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Are viroids escaped introns?

(split genes/RNA splicing/small nuclear RNAs/U1 RNA)

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ABSTRACT **A complex of considerable stability is possible between the 5′ end of U1 RNA and a specific nucleotide sequence of the potato spindle tuber viroid complement. Because base-pairing between the 5′ end of U1 RNA and the ends of introns is believed by some to be responsible for the precise alignment and correct excision of introns, the U1-related sequence may represent the two ends of a presumed intron ancestor of the viroid complement after circularization.**

Viroids—covalently closed circular single-stranded RNAs of low molecular weight ($1.1–1.3 \times 10^5$)—are the smallest known agents of infectious disease (for a review, see ref. 1). Their unique molecular structure has been elucidated to a large extent (2), and the complete nucleotide sequence of the potato spindle tuber viroid (PSTV) is known (3). Little is understood, however, of the mechanisms of viroid replication and pathogenesis (4). Viroid-encoded proteins do not appear to be involved (5, 6). Thus, replication seems to depend on preexisting host enzymes, and disease induction is most readily understood by postulating interference of the viroid with gene regulation (4). The origin of viroids is unknown but, with the discovery in eukaryotic organisms of split genes and RNA splicing (for review, see ref. 7), it has been suggested (4, 7) that viroids might have originated by circularization of excised intervening sequences (introns).

Originally, introns were regarded as "nonsense" sequences (7) and were believed to be degraded after excision, but more recently it has been shown that some introns, at least, code for portions of separate proteins that apparently exert regulatory functions (8). Also, circularization of introns has been demonstrated (8, 9), including some with the approximate size of viroids (8). One might speculate that, if such excised, circularized sequences would permit extensive intramolecular base-pairing (as do viroids), they might become stabilized and thus escape degradation. Furthermore, if such introns would comprise appropriate recognition sequences, they might be transcribed by a host enzyme capable of functioning as an RNA-directed RNA polymerase and thus escape the control mechanisms of the host cell.

Small nuclear RNAs (snRNAs) that are associated with ribonucleoprotein particles are believed by some to be involved in the processing of the primary transcription products of split genes (10–12). The 5′ end of one such RNA, U1, has been shown to exhibit complementarity with the ends of introns (11–13), and it is believed that this affords a mechanism ensuring correct excision of the intron sequences and accurate joining of the coding sequences (11–13).

Although the primary structures of higher plant snRNAs are unknown, the recent demonstration of a split gene in a higher plant species (14) and the similarities of its intron–exon boundary sequences with those of other eukaryotes (11) suggest that an snRNA homologous to mammalian U1 RNA (15) exists in higher plants and that its 5′-end sequence resembles that of the latter. If so, the intron theory of viroid origin predicts that a specific nucleotide sequence on viroids exhibits complementarity to the 5′ end of this putative plant snRNA, as well as to that of U1 RNA.

It therefore was of interest to determine whether the nucleotide sequence of PSTV contains stretches of significant complementarity with the 5′ end of U1 RNA, but a search for such sequences failed to reveal the possibility of stable complexes between the two RNAs.

However, because of the presence in infected plants of viroid-complementary RNA (16, 17) (from which, presumably, progeny viroid molecules are transcribed), it is equally plausible that viroid complements, not the viroids themselves, represent escaped introns and exhibit complementarity with U1 RNA.

Fig. 1 shows that a complex of considerable stability is indeed possible between the 5′ end of U1 RNA and nucleotides 257–279 of the PSTV complement. The hypothetical splice junction is located between nucleotides 262 and 263. The proposed intron deviates from a suggested consensus sequence (11) by ending with G-G instead of A-G. At least one mammalian intron, however, is known to end with G-G and not with A-G (11). The stability of the complex, as determined by the procedures of Tinoco *et al.* (18) and Salser (19), is about −21.6 kcal/mol (1 cal = 4.18 J) for the presumed acceptor sequence and −11.9 kcal for the donor sequence—a total of about −33.5 kcal. This value compares favorably with values reported for complexes between the splicing signals of two introns in the chicken α2-collagen (type 1) gene and the 5′ end of U1 RNA (−14.65 to −31.25 kcal) (13).

Although the striking complementarity possible between the 5′ end of U1 RNA and the PSTV complement may be a fortuitous coincidence, the high stability of the complex, compared with stabilities of genuine splicing sites, rather tends to support a functional role of this nucleotide sequence in the precise excision of a presumed intron ancestor of the PSTV complement.

This hypothetical model has several experimentally testable corollaries.

(*i*) There should exist in higher plants a RNA homologous to mammalian snRNA U1 with a nucleotide sequence at the 5′ end similar to that of the latter.

(*ii*) All viroid complements (or possibly viroids) should have in common a sequence complementary to the 5′ end of this snRNA.

(*iii*) Molecular hybridization between a probe capable of detecting PSTV sequences [such as PSTV cDNA (20) or cloned PSTV-complementary recombinant DNA (17)] and plant snRNA should yield hybrids of low stability.

(*iv*) If the PSTV complement is derived from an intron, PSTV-

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Abbreviations: PSTV, potato spindle tuber viroid; snRNA, small nuclear RNA.

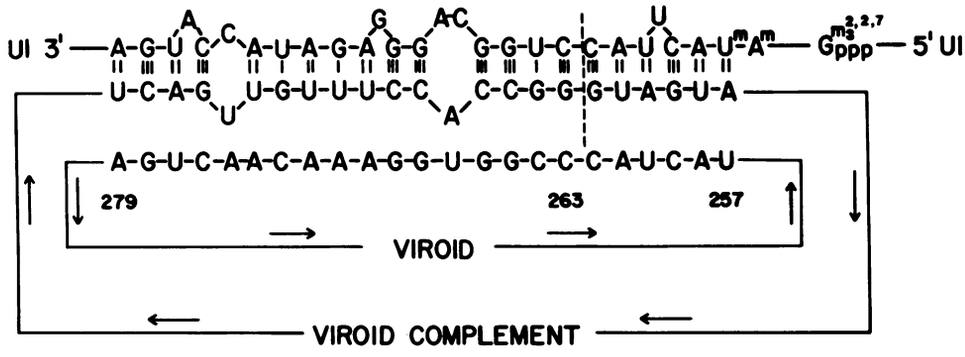

FIG. 1. Possible base-pairing interactions between the PSTV complement and the 5′ end of U1 RNA. -----, Hypothetical splice junction.

related sequences should exist in the cellular DNA of its original host. Although the presence of such sequences in the cellular DNAs of certain PSTV hosts has been reported (21), more recent work has shown that tomato DNA does not contain detectable PSTV-related sequences (22–24). It is possible, however, that a postulated intron ancestor of PSTV may have existed in a species that has not so far been tested for viroid-related DNA sequences.

The model suggests a mechanism of PSTV pathogenesis. Because PSTV contains a sequence that is homologous to the 5′ end of U1 RNA, it may interfere with the splicing process mediated by the latter's plant equivalent.

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