

TESIS DOCTORAL

“Novel insights of viroid biology and host responses to their infection”

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RESUMEN

Viroids are the simplest pathogens with autonomous replication and have only been found naturally infecting higher plants. A deeper understanding of the nature and mode of action of viroids have been the encompassing main goals of the thesis «Novel insights of viroid biology and host responses to their infection». A new efficient method for constructing infectious viroid clones was developed and tested with one viroid of each family: eggplant latent viroid (ELVd, *Avsunviroidae*) and hop stunt viroid (HSVd, *Pospiviroidae*). This procedure was based on type IIS restrictions enzymes that cut outside of the recognition site and supposes a universal procedure for obtaining infectious clones of a viroid independently of its sequence, with a high efficiency. Despite viroids have been considered as plant-pathogenic non-coding RNAs since their discovery, the computational analysis carried out in this research predicted small open reading frames in each of the HSVd and ELVd genomes. No significant similarities with proteins in the database of higher plants were found, but some of these predicted peptides were highly conserved among all HSVd and ELVd variants. Interestingly, the fusion of these conserved sequences to a fluorescent protein revealed a specific subcellular localization in the corresponding organelle where replication/accumulation takes place for each viroid: nucleolus and chloroplast for HSVd and ELVd, respectively. Mutations that truncate the nucleolar domain of HSVd were detrimental for the viroid while truncating any of the two ELVd ORF that contains a chloroplast transit signal also diminished (but to a lesser extent) viroid biological efficiency, maybe because of functional redundancy. Circular forms of both, HSVd and ELVd RNAs were found in polysome fractions, revealing their physical interaction with the translational machinery of the plant cell. Altogether, these experimental observations indicate that the coding capacity of viroids cannot be ruled out, although the definitive evidence is a technological challenge to be addressed in the future. Finally, to study the host changes that are produced during a symptomatic viroid infection, an integrative analysis of the timing and intensity of the genome-wide alterations in cucumber plants infected with HSVd was performed. Differential host transcriptome, sRNAome and methylome were integrated to determine the temporal response to viroid-infection. The results support that HSVd promotes the redesign of the cucumber regulatory-pathways predominantly affecting specific regulatory layers at different infection-phases. Overall, this research contributes to the first comprehensive map of the plant responses to a viroid infection.

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The examining board was formed by Miguel Aranda Regules (Centro de Edafología y Biología Aplicada del Segura CEBAS, CSIC), Marco de la Peña Del Rivero (IBMCP, CSIC-UPV) and Selma Gago Zachert (Institute of Biochemistry and Biotechnology, Martin Luther University), who granted the thesis as outstanding.

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