Nucleic Acids Symposium Series No. 33 143–144

Evidence for a model ancestral viroid

Frédéric Bussière, Daniel Lafontaine, Fabien Côté, Danièle Beaudry and Jean-Pierre Perreault*
Département de Biochimie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec J1H 5N4, Canada

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 that infect 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 monophylogenetic 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 hepatitus 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

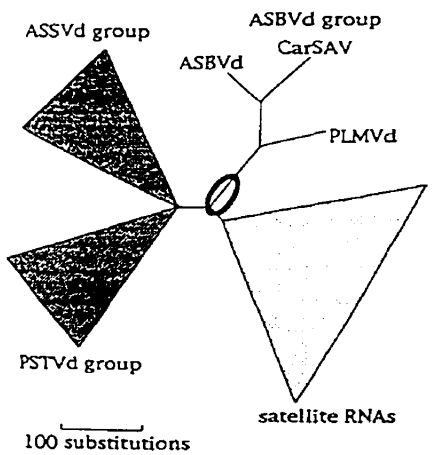

Figure 1.
Schematic representation of the proposed phylogenetic tree of viroids and viroid-like plant satellite RNAs. The triangles are the lineages of the PSTVd group and ASSVd group. The triangle size represents the diversity of these lineages. For ASBVd group, each member is specificaly identified. The circle (bold) defines the region where several taxa rooted the tree.

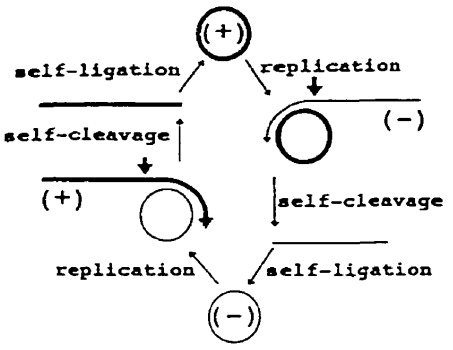

Figure 2.
Simplified rolling circle mechanism proposed for PLMVd (see text for description).

replication characteristics. We characterized the hammerhead self-cleavage of dimeric, monomeric and mutated transcripts derived from RNA of both polarities of PLMVd (9). Several experiments demonstrated that transcripts of both polarities derived from PLMVd self-cleave exclusively by single hammerhead structures. This observation contrasts with the case of ASBVd and CarSAV whose mechanisms involve mostly double hammerhead structures. Single hammerhead cleavage is associated with viroid-like satellite RNAs, and this PLMVd characteristic suggests that it may be the more likely candidate as a current "living fossil" dating from the precellular world.

Furthermore, we have demonstrated the non-enzymatic self-ligation of transcripts both polarities of PLMVd (10), the first such description of this process with viroid sequences. Self-ligation occurs when the 5'-hydroxyl and the 2'-3'-cyclic phosphate termini produced by the hammerhead self-cleavage of the viroid RNA are juxtaposed by the viroid rod-like structure, and a phosphodiester bond is formed between the two following hydrolysis of the cyclic phosphate. The intramolecular self-ligation of PLMVd transcripts could contribute to the production of properly circularized viroids during rolling circle replication. In addition, these results suggest that an ancestral viroid may not require the existence of a specific ligase to produce circularized progeny.

ROLLING CIRCLE MECHANISM

The discovery of both the self-ligation and the self-cleavage behavior of PLMVd transcripts suggests a reworking of the rolling circle mechanism of PLMVd replication (Fig. 2). Initially, the plus polarity circular RNA is copied to yield a RNA of minus polarity. In contrast to classical viroids (like PSTVd), the RNA polymerase II from wheat germ did not support the replication of PLMVd (unpublished data, F. Lareau and J.-P. Perreault) and ASBVd (11), in fact the RNA polymerase responsible for this step remains to be identified. Secondly, once the hammerhead motif is synthesized, the transcript rapidly self-cleaved. This reaction occurs concurrently with the polymerization step, hence no accumulation of long multimeric replicates is expected for PLMVd. Thirdly, the polymerization of the minus polarity RNA is persued, and, when a unit-length transcript is complete, the RNA folds into its stable rod-like structure which brings both termini into the close proximity required for self-ligation. This last reaction may be partly nonenzymatic, enhanced by a protein cofactor or catalyzed by a protein *in vivo*. The self-ligation has the advantage of favorizing 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 polymerisation 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.

ACKNOWLEDGEMENTS

This work was sponsored by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to J-PP. FL, DL and FC are in receipt of predoctoral fellowships from respectively Fondation George Phoenix, Fonds FRSQ-FCAR and Université de Sherbrooke. J-PP is a Scholar of the Medical Research Council of Canada (MRC).

REFERENCES

1. Symons,R.H. (1990) *Semin. Virol.*, **1**, 75-81.
2. Diener,T.O. (1993) *Trends Microbio.*, **1**, 289-294.
3. Diener,T.O. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 9370-9374.
4. Bussière, F., Lafontaine,D. and Perreault,J.-P. (1995) unpublished results.
5. Swofford,D.L. (1992) PAUP-Phylogenetic Analysis Using Parsimony. Version 3.0 s.
6. Elena,S.F., Dopazo,J., Flores,R., Diener,T.O. and Moya,A. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 5631-5634.
7. Hernandez,C. and Flores,R. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 3711-3715.
8. Hernandez,C., Elena,S.F., Moya,A. and Flores,R. (1992) *J. Gen. Virol.*, **73**, 2503-2507.
9. Beaudry,D., Bussière,F., Lareau,F., Lessard,C. and Perreault,J.-P. (1995) *Nucleic Acids Res.*, **23**, 745-752.
10. Lafontaine,D., Marquis,P., Beaudry,D. and Perreault,J.-P. (1995) submitted to *J. Virol.*
11. Lima,M.I., Fonseca,M.E.N., Flores,R. and Kitajima,E.W. (1994) *Arch. Virol.*, **138**, 385-390.

*To whom correspondence should be addressed