SUMMARY

Viroids, small circular RNAs (246-401 nt) with a high content in secondary structure that until recently have been detected only in higher plants, are the simplest infectious agents in the biological scale and do not encode any protein. Therefore, they depend on their genomic sequence and structural motifs to use (and even modulate) the transcription, processing, and trafficking machinery of their hosts in order to be replicated and invade them systemically, overcoming the defense barriers they mount and leading eventually to economically important diseases.

The secondary structure of nuclear viroids (family *Pospiviroidae*) is generally rod-like, while in some chloroplastic viroids (family *Avsunviroidae*) it is multi-branched. These conformations are supported by data: i) *in silico*, resulting from algorithms that predict the secondary structure with minimal free energy; ii) *in vitro*, using biophysical methods such as the analysis of electrophoretic mobility, electron microscopy and nuclear magnetic resonance; or biochemical approaches such as analysis in solution with RNases, bisulfite and dimethyl sulfate, and more recently, the acylation of the 2'-hydroxyl groups analysed by primer extension (SHAPE); and iii) *in vivo*, derived from the high genetic diversity of some viroids, site-directed mutagenesis of specific motifs, or UV irradiation.

The assumption that the conformation of the viroid RNAs *in vitro* is similar or even identical to that adopted *in vivo* is questionable due, among other reasons, to the different ionic conditions used in *in vitro* analyses with respect to those existing *in planta*, as well as to a number of interactions with the proteins or other factors in the host. Therefore, in the present Doctoral Thesis, the *in vivo* structures of three viroids have been studied, applying different approaches.

In the eggplant latent viroid (ELVd), taking advantage of its high genetic variability, co-variations and compensatory mutations have been screened in natural variants in order to confirm or refine *in vivo* the structures predicted *in silico* for both viroid strands and those obtained through *in vitro* SHAPE. The results of the three methodologies are consistent for ELVd (+) RNA and lead to a quasi-rod-like conformation with a bifurcation at each terminal domain. This structure, although similar, is not identical to that of ELVd (-) RNA, because its conformation has a central cruciform motif (confirmed *in vivo* by the presence of covariations therein) and because, in addition, both RNAs show different electrophoretic mobilities in native

polyacrylamide gels. The *in vitro* results for ELVd (-) RNA were less consistent with those obtained *in silico* and *in vivo*.

On the other hand, the high accumulation of the monomeric circular *(mc)* positive RNAs of potato spindle tuber viroid (PSTVd) and avocado sunblotch viroid (ASBVd) in *Nicotiana benthamiana,* and avocado respectively, allowed the determination of the *in vivo* structure of both RNAs by SHAPE, enabling their direct comparison with the conformations derived previously *in vitro* using the same technique, and those predicted *in silico*. The structures determined *in vivo* for *mc* PSTVd (+) and *mc* ASBVd (+) RNAs are very similar (but not identical) to those observed *in silico* and by *in vitro* SHAPE. These results provide the first direct evidence that, in their physiological context, the circular RNAs of two viroids, one nuclear and other chloroplastic, are essentially naked and not strongly associated with host proteins. However, we have observed that the conserved central region of *mc* PSTVd (+) RNA, particularly the loop-E involved in replication and other functions, shows a lower SHAPE reactivity *in vivo,* possibly due to interactions with one or more proteins mediating these functions or to structural changes induced by other factors of their natural habitat. The low accumulation of *mc* ASBVd (-) RNA in its host, only allowed for the examination of its structure *in silico* and by *in vitro* SHAPE, leading to a rod-like conformation similar to, but not identical, that of *mc* ASBVd (+) RNA, since the electrophoretic mobility of both RNAs in native polyacrylamide gels is slightly different.