (–)ASBV (26) and also of a short and a long RNA transcript equivalent to the full length of the ASBV molecule (23). The core sequence of the hammerhead structure of (+)sTRSV was used by Haseloff and Gerlach (27) to develop their *in vitro* system for targeting the cleavage of foreign RNAs. The reader is referred to a number of reviews and papers which summarize much of this earlier work (5–11,28–31). Of considerable recent importance is the determination of the crystalline

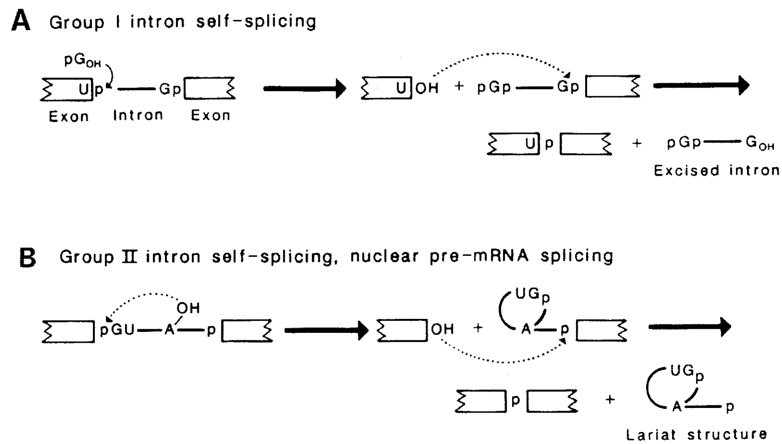

Figure 2. Group I and Group II intron self-splicing reactions. (**A**) Two-step self-splicing reactions of the Group I introns of nuclear rRNA genes, mitochondrial mRNA and rRNA genes, and chloroplast tRNA genes. (**B**) Two-step self-splicing reactions of the Group II introns and nuclear pre-mRNAs of structural genes of fungal and plant mitochondrial DNA and of structural and tRNA genes of chloroplasts.

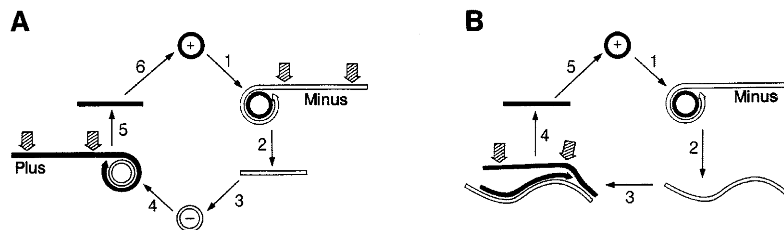

Figure 3. Rolling circle model for the replication of circular pathogenic RNAs (1). (**A**) Model where both the (+) and (–) multimeric RNAs are processed to monomers as indicated by arrows. Steps 3 and 6 involve the circularization of the linear monomers. (**B**) Model where only the linear multimeric (+) RNA is processed. The unprocessed linear (–) strand is copied to give the linear (+) strand.

structure of the hammerhead, initially with modifications to prevent self-cleavage (32–34), and more recently in an unmodified form (35).

The hairpin ribozyme structure

Although the hairpin self-cleavage reaction of (–) sTRSV was first identified in 1986 (36,37), the same year as the hammerhead reaction in (+)sTRSV and ASBV (12,24,36,37), it has received less attention than the hammerhead. However, there is a greatly increasing effort into the 50 nt hairpin structure (38–42). So far only three satellite RNAs have been found where the (–) RNA self cleaves by the hairpin structure (Fig. 4B) and the helper viruses all belong to the Nepovirus family (Table 1). In contrast to the hammerhead self-cleavage reaction, that of the hairpin ribozyme is readily reversible. As for the hammerhead structure, it lends itself readily to mutational analysis and reactions carried out both *in cis* and *in trans* (38,41,42) and such experiments together with phylogenetic comparisons have allowed the establishment of the secondary structure shown in Figure 4B for (–)sTRSV. One can foresee the RNA crystallographers turning their attention to determining the three-dimensional structure of the active hairpin ribozyme and an increasing interest in exploring the potential for *in trans* cleavage of foreign RNAs both *in vitro* and *in vivo* (43).

Of historical interest is that the self-cleavage structure was originally called a paperclip, so named by the Bruening laboratory which discovered the reactions (10,36). However, the introduction and continued use by others of the alternative name of hairpin has led to general acceptance of the latter term.

Does the *in vitro* hammerhead self-cleavage reaction have its predicted role *in vivo*?

The two viroids and the four viroid-like satellite RNAs or virusoids associated with the four helper Sobemoviruses (Table 1) are isolated from infected plants in the circular form. Hence, the role of the self-cleavage reaction in the rolling circle replication of these RNAs is inferred from the absence or presence in infected plants of a high molecular weight minus strand. For example, in the case of vLTSV where both (+) and (–) strands self-cleave *in vitro* (21–23), mostly monomeric (+) and (–) strands, together with some lower multimeric (+) forms, were found in infected plants and no high molecular weight (–) forms, evidence consistent with specific cleavage of the (–) strand *in vivo* (44). Likewise, the presence of high molecular weight (–) forms of vSNMV, vSCMoV and vVTMoV in infected plants and the absence of monomeric (–) forms (44,45) is consistent with the *in vitro* self-cleavage data (Table 1).

In order to provide more definitive evidence, three full length cDNA clones of vLTSV, mutated at sites that inactivate *in vitro* self-cleavage of the (–) RNA were inoculated as excised plasmid inserts, together with helper virus LTSV, on susceptible host plants (46). As predicted if hammerhead self-cleavage is involved in *in vivo* cleavage of (–) RNAs, high molecular weight (–)vLTSV

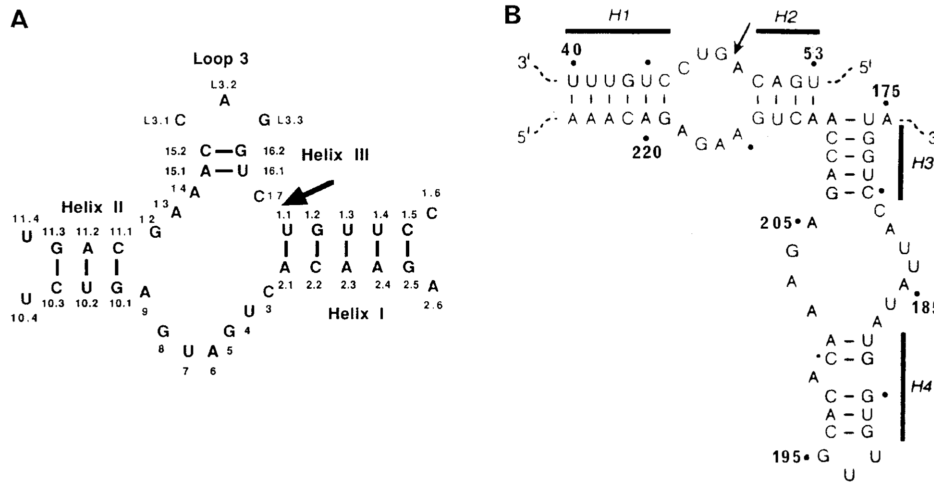

Figure 4. Hammerhead and hairpin self-cleavage structures. (**A**) Hammerhead self-cleavage structure of (+) ASBV. The residue numbering system was introduced in order to simplify the comparison of data from different laboratories (25). (**B**) Hairpin self-cleavage structure of (–) sTRSV (38,39). Residue numbers are those of the (+) RNA and hence run in the 3′ to 5′ direction. H1–H4 are the four helices. The arrow indicates the self-cleavage site in both structures.

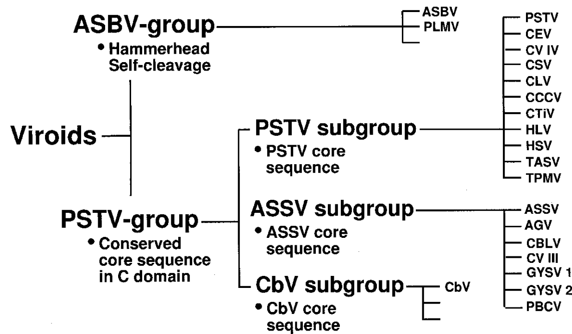

Figure 5. Classification of viroids. All viroids sequenced so far can be divided into two groups on the basis of comparative sequence analysis and the ability to show hammerhead self-cleavage; the avocado sunblotch viroid (ASBV) group and the potato spindle tuber viroid (PSTV) group. Subdivision of the PSTV group into the PSTV subgroup, the apple scar skin viroid (ASSV) subgroup and the coleus blumei viroid (CbV) subgroup is based on a conserved core sequence (Fig. 7) in the central conserved domain C (Fig. 6) (48,50). Full viroid names are listed in ref. 47; note that two of the viroids listed, GYSV and G1BV, should be grapevine yellow speckle viroids 1 and 2, respectively; GYSV1 and GYSV2. Also, vLTSV satellite RNA should be in the Nepovirus, and not the Sobemovirus, group as in Table 1 here.

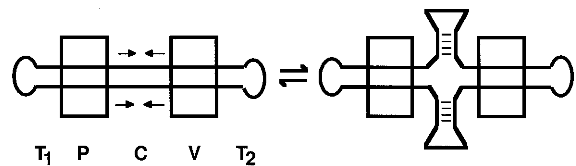

Figure 6. Model of the five domains in the PSTV group of viroids (48). Left hand side: T1 and T2, terminal domains; P and V, pathogenic and variable domains; C, central conserved domain. Inverted repeat sequences in the C domain are indicated by arrows. Right hand side: potential stem–loop structure formed in the C domain involving the inverted repeat sequences.

RNAs were present in total RNA extracts of these mutant inoculated plants but not in wild-type inoculated plants. Surprisingly, the mutated virusoids also produced monomeric (–) RNAs *in vivo*; sequence analysis of these RNAs indicated reversions or pseudoreversions of the introduced mutations which most likely restored some *in vivo* self-cleavage activity in the (–) RNAs. In spite of the complications of these reversions, the results provide strong support for the proposed role of the hammerhead reaction in the processing *in vivo* of multimeric (–)vLTSV RNA.

The PSTV group of viroids—potential for a new ribozyme?

The PSTV (potato spindle tuber viroid) group of viroids contains about 20 different viroids (Fig. 5) and new ones are being described at regular intervals. Relevant to further discussion, a brief description of viroid classification is important. Viroids can be classified into two separate groups based on comparative sequence analysis and self-cleavage ability (5,48–50). There are currently only two members in the ASBV group (Table 1, Fig. 5) and both (+) and (–) RNAs self-cleave *in vitro* via the hammerhead structure. All other viroids identified so far fall into the PSTV group and none contain the sequence motifs required for hammerhead or hairpin self-cleavage nor have any been shown so far to be capable of a specific self-cleavage reaction *in vitro* (see further discussion below).

The domain model for the PSTV group of viroids was developed in 1985 (48) and has stood the test of time as more viroids were discovered and sequenced. Comparative pairwise sequence analysis of members of the PSTV subgroup of viroids (Fig. 5) indicated the presence of five domains (Fig. 6), the boundaries of which were defined by sharp changes in sequence homology, from high to low or vice versa. Different pairwise sequence comparisons were consistent in defining the exact position of the boundaries. The domains are: T1 and T2, the left and right terminal domains; P, the pathogenic domain; C, the central conserved domain; and V, the variable domain. By convention, residue numbering starts in the middle of the end loop of the T1 domain.

The subdivision of the PSTV group into three subgroups (50) was based in part on the grouping of viroids with shared sequences but mainly on the presence of highly conserved

Figure 7. Conserved sequences in the C domain of the three subgroups of the PSTV group of viroids. These sequences provide the basis for the subdivision within the PSTV group of viroids. The sequences given for the PSTV and ASSV subgroup are those of PSTV and ASSV, respectively. The variation of sequence in one or more of the 11 viroids of the PSTV subgroup or of the seven viroids of the ASSV subgroup are given above or below the relevant residue. O indicates a single base deletion. Arrows represent the inverted repeat sequences. Only one member of the CbV subgroup has been identified so far.

sequences within the C domain or central conserved region (CCR) (Fig. 7). Thus, we have the PSTV subgroup, the apple scar skin viroid (ASSV) subgroup and the coleus blumei viroid (CbV) subgroup. Although there is currently only one member of the latter group, the sequence of the CbV region corresponding to the CCR in the other two groups clearly defines a new subgroup.

A fascinating aspect of 12 viroids, including ASBV and PLMV, and of the human hepatitis delta RNA, a 1700 nt single stranded circular RNA with some viroid-like features (51), is the pattern of purine and pyrimidine tracts which make up substantial portions of their genomes (52). In contrast, such tracts are not common in the four virusoids and sTRSV (Table 1). The significance of these polypurine and polypyrimidine tracts in viroids and hepatitis delta RNA is not known but the authors consider (52) that they may be related to the differences in replication strategies of the two groups of RNAs, the non-viroid RNAs being dependent on a specific helper virus whereas viroids are replicated independently of any such virus. Relevant to this aspect is the possibility that such tracts may function as organelle localisation signals (53), for the nucleus in the case of PSTV, CEV and CCCV (13–15) and hepatitis delta RNA (54), and the chloroplast for ASBV (13). Accumulating evidence, usually based on inhibition studies with α-amanitin, indicates a central role for nuclear DNA-dependent RNA polymerase II in the replication of PSTV and CEV (55–57, D.Warrilow and R.H.Symons, unpublished data) and hepatitis delta RNA (54).

The other most important aspect is the potential ability of the CCR of all members of the PSTV group to form two hairpin structures as indicated in Figure 6; the formation of these requires inverted repeat sequences as indicated by the arrows. The CCR of PSTV is shown in more detail in Figure 8 with the first residue of each hairpin stem corresponding to the base of each arrow. An interesting aspect of the CCR of PSTV is the cross-linking of G98 to U260 (Fig. 8) on irradiation of the purified viroid with UV-light (58) which indicates a structural juxtaposition of these two residues for the cross-linking to occur.

With one exception (59; see below), there have been no other published reports of self-cleavage reactions for any members of the PSTV group of viroids so that emphasis has been placed by some groups on searching for an enzyme catalysed processing reaction that would be involved in the rolling circle replication (60,61). Since only the (+) strand of PSTV and related viroids is processed after synthesis on a multimeric (–) strand, work needs only to concentrate on the processing of the (+) strand.

The CCR of these viroids is a highly prospective site for any type of specific processing reaction. On the basis of mutagenesis studies on longer-than-unit-length RNA transcripts of cDNA clones of citrus exocortis viroid (CEV), we identified a potential *in vivo* processing site in the upper strand of the CCR (62) which corresponds to G96–G98 of the PSTV sequence (Fig. 8). However, our further characterization of the effect of cloning site of the CEV cDNA and the sequences, both viroid and vector, around each cloning site on infectivity of RNA transcripts showed that the basic requirement for infectivity appeared to be the ability of the RNA transcripts to form a short double-stranded region of viroid and vector sequences at the junction of the two termini (63). cDNA clones of CEV prepared at seven different

Figure 8. Sequence of the C domain of PSTV in the rod-like structure (left) and the potential stem–loop structure that can be formed from it (right). The four arrows represent the inverted repeat sequences; the base residues of these sequences are numbered. The UV-induced cross-linking between G98 and U260 (Warrilow,D. and Symons,R.H., unpublished data) is shown by double-headed arrows.

sites within the viroid sequence provided infectious RNA transcripts showing that the site of cloning was not important for infectivity. In addition, non-viroid sequences were absent from the progeny viroids so it is feasible that some type of crossing-over occurred *in vivo* between the two terminal sequences to produce viable wild-type progeny. Hence, these indirect approaches have not been fruitful in defining the processing site, let alone the mechanism involved, but at least they have served to emphasize the difficulty in interpreting mutation data and infectivity.

One of the earliest indications that an RNA catalysed cleavage reaction can occur in PSTV was shown by the incubation of a dimeric transcript of PSTV under conditions used for Group I splicing reactions (59). There was ~1–5% conversion of the dimeric RNA to a product which comigrated on electrophoresis in an acrylamide gel with linear monomeric PSTV as well as two bands of ~190 and 210 nt. The results placed the cleavage site between residues 250–270 which is within the bottom strand of the central conserved region of PSTV in a highly prospective region for a processing site. Unfortunately, these initial observations were not followed up.

In another direct approach, we have spent considerable effort following the techniques that we used successfully in the discovery and characterisation of the hammerhead self-cleavage reaction in ASBV and the four viroid-like RNAs or virusoids (12,21–23,29). The overall approach was to prepare full-length, and longer than full-length, cDNA clones of members of the PSTV group of viroids and to use RNA transcripts from various regions of each clone to test for self-cleavage activity under a whole range of different conditions. For this work we used cDNA clones of citrus exocortis viroid (CEV) which is 371 nt long and of coconut cadang cadang viroid (CCCV) which is one nucleotide shorter than ASBV at 246 nt.

In spite of much effort, we have had little success in finding a convincing self-cleavage reaction in CEV and CCCV (McInnes,J.L., Couch,T., Hodgson,R. and Symons, R.H., unpublished data). A major criterion for success in identifying a processing site is that there can be only one such site per viroid monomer and that it is in a region highly conserved within the members of the viroid subgroup. No unique self-cleavage site was identified but we did identify non-unique sites which occurred at CpA sequences, indicating a natural lability of this linkage. It is of interest here that Kikuchi *et al*. (64) reported that natural linear PSTV RNA of 359 nt has nicks between C181/A182 and between C348/A349, which have presumably arisen during the purification and manipulation of the RNA.

The most likely cause of our lack of success to date, and that of others, is the ability of single-stranded RNA to fold into multiple conformations. This is best illustrated from our own experience (21–23) on the hammerhead self-cleavage reaction using the (+) and (–) strands of the virusoid or viroid-like satellite RNA of lucerne transient streak virus (vLTSV) (Table 1). Various length RNA transcripts containing the self-cleavage site only self-cleaved upon the addition of Mg^{2+} after heat denaturation and snap cooling and assembly of the reaction mixture on ice (21). Heating and slow cooling or allowing the snap-cooled RNA to warm up before the addition of Mg^{2+} gave inactive RNA. This led to the concept of active and inactive self-cleavage conformations within an RNA population. In the case of our work on CEV and CCCV, it is feasible that, in spite of our best efforts at manipulating RNA transcripts of various lengths, the RNA always folded into a variety of conformations all of which were inactive for self-cleavage.

Given that an RNA catalysed processing reaction is involved in the rolling circle replication *in vivo* of members of the PSTV group of viroids, the question arises as to why such processing is so obviously efficient *in vivo* and impossible so far to reproduce *in vitro*. The synthesis of longer-than-unit length (+) RNA from similar (–) RNA *in vivo* presumably provides the RNA conformation and environment for the putative RNA catalysed processing of the (+) RNA. The host nuclear RNA polymerase II is most likely responsible for viroid synthesis *in vivo* for members of the PSTV group (55–57, D.Warrilow and R.H.Symons, unpublished data) and is a large, complex and multi-component enzyme. It is feasible that it could provide the right folding environment for the processing reaction to occur during viroid synthesis such that a protein-catalysed processing reaction is not involved.

What is the UV-induced cross-linking between RNA strands telling us about local tertiary structure and RNA processing?

As mentioned above, UV-irradiation of PSTV cross-links G98 and U260 in the CCR of the native molecule (58) and in a region highly prospective for RNA processing during rolling circle replication. Two other RNAs also show similar UV-induced cross-linking. The (–) sTRSV RNA is cross-linked between G204 and U183 (Fig. 4B) and close to the self-cleavage site in the hairpin structure (42,65). A similar situation also exists in the single-stranded, circular self-cleaving hepatitis delta RNA, a 1700 nt rod-like molecule where both the genomic and antigenomic RNAs show self-cleavage via a similar structure (66). This self-cleavage occurs at one end of the molecule which shows viroid-like features and near to the site where the genomic RNA is cross-linked by UV-irradiation (67).

The UV-induced cross-linking indicates a tertiary structure motif where juxtapositioning occurs of two bases from opposite strands. It is feasible that such a tertiary element may play a role in aiding the formation of an active self-cleavage structure in these molecules.

Future prospects

The single-stranded circular plant pathogenic RNAs and the more complex hepatitis delta RNA have provided us so far with most of the naturally occurring self-cleaving RNAs. As considered above, it is predicted that the PSTV group of viroids will provide another source of such RNAs. The carnation small viroid-like RNA (CarSV RNA) appears part of a more complex system (17,18) which is still to be resolved and it may provide some more surprises in RNA catalysis.

It is feasible that there are more single-stranded circular pathogenic RNAs waiting to be discovered and characterised in both the animal and plant kingdoms and these could be prospective for new types of self-cleavage reactions. The possibility should be considered that the transcription of circular single-stranded and double-stranded DNA may, in some cases, depend on self-cleavage reactions for processing of the transcripts. A good example here is the self-cleavage of a multimeric RNA transcript of the circular 881 bp *Neurospora* mitochondrial DNA plasmid to produce a linear 881 nt RNA which is then

circularised (68). The self-cleaving domains in this RNA are different to those found for the hammerhead, hairpin and hepatitis delta RNA ribozymes (69).

And, finally, the transcript of satellite 2, a 300–350 bp repetitive DNA that is highly conserved in salamanders, contains a hammerhead self-cleavage structure that provides monomeric transcripts (70,71). This is the only example of a hammerhead-like RNA that has been found in an animal. This example, and that of the *Neurospora* mitochondrial DNA plasmid, indicate that non-pathogen sources may also provide a rich source of RNA catalysed cleavage reactions.

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REFERENCES

1 Branch, A.D. and Robertson, H.D. (1984) *Science* **223**, 450–455.
2 Cech, T.R. (1990) *Annu. Rev. Biochem.* **59**, 543–568.
3 Cech, T.R. and Herschlag, D. (1996) In Eckstein, F. and Lilley, D.M.J. (eds), *Nucleic Acids and Molecular Biology*, volume 10. Springer-Verlag, Berlin, pp. 1–17.
4 Pyle, A.M. (1996) In Eckstein F. and Lilley, D.M.J. (eds), *Nucleic Acids and Molecular Biology*, volume 10. Springer-Verlag, Berlin, pp. 75–107.
5 Keese P., Visvader, J.E. and Symons, R.H. (1988) In Domingo, E., Holland, J. and Ahlquist, P. (eds) *RNA Genetics*, *volume 3; RNA Replication*. CRC Press, Boca Raton, pp. 71–98.
6 Symons, R.H. (1989) *Trends Biochem. Sci.* **14**, 445–450.
7 Symons, R.H. (1991) *Crit. Rev. Plant Sci.* **10**, 189–234.
8 Symons, R.H. (1992) *Annu. Rev. Biochem.* **61**, 641–671.
9 Symons, R.H. (1994) *Curr. Opin. Struct. Biol.* **4**, 322–330.
10 Bruening, G. (1990) *Semin. Virol.* **1**, 127–134.
11 Bruening, G., Passmore, B.K., van Tol, H., Buzayan, J.M. and Feldstein, P.A. (1991) *Mol. Plant–Microbe Interact.* **4**, 219–225.
12 Hutchins, C.J., Rathjen, P.D., Forster, A.C. and Symons, R.H. (1986) *Nucleic Acids Res*. **14**, 3627–3640.
13 Bonfiglioli, R.G., McFadden, G.I. and Symons, R.H. (1994) *Plant J.* **6**, 99–103.
14 Bonfiglioli, R.G., Webb, D.R. and Symons, R.H. (1996) *Plant J*. **9**, 457–465.
15 Harders, J., Lukacs, N., Robert-Nicoud, M., Jovin, T.M. and Riesner, D. (1989) *EMBO J*. **8**, 3941–3949.
16 Hernandez, C. and Flores, R. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3711–3715.
17 Hernandez, C., Davos, J.A., Elena, S.F., Moya, A. and Flores, R. (1992) *Nucleic Acids Res*. **20**, 6323–6329.
18 Daros, J.A. and Flores, R. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 6856–6860.
19 Allen, R.N., Palukaitis, P. and Symons, R.H. (1981) *Aust. Plant Pathol.* **10**, 31–32.
20 Epstein, L.M. and Gall, J.G. (1987) *Cell* **48**, 535–543.
21 Forster, A.C. and Symons, R.H. (1987) *Cell* **49**, 211–220.
22 Forster, A.C. and Symons, R.H. (1987) *Cell* **50**, 9–16.
23 Forster, A.C., Jeffries, A.C., Sheldon, C.C. and Symons, R.H. (1987) *Cold Spring Harbor Symp. Quant. Biol.* **52**, 249–259.
24 Prody, G.A., Bakos, J.T., Buzayan, J.M., Schneider, I.R. and Bruening, G. (1986) *Science* **231**, 1577–1580.
25 Hertel, K,J., Pardi, A., Uhlenbeck, O.C., Kozumi, M., Ohtsuka, E., Uesugi, S., Cedergren, R., Eckstein, F., Gerlach, W.L., Hodgson, R., and Symons, R.H. (1992) *Nucleic Acids Res*. **20**, 3252.
26 Uhlenbeck, O.C. (1987) *Nature* **328**, 596–600.
27 Haseloff, J. and Gerlach, W.L. (1988) *Nature* **334**, 585–591.
28 Long, D.M. and Uhlenbeck, O.C. (1993) *FASEB J.* **7**, 25–30.
29 Sheldon, C.C. Jeffries, A.C., Davies, C. and Symons, R.H. (1990) In Eckstein, F. and Lilley, D.M.J. (eds), *Nucleic Acids and Molecular Biology*, volume 4. Springer-Verlag, Berlin, pp. 227–242.
30 Tuschl, T., Thomson, J.B. and Eckstein, F. (1995) *Curr. Opin. Struct. Biol*. **5**, 296–302.
31 Thomson, J.B., Tuschl, T. and Eckstein, F. (1996) In Eckstein, F. and Lilley, D.M.J. (eds), *Nucleic Acids and Molecular Biology*, volume 10. Springer-Verlag, Berlin, pp. 173–196.
32 Pley, H.W., Flaherty, K.M. and McKay D.B. (1994) *Nature* **372**, 68–74.
33 McKay, D.B., In Eckstein, F. and Lilley, D.M.J. (eds) (1996) *Nucleic Acids and Molecular Biology* volume 10. Springer-Verlag, Berlin, pp. 161–172.
34 Scott, W.G., Finch, J.T. and Klug, A. (1995) *Cell* **81**, 991–1002.
35 Scott, W.G., Murray, J.B., Arnold, J.R.P., Stoddard, B.L. and Klug, A. (1996) *Science* **274**, 2065–2069.
36 Buzayan, J.M., Gerlach, W.L. and Bruening, G. (1986) *Nature* **323**, 349–353.
37 Buzayan, J.M., Gerlach, W.L. and Bruening, G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8859–8862.
38 Haseloff, J. and Gerlach, W.L. (1989) *Gene* **82**, 43–52.
39 Feldstein, P.A., Buzayan, J.M. and Bruening, G. (1989) *Gene* **82**, 53–61.
40 Feldstein, P.A., and Bruening, G. (1993) *Nucleic Acids Res.* **21**, 1991–1998.
41 Hegg, L.A. and Fedor, M.J. (1995) *Biochemistry* **34**, 15813–15828.
42 Burke, J.M., Butcher, S.E. and Sarguiel, B. (1996) In Eckstein, F. and Lilley, D.M.J. (eds), *Nucleic Acids and Molecular Biology*, Springer-Verlag, Berlin, volume 10, pp. 129–143.
43 Couture, L.A. and Stinchcomb, D.T. (1996) *Trends Genet.* **12**, 510–515.
44 Hutchins, C.J., Keese, P., Visvader, J.E., Rathjen, P.D., McInnes, J.L and Symons, R.H., (1985) *Plant Mol. Biol.* **4**, 293–304.
45 Davies, C., Haseloff, J. and Symons, R.H. (1990) *Virology* **177**, 216–224.
46 Sheldon, C.C. and Symons, R.H. (1993) *Virology* **194**, 463–474.
47 Bussière, F., Lafontaine, D. and Perreault, J.P. (1996) *Nucleic Acids Res.* **24**, 1793–1798.
48 Keese, P. and Symons, R.H. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4582–4586.
49 Symons, R.H. (1990) *Semin. Virol.* **1**, 75–81.
50 Koltunow, A.M. and Rezaian, M.A. (1989) *Intervirology* **30**, 194–201.
51 Branch, A.D., Levine, B.J. and Robertson, H.D. (1990) *Semin. Virol.* **1**, 143–152.
52 Branch, A.D., Lee, S.E., Neel, O.D. and Robertson, H.D. (1993) *Nucleic Acids Res.* **21**, 3529–3535.
53 Grimm, C., Lund, E. and Dahlberg, J.E. (1997) *EMBO J.* **16**, 793–806.
54 MacNaughton, T.B., Gowans, E.J., McNamara, S.P. and Burrell, C.J. (1991) *Virology* **184**, 387–390.
55 Flores, R. and Semancik, J.S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6285–6288.
56 Mühlbach, H.P. and Sänger, H.L. (1979) *Nature* **278**, 185–188.
57 Schindler, I.M. and Mühlbach, H.P. (1992) *Plant Sci.* **84**, 221–229.
58 Branch, A.D., Benenfeld, B.J. and Robertson, H.D. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6590–6594.
59 Robertson, H.A., Rosen, D.L. and Branch, A.D (1985) *Virology* **142**, 441–447.
60 Steger, G., Baumstark,T., Mörchen, M., Tabler, M., Tsagris, M., Sänger, H.L., and Riesner, D. (1992) *J. Mol. Biol.* **227**, 719–737.
61 Baumstark, T., Riesner, D. (1995) *Nucleic Acids Res.* **23**, 4246–4254.
62 Visvader, J.E., Forster, A.C. and Symons, R.H. (1986) *Nucleic Acids Res.* **13**, 5843–5856.
63 Rakowski, A.G and Symons, R.H. (1994) *Virology* **203**, 328–335.
64 Kikuchi, Y., Tyc, K., Filipowicz, W., Sänger, H.L. and Gross, H.J. (1982) *Nucleic Acids Res*. **10**, 5721–7529.
65 Butcher, S.E. and Burke, J.M. (1994). *Biochemistry* **33**, 992–999.
66 Tanner, N.K. (1995) In Dinton-Gottlieb, G. (ed.), *The Unique Hepatitis Virus*. R.G. Landes Company, Austin, Texas, pp. 11–31.
67 Branch, A.D., Benenfeld, B.J., Baroudy, B.M., Wells, F.V., Gerin, J.L. and Robertson, H.D. (1989) *Science* **243**, 649–652.
68 Saville, B.J. and Collins, R.A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8826–8830.
69 Rastogi, T., Beattie, T.L., Olive, J.E. and Collins, R.A (1996) *EMBO J.* **15**, 2820–2825
70 Epstein, L.M. and Gall, J.G. (1987) *Cell* **48**, 535–543.
71 Garrett, T.A., Pabon-Pena, L.M., Gokaldas, N. and Epstein, L.M. (1996) *RNA* **2**, 699–706