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Poster Abstract – E.13

TRANSPLASTOMIC EXPRESSION IN *NICOTIANA TABACUM* OF A NON-PROTEIN-CODING VIROID RNA

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Viroids are small non-protein-coding circular RNAs infecting plants. They directly interact with their host transcription and RNA trafficking machineries for replication, subcellular localization and spreading and, as a side effect, they frequently elicit diseases. Viroids are useful tools for dissecting structural-functional relationships of RNA in plants. While members of the family *Pospiviroidae* replicate and accumulate in the nucleus, *Peach latent mosaic viroid* (PLMVd) and the other members of the family *Avsunviroidae* replicate and accumulate in the chloroplast. Interestingly, chloroplast-replicating viroids are some of the few RNAs able to cross the plastid double membrane, suggesting that RNA trafficking pathways remain to be discovered in plants. Further progress into the underlying molecular mechanisms is hampered by the host range of chloroplast-replicating viroids, which is restricted to the very few plants wherein they have been isolated. Because none of these plants is a model organism, we are exploring alternative systems and, more specifically, the possibility of using for this purpose the non-host *Nicotiana tabacum.* As a first step in this direction, we have generated transplastomic plants with head-to-tail dimeric constructs of PLMVd under the control of a promoter that can drive transcription by the two known chloroplastic RNA polymerases: the plastid-encoded polymerase (PEP) and the nuclear-encoded polymerase (NEP). Analysis by Southern-blot hybridization has identified several homoplasmic lines, and preliminary results from Northern-blot hybridization and RT-PCR indicate that the corresponding transcripts are expressed in some of them. Additional work should clarify whether these primary transcripts are correctly processed into the viroid circular forms, and whether they then act as templates for RNA-RNA transcription like in the natural host. This information may be relevant for understanding the limited host range, which could result from specific access into the chloroplast or from specific factors needed for replication. Transplastomic systems may therefore serve not only for expressing foreign proteins but also for getting insights into the behaviour of non-protein-coding RNAs.