Evidence for a model ancestral viroid

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ABSTRACT
The generation of a phylogenetic tree of viroids and viroid-like plant satellite RNAs via computer analysis, coupled with several conspicuous biochemical characteristics of the rolling circle replication of these RNAs -including both self-cleavage and self-ligation- leads us to propose the peach latent mosaic viroid (PLMVd) as a current "living fossil" dating from a precellular world. Incorporated within this proposal is a revised mechanism of PLMVd rolling circle replication which requires a minimal protein involvement.

INTRODUCTION
Viroids are small (246-375 nt) single-stranded circular RNAs infecting higher plants causing diseases in numerous crop species which result in significant economic losses in the agricultural industry (1,2). Both viroids and "viroid-like" plant satellite RNAs have been proposed as examples of "living fossils" from a precellular world (see Ref. 3). The fundamental belief of this hypothesis is that these biological macromolecules exhibit both genotypical and phenotypical functions.

Using the numerous viroid sequences published during the last five years, we have refined the phylogenetic analysis of viroids and viroid-like plant satellite RNAs (4). This modification of the phylogenetic tree was achieved using the maximum parsimony algorithms included in the PAUP package (5). This analytical method, coupled with the use of a larger number of species, permitted the prediction of a more parsimonious tree than any published to date (6-8). The resulting tree, which is summarized in figure 1, supports a monophylegetic origin of these RNAs as proposed previously (3). As illustrated in figure 1, the viroid sequences are clustered in three groups (potato spindle tuber viroid (PSTVd) group, apple scar skin viroid (ASSVd) group and avocado sunblotch viroid (ASBVd) group), while satellite RNAs are clustered together in a fourth group. The cluster formed by the ASBVd, the PLMVd and the carnation stunt associated viroid (CarSAV, which is not yet formally classified as a viroid) supports the existence of the ASBVd group. The members of this group, which appears as an evolutionary link between the classical viroids and the related satellite RNAs, have the capacity to self-cleave by hammerhead structures.

Here, we report several experiments designed to identify the member of the ASBVd group that most closely resembles the potential ancestral organism of both classical viroids and satellite RNAs.

PHYLOGENETIC EXPERIMENTS
Two different approaches were used for rooting the proposed tree. Firstly the satellite RNAs were considered as the outgroup taxa; and, secondly, a minimal number of species, including members of each group, were used to computer generate phylogenetic trees by the exhaustive method using various outgroup taxa (including viroid-like domains of either the mitochondria transcripts from satellite 2 DNA or the human delta hepatitis virus, RNA replicon domains, etc). These analyses produced no precise root as no selection of outgroup taxa was perfect. However, all tested outgroups permitted the rooting of the tree in the first part of the ASBVd group branch (fig. 1). These results support the hypothesis that ASBVd group is an evolutionary link between the classical viroids and the satellite RNAs. Furthermore, these results suggest that the hammerhead motif was part of the ancestral viroid world as it is found in the members of ASBVd group.

BIOLOGICAL CHARACTERISTICS
To determine whether a viroid from the ASBVd group may be a current "living fossil", we considered their known

Figure 1.
Schematic representation of the proposed phylogenetic tree of viroids and viroid-like plant satellite RNAs. The triangles are the lineages of the PSTVd group and ASSVd group. The triangle size represents the diversity of these lineages. For ASBVd group, each member is specifically identified. The circle (bold) defines the region where several taxa rooted the tree.
RNA of PSTVd), ROLLING circle mechanism of viroids has been proposed that involves self-ligation and self-cleavage. This mechanism suggests that the RNA folds into a stable rod-like structure which brings both termini into the close proximity required for self-ligation. This reaction may be catalyzed by a protein cofactor or catalyzed by a protein in vivo. The self-ligation has the advantage of favoring the formation of a 2',5'-phosphodiester bond which prevents further intramolecular self-cleavage. The same three steps are repeated to yield the progeny viroids. In contrast to the proposed replication mechanism for classical viroids, which requires different enzymes for each step, only the polymerization described here requires the involvement of an exogenous enzymatic activity. This simplified rolling circle mechanism could be important in the replication of ancestral viroids because of the minimal requirement for exogenous enzymatic activity.

The sequences required for both the self-cleavage and self-ligation of PLMVd are localized in the left arm region and concentrated in ~100 nt of this viroid. This region is the replicational domain of PLMVd and could have been shared with an ancestral viroid.

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