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Minireview

Viroids: the minimal non-coding RNAs with autonomous replication

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Abstract Viroids are small (246–401 nucleotides), non-coding, circular RNAs able to replicate autonomously in certain plants. Viroids are classified into the families *Pospiviroidae* and *Avsunviroidae*, whose members replicate in the nucleus and chloroplast, respectively. Replication occurs by an RNA-based rolling-circle mechanism in three steps: (1) synthesis of longer-than-unit strands catalyzed by host DNA-dependent RNA polymerases forced to transcribe RNA templates, (2) processing to unit-length, which in family *Avsunviroidae* is mediated by hammerhead ribozymes, and (3) circularization either through an RNA ligase or autocatalytically. Disease induction might result from the accumulation of viroid-specific small interfering RNAs that, via RNA silencing, could interfere with normal developmental pathways.

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1. Introduction

Viroids are not supramolecular aggregates such as viruses, but instead are only composed of small (246–401 nt), single-stranded, circular RNA with the ability to infect certain plants and in most cases induce disease [1,2]. Additionally viroids, in contrast to viruses: (1) do not code for any protein, (2) some are catalytic RNAs with ribozyme activity, and (3) have presumably an extremely old origin that traces back to the pre-cellular RNA world postulated to have preceded our present world based on DNA and proteins [3]. Whereas RNA viruses can essentially be regarded as parasites of the translation machinery of their hosts, the lack of coding capacity of viroids entails that they can essentially be regarded as parasites of the transcription apparatus of their hosts. The catalytic activity of some viroids resides in their capacity to form hammerhead ribozymes, the simplest known ribozymes [4–6, for a review see 7]. This property is the most solid argument supporting that viroids have an evolutionary origin very old and independent from that of viruses. In this minireview, we will focus on some specific aspects related to the structure and replication of viroids and the interactions of these minimal pathogens with their hosts.

2. Structure of viroids as related to their function

The 26 members of the family *Pospiviroidae*, whose type species is *Potato spindle tuber viroid* (PSTVd), the first described viroid [8,9], have a characteristic central conserved region (CCR) and either a terminal conserved region (TCR) or a terminal conserved hairpin (TCH) [2] (Fig. 1). The sequence of the CCR and the presence or absence of the TCR and TCH serve to allocate members of this family into five genera. The other four viroids do not have these conserved motifs and are classified within the family *Avsunviroidae*, whose type species is *Avocado sunblotch viroid* (ASBVd), on the basis of their ability to form hammerhead structures (a particular class of ribozymes) that mediate the self-cleavage of their multimeric replicative RNA intermediates of both polarities [2,10]. Other demarcating criteria also support this classification (see below). There is sound evidence that PSTVd, and by extension the other members of its family, adopt in vitro a typical rod-like (or quasi-rod-like) secondary structure formed by alternating short double-stranded regions and single-stranded loops. Moreover, the repetitions and deletions observed in certain viroids always preserve the rod-like structure indicating that, most likely, it is also significant in vivo. From sequence comparisons, the rod-like structure has been divided into five structural/functional domains: central (C), pathogenic (P), variable (V) and terminal right (T_R) and left (T_L) (Fig. 1) [11]. The CCR is located within the C domain, and the TCR and TCH within the T_L domain. Some of these structural domains have been related to specific functions: the C domain, particularly the upper strand of the CCR, has been involved in the cleavage and ligation of the multimeric PSTVd RNA intermediates generated in the replication cycle [12], and the P domain in pathogenicity in PSTVd and closely related viroids. However, the situation is probably not so simple, with some roles being concurrently regulated by determinants located in different domains [13]. On the other hand, some conserved regions, like the TCR and the TCH, still await a candidate function.

Within the family *Avsunviroidae* [2,10], ASBVd and *Eggplant latent viroid* (ELVd) adopt quasi-rod-like secondary structures, but *Peach latent mosaic viroid* (PLMVd) and *Chrysanthemum chlorotic mottle viroid* (CChMVd) fold into clearly branched

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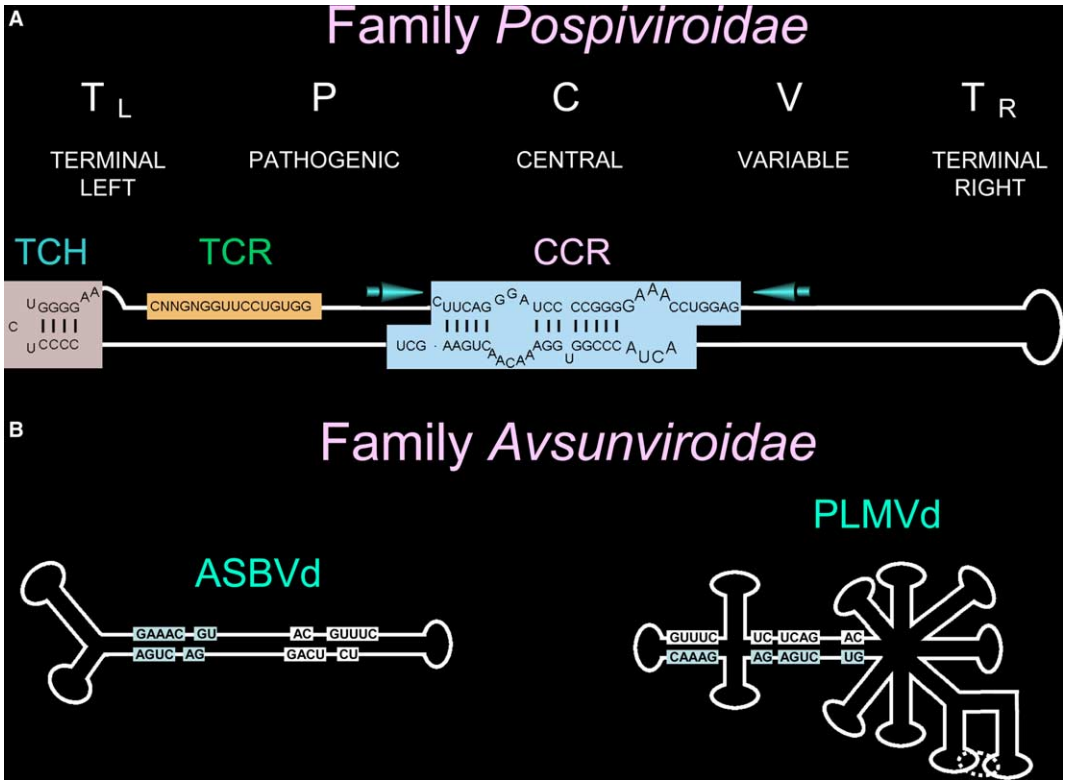

Fig. 1. Structure of viroids. (A) Rod-like secondary structure proposed for members of the family *Pospiviroidae*. The structural domains C, P, V, and T_L and T_R, respectively, are indicated. The motifs CCR (here displayed that of genus *Pospiviroid*), TCR (present in genera *Pospi-* and *Apscaviroid*, and in the two largest members of genus *Coleviroid)* and TCH (present in genera *Hostu-* and *Cocadviroid*) are shown. Arrows indicate flanking sequences that, together with the upper CCR strand, form imperfect inverted repeats. (B) Quasi-rod-like and branched secondary structures proposed for ASBVd and PLMVd, respectively, of the family *Avsunviroidae*. Nucleotide residues strictly or highly conserved in natural hammerhead structures are shown within boxes with blue and white backgrounds for plus and minus polarities, respectively. Broken lines in PLMVd denote a pseudoknot element of the kissing loop class.

secondary structures (Fig. 1). These complex structures, composed of multiple hairpins and internal loops, are most likely biologically relevant because the sequence heterogeneity found in a number of natural variants, particularly of CChMVd, preserve their stability (changes map at the loops or, when in the stems, they are co-variations or compensatory mutations). As with other RNAs, non-Watson–Crick base pairs and certain RNA structural motifs also exist in viroids. Examples include: (i) the so-called loop E, initially identified in 5S rRNA, which has been also mapped at the CCR of PSTVd [14] and proposed to play a role in the final ligation step of the PSTVd replication cycle [12], (ii) thermodynamically stable tetraloops of the classes GNRA and UNCG (where N represents any nucleotide and R a purine), which have been involved in cleavage of the multimeric RNA intermediates generated in PSTVd replication [12] and as determinants of CChMVd pathogenesis [15], respectively, (iii) the hammerhead structures that mediate self-cleavage of the multimeric RNA intermediates generated in the replication of members of the family *Avsunviroidae* (see below), and (iv) pseudoknot elements of the kissing loop class, which have been identified in PLMVd by in vitro chemical and enzymatic probing [16] and in CChMVd by site-directed mutagenesis and bioassays (Gago, De la Peña and Flores, unpublished results), where they may contribute to stabilizing the branched conformation of these two viroids (Fig. 1).

3. Viroid replication: rolling-circle mechanism

The circular nature of viroids determines their replication mode, which occurs through a rolling-circle mechanism [17], with only RNA intermediates [18], in which the infecting monomeric (+) circular RNA (this polarity is assigned arbitrarily to the in vivo most abundant strand) is transcribed by an RNA polymerase into head-to-tail (−) multimers that serve as templates for a second RNA–RNA transcription step. The resulting head-to-tail (+) multimers are cleaved into unit-length strands and subsequently ligated to the final progeny of monomeric (+) circular RNAs via RNase and RNA ligase activities, respectively (Fig. 2). This asymmetric pathway of the rolling-circle mechanism is followed by PSTVd and other members of the family *Pospiviroidae*, which replicate in the nucleus [19,20]. In contrast, ASBVd and other members of the family *Avsunviroidae*, which replicate in the chloroplast, follow a symmetric pathway in which the (−) multimers are processed to the monomeric (−) circular forms, the template for the second half of the replication cycle that is symmetric to the first [21]. As already indicated, cleavage of (+) and (−) multimers is autocatalytic in the family *Avsunviroidae* and mediated by hammerhead ribozymes [4–6]. The RNA ligase catalyzing circularization of linear monomeric forms is presumably a host enzyme [12,22,23], although for a member of the family *Avsunviroidae* not only

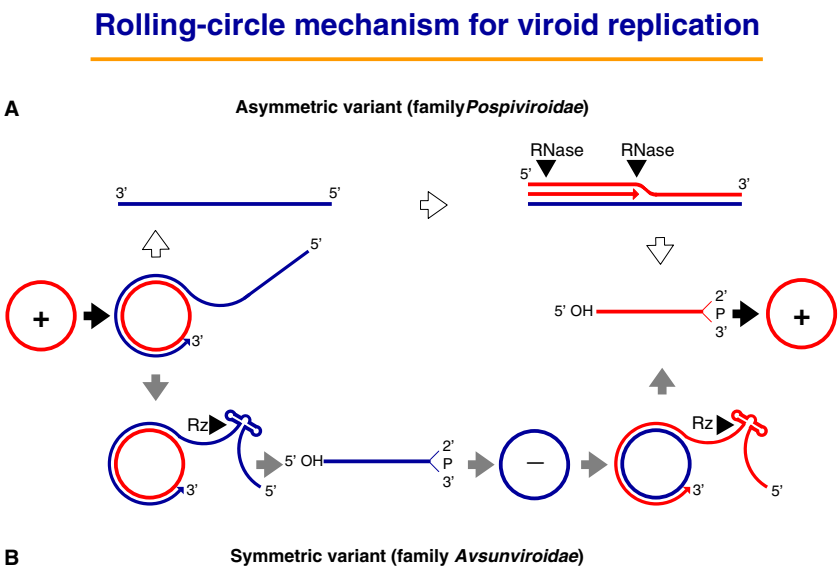

Fig. 2. Replication of viroids through a rolling-circle mechanism. (A,B) Asymmetric and symmetric pathways with one and two rolling circles proposed to operate in members of the families *Pospiviroidae* and *Avsunviroidae*, respectively. White and gray arrows indicate the asymmetric and symmetric pathways, respectively (the first and the last step, indicated with black arrows, are common to both pathways). Red and blue lines represent plus (+) and minus (−) polarities, respectively, and cleavage sites are marked by arrowheads. Self-cleavage mediated by hammerhead ribozymes (Rz) leads to linear monomeric RNAs with 5′-hydroxyl and 2′–3′-cyclic phosphodiester termini, the same termini being also most likely generated in cleavage catalyzed by a host RNase.

cleavage, but also ligation, has been proposed to occur autocatalytically and lead to atypical 2′–5′ phosphodiester bonds [24].

4. Viroid replication: enzymes involved in RNA polymerization and cleavage

In the first step of their replication cycle, the polymerization of RNA strands, viroids must cope with the fact that cellular RNA polymerases catalyze transcription of DNA templates. Although RNA-dependent RNA polymerases have been identified and cloned in plants, their subcellular localization in the cytoplasm is incompatible with playing a role in viroid replication that, as already indicated, occurs in the nucleus (family *Pospiviroidae*) or in the chloroplast (family *Avsunviroidae*). Therefore, viroids need to subvert the template specificity of certain host DNA-dependent RNA polymerases and force them to transcribe RNA; how they accomplish this template switch, which most probably requires the recruitment of specific transcription host factors, is one of the most intriguing questions that remain to be solved.

Two lines of evidence support that the enzyme catalyzing polymerization of both strands of PSTVd and other members of the family *Pospiviroidae* is the nuclear RNA polymerase II. First, experiments in vivo [25] and in vitro [26,27] have shown that replication of PSTVd, *Citrus exocortis viroid* (CEVd) and *Hop stunt viroid* (HSVd), is inhibited by the low concentrations of the fungal toxin α-amanitin that characteristically inhibits the synthesis of *bona fide* RNA polymerase II transcripts. And second, immunoprecipitation assays with a monoclonal antibody against a domain conserved in the largest subunit of RNA polymerase II have shown that this subunit co-precipitates with CEVd (+) and (−) strands present in nuclear-enriched preparations from infected tissue with CEVd RNA synthesis activity [28]. Parallel studies with members of the family *Avsunviroidae* are less advanced, but the effects of the bacterial inhibitor tagetitoxin on a chloroplastic transcription system from ASBVd-infected tissue are consistent with the involvement in the synthesis of viroid strands of a nuclear encoded polymerase structurally similar to the T7 phage RNA polymerase [29], although other studies using PLMVd and *Escherichia coli* RNA polymerase suggest the participation of the eubacterial-like plastid encoded polymerase [30].

Does transcription of viroid strands start at random – a plausible alternative considering that the circular or the oligomeric nature of the templates allows complete transcription regardless of the initiation site – or at specific sites? In chloroplasts, the 5′ termini of primary transcripts, but not those resulting from their processing, have a free triphosphate group that can be specifically labeled in vitro with [α-^{32}P]GTP and guanylyltransferase. Application of this labeling to the linear monomeric (+) and (−) ASBVd RNAs isolated from infected avocado tissue, in combination with RNase protection assays using viroid-specific riboprobes, has revealed that both ASBVd strands begin with a UAAAA sequence that maps to similar A + U-rich right terminal loops in their predicted quasi-rod-like secondary structures, a result that has been confirmed by primer-extension [29]. Identification by primer-extension of the 5′ termini of certain PLMVd subgenomic RNAs, presumed to result from replication, suggests that the initiation sites of this viroid also occur in terminal loops [30]. Regarding members of the family *Pospiviroidae* with nuclear replication, it is possible that the 5′ triphosphate of the primary transcripts could be capped in vivo. If so, this would mark the transcription initiation sites in this family of viroids, an issue that remains unanswered.

How are the oligomeric viroid strands of one or both polarities specifically cleaved to their unit-length counterparts?

Here again different mechanisms operate in the two families, with the family *Avsunviroidae* following a ribozyme-mediated self-cleavage (see below). In contrast, one or more host RNases appear to catalyze cleavage of the oligomeric (+) strands in members of the family *Pospiviroidae*. In vitro, potato nuclear extracts are able to process an oligomeric (+) PSTVd RNA [23], or a monomeric (+) PSTVd RNA with a short repeat of the CCR upper strand [12], into the infectious monomeric circular forms. Surprisingly, a non-specific fungal RNase can also catalyze the same in vitro cleavage and ligation reactions [31], suggesting that the specificity of the cleavage reaction (and of the subsequent ligation) is most probably determined by a defined conformation(s) of the oligomeric viroid RNA. More specifically, the enzymatic cleavage and ligation of the PSTVd (+) strand has been advanced to be driven by a switch from a branched structure containing a tetraloop to an extended conformation with an E loop [12]. However, other data obtained with in vivo approaches provide circumstantial support for the existence of alternative cleavage sites in PSTVd and CEVd. More recently, correct processing to the monomeric (+) circular forms has been observed in *Arabidopsis thaliana* transformed with cDNAs expressing dimeric (+) transcripts of five representative species of the family *Pospiviroidae*, showing that this model plant has the appropriate RNase and RNA ligase activities. Conversely, a dimeric (−) transcript of HSVd expressed transgenically in *A. thaliana* failed to be processed, thus indicating that processing of dimeric transcripts is a polarity intrinsic property which, through the adoption of particular conformations, dictates the susceptibility to and the specificity of the reactions mediated by the host enzymes (Daròs and Flores, unpublished results). The finding that in infected cultured cells and plants PSTVd (−) strands accumulate in the nucleoplasm whereas the (+) strands are localized in the nucleolus as well as in nucleoplasm, provides an explanation for this different behavior and suggests that processing of the (+) strands occurs in the nucleolus [20], where processing of the precursors of rRNAs and tRNAs also takes place.

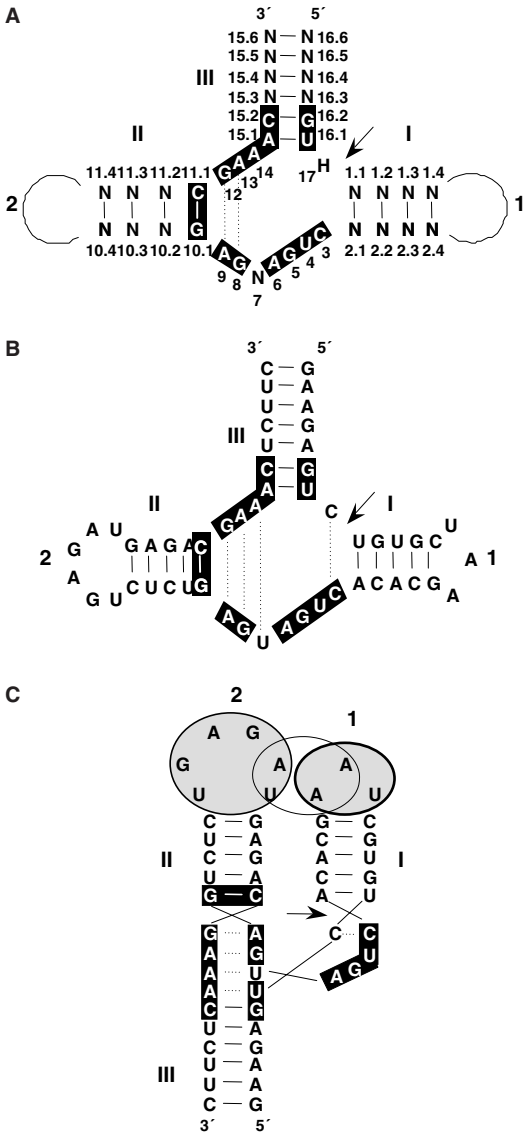

Fig. 3. Hammerhead structures. (A) Consensus hammerhead structure schematically as originally proposed with its numbering system. Residues strictly or highly conserved in natural hammerhead structures are shown within boxes on a black background. Arrow marks the self-cleavage site, N indicates any residue and H any residue except G, and continuous and broken lines denote Watson–Crick and non-canonical pairs, respectively. The central core is flanked by helices I–III, with helices I and II being closed in most natural hammerhead structures by short loops 1 and 2, respectively. (B) and (C), PLMVd (+) hammerhead structure represented according to the original scheme and to X-ray crystallography data derived from an artificial hammerhead structure, respectively. The proposed tertiary interaction between loops I and II, that facilitates the catalytic activity at the low magnesium concentration existing in vivo, is indicated in gray.

5. Viroid replication: ribozyme involvement in RNA self-cleavage

The discovery of the hammerhead ribozyme in ASBVd [4] and in a satellite RNA, structurally similar to viroids but functionally dependent on a helper virus [5], is regarded as a milestone in molecular virology with major consequences on the replication and evolutionary origin of these RNAs [3,6]. Moreover, this ribozyme, being the structurally simplest one, has sparked much interest on understanding its catalytic mechanism and potential use as a therapeutic tool. The hammerhead ribozyme is a small RNA motif that at room temperature, neutral pH and in the presence of a divalent metal ion (generally Mg^{2+}), self-cleaves at a specific phosphodiester bond producing through a transesterification 2′,3′ cyclic phosphodiester and 5′ hydroxyl termini (Fig. 3).

Comparative analysis of natural hammerhead structures [see for a review 7] has revealed a central core of strictly conserved nucleotide residues flanked by three double-helix regions (I–III) with loose sequence requirements except for positions 15.1 and 16.1, which form an A–U pair, and 15.2 and 16.2, and 10.1 and 11.1, which usually form a C–G and a G–C pair, respectively (Fig. 3). X-ray crystallography of a model hammerhead structure [32] has shown a complex array of interactions between the residues of the central core, particularly three non-Watson–Crick pairs (involving A9 and G12, G8 and A13, and U7 and A14 that extend helix II) and a uridine turn motif, the tetranucleotide CUGA (positions 3–6), which form the catalytic pocket surrounding the cleavage site (Fig. 3).

Evidence of different nature supports the in vivo significance of hammerhead ribozymes in the processing of the oligomeric viroid RNAs containing these catalytic domains. First, linear monomeric RNAs of one or both polarities with 5′ termini identical to those produced in the in vitro self-cleavage reactions have been identified in tissues infected by ASBVd [21,29] and CChMVd [33]. Second, compensatory mutations or covariations that preserve the stability of the hammerhead structures have been found in sequence variants of PLMVd [34], CChMVd [15,33] and ELVd [10]. Third, a correlation has been established between the infectivity of different CChMVd and PLMVd variants and the extent of their self-cleavage during in vitro transcription [15,34]. And fourth, a 2′ phosphomonoester, 3′,5′ phosphodiester bond (the presumed signature of an RNA ligase, see below) has been found in a viroid-like satellite RNA in a position coincidental with the self-cleavage site predicted by its hammerhead structure [35], and indirect data, consistent with an extra 2′ phosphomonoester at the nucleotide preceding the predicted self-cleavage/ligation site, have also been obtained for some viroids [15,34]. Altogether, these results provide a solid basis for the in vivo functional role of hammerhead ribozymes.

6. In vivo activity and regulation of viroid hammerheads

Despite the previously described evidence, one critical aspect challenging this view has remained unsolved until very recently. Many data from studies with model hammerheads acting in *trans* (the design of ribozymes targeted against specific RNAs requires this artificial format that, additionally, facilitates the kinetic analysis in protein-free media) show that efficient in vitro cleavage requires Mg^{2+} concentrations of 5–10 mM, whereas the in vivo concentration of this cation is only about 0.5 mM. What is the explanation for this discrepancy? In vitro and in vivo results published last year [36,37] demonstrate that natural *cis*-acting hammerheads self-cleave much faster than their *trans*-acting derivatives, and that modifications of the peripheral loops 1 and 2 of natural hammerheads induce a severe reduction of their self-cleavage constants. These data indicate that, in contrast to the established view, regions external to the central conserved core of natural hammerheads play a critical role in catalysis, and strongly suggest the presence of tertiary interactions between these peripheral loops that may help the positioning and rigidity within the active site, thus optimizing the catalytic activity at the low magnesium concentration existing under physiological conditions (Fig. 3). Moreover, the interactions could be stabilized by proteins as suggested by the finding that a chloroplastic protein facilitates in vitro, and presumably in vivo, the hammerhead-mediated self-cleavage of a viroid RNA [38]. Dissecting these interactions will most likely be a focus of interest in the next years.

On the other hand, hammerhead ribozymes must be exquisitely regulated during viroid replication, with their activity being turned on to catalyze self-cleavage of oligomeric RNAs and then turned off to preserve a certain level of monomeric circular RNAs required as templates for the successive replication rounds. To this aim, two different mechanisms appear to operate. First, some hammerhead structures, such as those of both ASBVd RNAs, are thermodynamically unstable because they have a stem III of only two base pairs closed by a short loop of two or three residues [4]. Accordingly, in vitro self-cleavage of these monomeric RNAs is very inefficient. However, in their corresponding dimeric or oligomeric replicative intermediates, the sequences of two single-hammerhead structures can form a stable double-hammerhead structure with an extended helix III that promotes efficient self-cleavage in vitro and most likely in vivo [39]. A second mechanism has been proposed for PLMVd, CChMVd and ELVd, in which the monomeric plus and minus RNAs self-cleave efficiently in vitro because they can adopt stable single-hammerhead structures. However, the formation of these hammerhead structures in vivo is most likely hampered because the conserved sequences of both polarity hammerhead structures, due to their extensive complementarity, are involved in an alternative stable folding that does not promote self-cleavage of the monomeric RNAs. The catalytically active hammerhead structures may only form transiently during transcription, inducing self-cleavage of the oligomeric RNAs [6]. Therefore, there seems to be a switch between two conformations, one with the hammerhead structure promoting self-cleavage, and another blocking self-cleavage and favoring circularization.

7. Viroid replication: RNA ligase-mediated circularization or self-ligation?

Although certain RNases can catalyze in vitro the correct cleavage and ligation of an oligomeric (+) PSTVd RNA [31], the second reaction is most likely catalyzed in vivo by an RNA ligase. The atypical 2′ phosphomonoester, 3′,5′ phosphodiester bond found at the ligation site in a viroid-like satellite RNA [35] is the expected mark left by a plant RNA ligase, such as that from wheat germ with low substrate specificity and only requiring 5′-OH and 2′,3′ cyclic phosphodiester termini. In fact, the wheat germ RNA ligase can catalyze the in vitro circularization of the monomeric linear PSTVd forms isolated from infected tissue [22], and *A. thaliana* appears to have a similar enzyme with the ability to circularize in vivo the monomeric linear forms of five representative species of the family *Pospiviroidae* (Daròs and Flores, unpublished results). What is the situation with members of the family *Avsunviroidae?* No significant reversibility of hammerhead-mediated self-cleavage reactions has been observed, with the exception of PLMVd for which in vitro self-ligation of the resulting linear monomers has been reported. However, the generated phosphodiester bonds are mostly 2′,5′ instead of 3′,5′ and, although this atypical 2′,5′ bond has been advanced to exist in circular PLMVd RNAs isolated from infected tissue [24], these data should be regarded with care because: (1) there are no previous reports on the existence of natural RNAs with 2′,5′ bonds serving as transcription templates, (2) in vitro self-ligation has also been observed in PSTVd [12], showing that this is not a peculiarity of members of the family *Avsunviroidae*, and (3) other natural ribozymes endowed with RNA ligase activity, like the hairpin ribozyme, lead to the formation of the conventional 3′,5′ bonds. Therefore, the involvement of a chloroplast RNA ligase should be alternatively entertained. Even if no such enzyme has been annotated in the chloroplast genomes that have been sequenced, the possibility of a nuclear encoded RNA ligase targeted to the chloroplast cannot be

discarded. Moreover, since no plant RNA ligase has been yet cloned, it is difficult to predict its molecular properties.

8. Viroids and post-transcriptional gene silencing

Space limitations inherent to a minireview impede a full treatment of viroid–host interactions. For that reason, we will focus on one specific aspect that has emerged recently: the possibility that viroids could be inducers (and targets) of post-transcriptional gene silencing (PTGS), and that phenomena of this kind could mediate viroid pathogenesis. PTGS is a mechanism for regulating gene expression in eukaryotes that results in the sequence-specific degradation of single-stranded RNAs (ssRNAs) of internal or foreign origin [40]. PTGS appears to be triggered by double-stranded RNA (dsRNA), an intermediate of the replication of ssRNA viruses that can also be formed in systems expressing multiples copies of a transgene [41,42], which is subsequently processed into 21–25 nt fragments called small interfering RNAs (siRNAs) by an RNase III-like enzyme (Dicer). The siRNAs guide a second RNase (RISC, from *R*NA *i*nduced *s*ilencing *c*omplex) for degrading their cognate ssRNA. The presence of siRNAs homologous and complementary to a targeted ssRNA is regarded as a marker for PTGS. In plants, PTGS has been reported for cytoplasmic ssRNAs from endogenous nuclear genes, transgenes and RNA or DNA viruses [43]. Recently, PSTVd-specific siRNAs have been detected in infected plants [44,45], strongly suggesting that this viroid can also induce PTGS and that the process may take place in the cell nucleus, where some Dicer isoforms are located. Intriguingly, siRNAs derived from PLMVd and CChMVd, which replicate and accumulate in the chloroplast, have also been identified in plants infected by these two viroids [46]. Since it is unlikely that PTGS-like processes may occur in the chloroplast, the most direct interpretation is that these siRNAs are generated in the cytoplasm during viroid movement from cell to cell, a possibility that could also apply to nuclear viroids. This entails that the viroid RNA itself, or some aberrant derivatives thereof resulting from the action of a cytoplasmic RNA-dependent RNA polymerase [43], would be the substrate for generating the siRNAs.

Do viroid-specific siRNAs play any physiological role? Since the accumulation levels of the siRNAs induced by symptomatic and asymptomatic strains of PSTVd and CChMVd are essentially the same [44,46], the in vivo concentration of the siRNAs cannot explain the differences in symptom development. However, an inverse correlation has been found in chloroplastic viroids between the accumulation levels of the mature viroid forms and their corresponding siRNAs: PLMVd and CChMVd reach low in vivo concentrations but their siRNAs are easily detectable, whereas in tissues where the in vivo concentration of ASBVd is very high, the corresponding siRNAs are undetectable [46] or accumulate to low levels [47]. This inverse correlation is consistent with the involvement of the siRNAs in a PTGS defense response of the host that would attenuate the detrimental effect of viroids by lowering their in vivo titer [46], but the reasons of the weak PTGS reaction induced by ASBVd in avocado remain to be determined.

The molecular basis for disease induction by viroids is an enigma and, until very recently, this was also the situation for RNA viruses. However, data obtained with *Turnip mosaic virus* (TuMV), which incites in *Arabidopsis* developmental defects in vegetative and reproductive organs resembling those observed in micro-RNA (miRNA)-deficient *dicer-like1* mutants, show that these defects are due to a TuMV-encoded RNA-silencing suppressor (P1/HC-Pro) [48]. Suppression of RNA silencing is a counterdefense strategy developed by many viruses that in this particular case enables TuMV to infect *Arabidopsis* systemically. On the other hand, miRNAs are small non-coding RNAs of a size similar to the siRNAs and also produced by Dicer – acting on precursor RNAs with a typical hairpin secondary structure – that participate in the regulation of endogenous gene expression in a number of developmental processes [49]. P1/HC-Pro suppressor acts by inhibiting the miRNA-guided cleavage of several mRNAs coding for a family of transcription factors [50], suggesting that interference with miRNA-regulated developmental pathways that share mechanistic links with the antiviral RNA-silencing machinery may explain some of the virus-induced symptoms in plants [48]. A variant of this mechanism could also explain viroid pathogenicity if it is assumed that certain viroid-specific siRNAs, acting like endogenous miRNAs, might target host mRNAs and promote their degradation [44,47]. This possibility is consistent with the observation that minimal changes affecting approximately 1% of the sequence of representative members of both viroid families are sufficient to transform a severe into a latent strain. Moreover, the attenuation for a variable time of the viroid titer and the characteristic symptoms induced by a severe strain in plants that have been pre-inoculated with a mild strain of the same or a closely related viroid (the so-called cross-protection phenomena), may also be explained if the siRNAs generated by the pre-inoculated mild strain target the RNA of the challenging severe strain for its degradation.

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