

## SUMMARY

Viroids represent the lowest step of the biological scale: they consist of unencapsidated, circular, single-stranded, non-protein-coding RNAs of about 250 to 400 nucleotides (nt). Despite their structural simplicity, viroids replicate autonomously in their host plants and elicit symptoms in most instances. Infections by representative viroids produce the accumulation of viroid-derived small RNAs (vd-sRNAs) of 21-24 nt with the characteristics of the small interfering RNAs, the hallmarks of RNA silencing. Identification of vd-sRNAs supports their targeting by the first host RNA silencing barrier formed by Dicer-like RNases. To examine whether vd-sRNAs are loaded by AGO proteins —at the core of the RNA-induced silencing complex (RISC) that forms the second RNA silencing barrier— leaves of *Nicotiana benthamiana* infected by potato spindle tuber viroid (PSTVd) were agroinfiltrated with nine of the ten AGOs from *Arabidopsis thaliana*. Immunoprecipitations from the agroinfiltrated halos and Western- and Northern-blot analyses revealed that all AGOs were expressed and, except AGO6, AGO7 and AGO 10, bound vd-sRNAs: AGO1, AGO2 and AGO3 those of 21 and 22 nt, while AGO4, AGO5 and AGO9 bound additionally those of 24 nt. Deep sequencing revealed that the agroexpressed AGO1, AGO2, AGO4 and AGO5 bind vd-sRNAs according to their size and 5'-terminal nucleotide, with the profiles of the corresponding AGO-loaded vd-sRNAs adopting specific hot spot distributions along the viroid genome. Agroexpression of AGO1, AGO2, AGO4 and AGO5 on PSTVd-infected tissue attenuated the level of the genomic RNAs, indicating that they, or their precursors, are RISC-targeted. In contrast to RNA viruses, PSTVd infection of *N. benthamiana* did not affect miR168-mediated regulation of the endogenous AGO1, which loaded vd-sRNAs with specificity similar to its *A. thaliana* counterpart.

While biogenesis of viroid RNAs is well-known, how they decay is restricted to data involving host RNA silencing. In the course of our studies on PSTVd, we have consistently observed a pattern of 6-7 (+) subgenomic RNAs (sgRNAs) that appear together with the genomic circular and linear RNAs in eggplant, an experimental host of this viroid. Northern-blot hybridizations with full- and partial-length probes revealed that the PSTVd (+) sgRNAs derive from different regions of the genomic RNA, and that some are partially overlapping. Part of the PSTVd (+) sgRNAs were also observed in *N. benthamiana* and tomato, wherein they have been overlooked due to their low accumulation. Analysis by primer extension of representative (+) sgRNAs showed the lack of a common 5' terminus, excluding that they

could result from aborted transcription initiated at one specific site. Further examination by 5'- and 3'-RACE indicated that the (+) sgRNAs have 5'-OH and 3'-P termini, most likely resulting from endonucleolytic cleavage of longer precursors catalyzed by RNases that typically generate these termini. Analysis of PSTVd (-) sgRNAs, which also accumulate in infected eggplant, showed that they present features very similar to those of their (+) counterparts. Our results provide a novel insight on how viroid decay proceeds *in vivo*, possibly during replication, and suggest that synthesis and decay of PSTVd strands might be connected as observed in mRNA.