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The DNA form of a retroviroid-like element is involved in recombination events with itself and with the plant genome

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Abstract

Carnation small viroid-like RNA (CarSV RNA) is unique among plant viroid-like RNAs in having a homologous DNA counterpart. In the present study, we found the most abundant CarSV DNA form (275 nt) coexisting with other smaller and longer-than-unit forms. Further analysis of PCR-amplified products revealed the presence of CarSV DNA-related sequences integrated in the plant genome, fused to microsatellite-like genomic sequences. Six to seven nucleotides at the boundaries in the CarSV DNA sequence could be found in the genomic sequences and also delimiting the boundaries of an enlarged version with partial duplication. This suggests that a common mechanism might have played a role in their emergence, namely, polymerase pausing and switching between stretches of homologous sequences. These plants also contained deleted CarSV DNA mutants with boundaries near those observed with fused sequences.
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Keywords: CarSV RNA–DNA; Retroviroid-like element; Microhomology; Recombination; Integration; Secondary structure

Introduction

Carnation small viroid-like (CarSV) RNA is a unique member among subviral RNA molecules as it lacks infectivity, characteristic of viroid molecules, and also exists as a homologous DNA counterpart (Daròs and Flores, 1995b; Hegedűs et al., 2001). CarSV DNA is organized as a series of head-to-tail multimers forming part of extrachromosomal elements in which CarSV DNA sequences are fused to sequences of carnation-etched ring virus (CERV), a plant pararetrovirus (Vera et al., 2000). The term retroviroid-like element was proposed for the CarSV RNA–DNA system, which is so far the only such system described.

The RNA form of the element shares some structural features with viroids and viroid-like satellite RNAs (reviewed in Diener, 2001): it exists as a covalently closed, circular molecule and replicates autonomously by adopting hammerhead structures in both polarity strands that self-cleave accordingly (Hernández et al., 1992). In addition to the predominant 275-nt CarSV RNA, several coexisting RNA forms with duplications and deletions have been observed and divided into groups. Each group showed heterogeneities in the exact positions of deletions or duplications. In addition to major deletions and duplications, minor sequence heterogeneities were observed between the cDNA clones. A copy-choice model was proposed for the emergence of deletions and duplications, in which the RNA polymerase with the nascent strand dissociates from the template at regions rich in secondary structure and reinitiates synthesis at different positions (Daròs and Flores, 1995a). Sequence repetitions, but not deletions, have been found to occur naturally in two other small circular RNAs: coconut cadang–cadang viroid (CCCVd) (Haseloff et al., 1982; Keese and Symons, 1985) and citrus exocortis viroid (CEVd) (Fadda et al., 2003; Semancik et al., 1994). The structure and sequence of the CarSV RNA (+) hammerhead show extensive similarities with that of the genomic satellite 2 transcript of the newt *Notophthalmus viridescens* (Daròs and Flores, 1995b) that, together with the schistosome satellite DNA transcript and the satellite pDo500 family transcript of certain cave crickets, is the only animal RNA known to have a hammerhead structure; these RNAs are transcribed from tandemly repeated DNA sequences (Epstein and Gall, 1987; Ferbeyre et al., 1998; Rojas et al., 2000). The VS RNA found in the mitochondria of certain

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Neurospora isolates, which self-cleaves and ligates although through a nonhammerhead ribozyme, is also transcribed from a low-copy double-stranded circular VS plasmid DNA (Kennell et al., 1995). The models for the maintenance and propagation of the repeated genomic DNA copies of satellite 2 from the newt and satellite α DNA from schistosomes involve the action of a reverse transcriptase, with the resulting transcripts being then integrated into the genome (Ferbeyre et al., 1998; Green et al., 1993). The existing evidence indicates that the *Neurospora* VS plasmid DNA also replicates via reverse transcription (Kennell et al., 1995). A similar mechanism was proposed for the emergence of CarSV DNA molecules in plants coinfected by CarSV RNA and CERV, probably making use of the reverse transcriptase encoded by the virus. There are short stretches of nucleotides common to both CarSV and CERV in the extrachromosomal fusion element that suggest a polymerase-driven mechanism for their origin, most likely involving the viral reverse transcriptase. Some of the DNA forms of previously characterized CarSV RNAs with sequence dele-

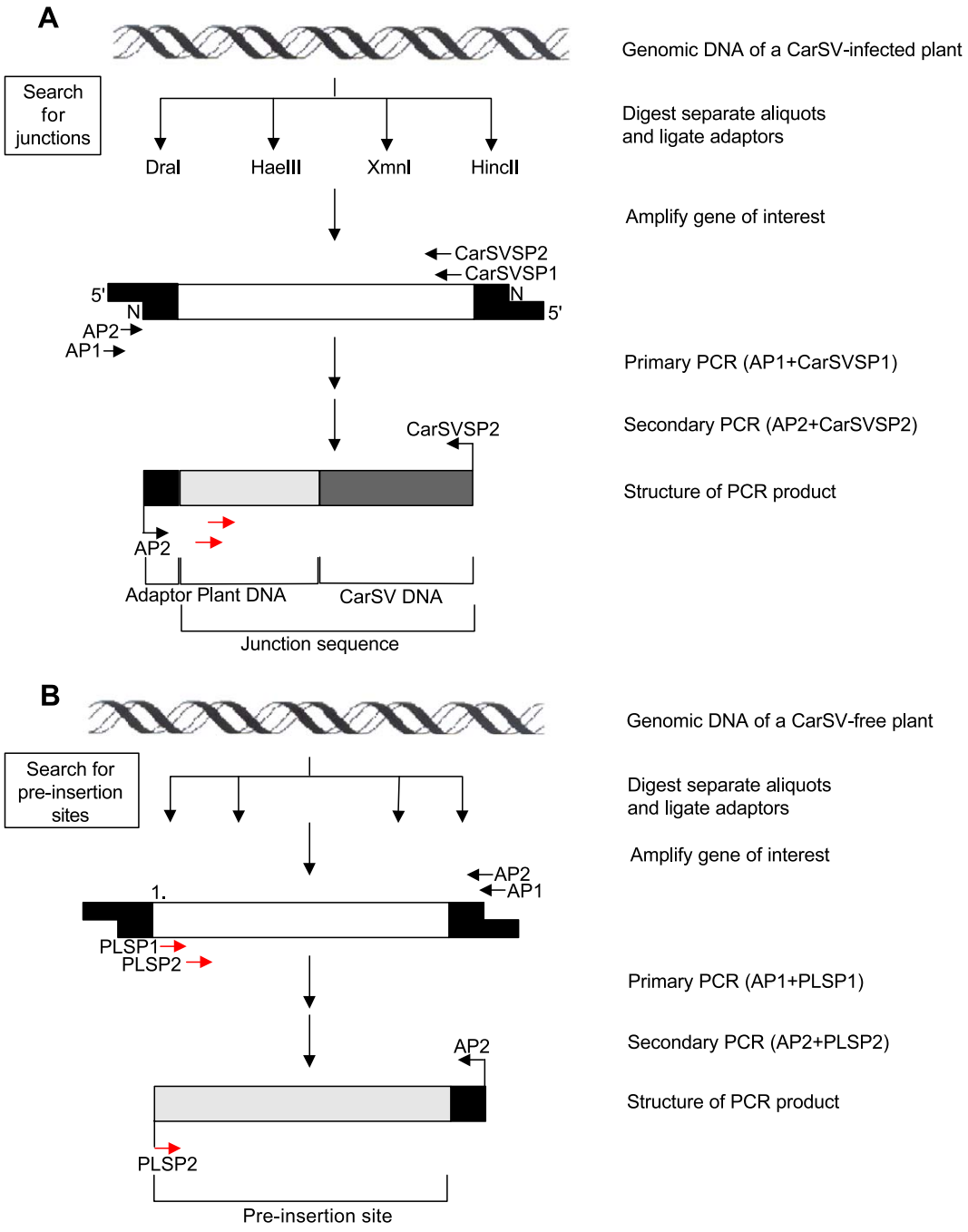


Fig. 1. Flow chart of the isolation of CarSV–plant junctions (A) and preinsertion sites (B). Number 1 refers to the numbering in Figs. 2C and 3B. AP: adaptor primer. CarSVSP: CarSV-specific primer. PLSP: plant-specific primer (red arrows in A and B).

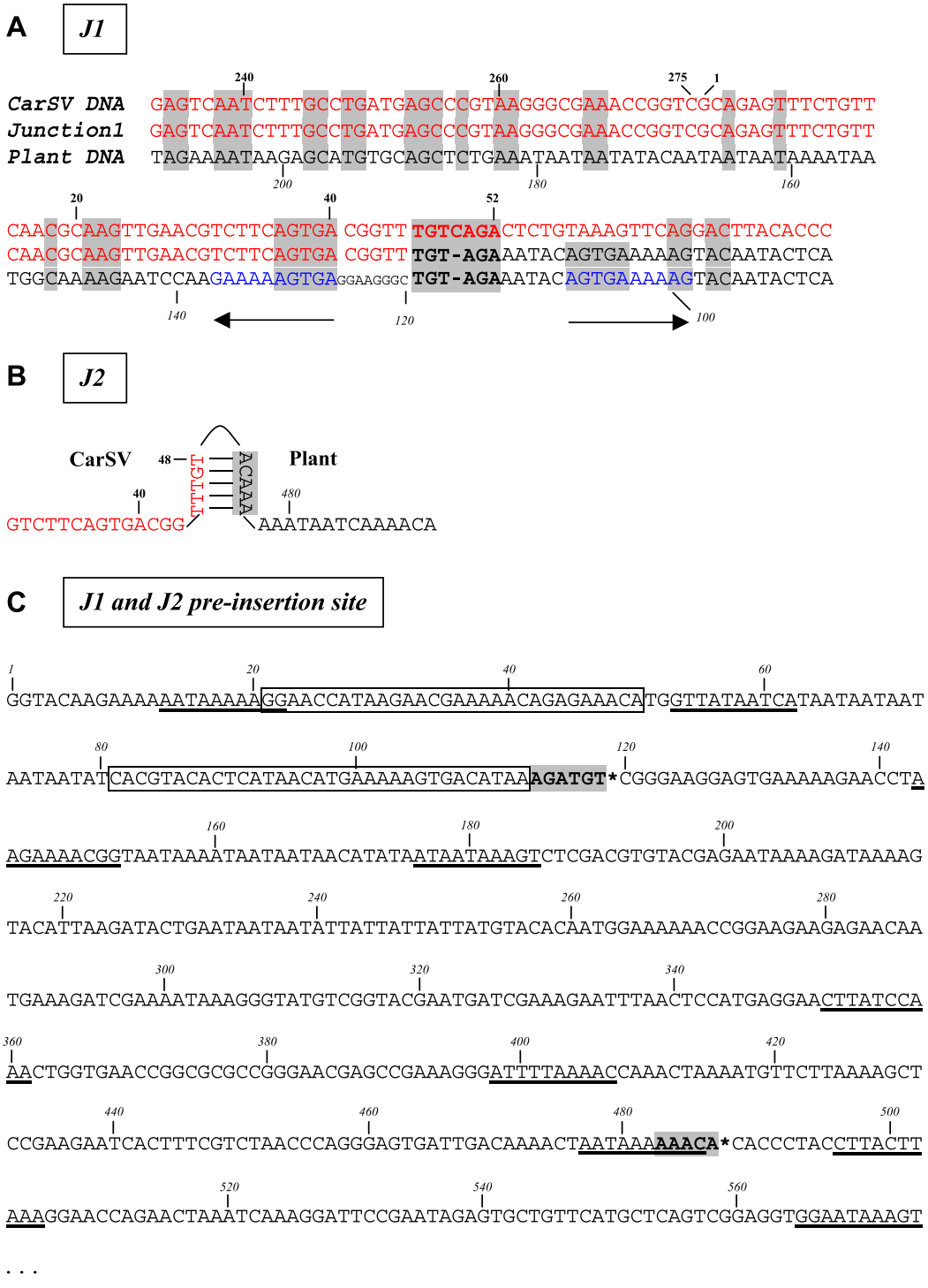


Fig. 2. Characteristics of CarSV–plant junction sequences (J1 and J2) and their preinsertion sites. (A) J1 at the site of recombination is represented in three strands shown in the 5′–3′ orientation. The middle strand is the CarSV–plant DNA junction sequence, which is aligned with CarSV DNA (red) and plant DNA (black) sequences of the same region. Grey-shaded regions indicate sequence identity with that at the junction site being shown in bold. Blue letters and arrows represent an inverted repeat. CarSV DNA is numbered in bold with respect to the reference sequence (GenBank accession no. X68034) and numbers in italics refer to plant DNA numbers in C. Sequences are aligned to obtain the best fit. (B) J2 at the site of recombination, a hairpin-like structure probably involved in junction formation. (C) Preinsertion site of J1 and J2. Junction sites are shown by two consecutive bold asterisks and the nucleotides involved in junction formation are shaded grey. Nested primers used to isolate and clone this site are boxed. TATA-like patterns are underlined.

tions, but not duplications, were identified together with other CarSV DNA forms with deletions not found in any RNA species so far (Vera et al., 2000).

The observation of a growth abnormality consisting of extensive shoot proliferation in cultivated carnations in Hungary prompted the molecular analysis of these plants, in which both CarSV RNA and DNA forms were detected. Several CarSV DNA sequences were characterized in various *Dianthus caryophyllus* cultivars that were symptomless or showed different symptoms. CarSV DNA forms showing minor sequence heterogeneities and deletions occurred in the same plant, and unit-length CarSV DNA sequences were found to accumulate in the plant cell nucleus (Hegedűs et al., 2001). Our present study, aimed at the localization of CarSV DNA forms within the plant cell nucleus, has revealed the presence of short nucleotide stretches common to both CarSV DNA and the plant genome that might have promoted an integration event, a situation similar to that proposed for the CarSV–CERV junctions (Vera et al., 2000). Microsimilarity-mediated nonhomologous recombination between the plant genome and sequences of various sources including T-DNA sequences (Brunaud et al., 2002; Kumar and Fladung, 2002) and transgenes (Kohli et al., 1999; Takano et al., 1997) has been previously reported, and a similar process appears to occur also in mammalian cells (Roth and Wilson, 1986).

Results

Strategy for cloning CarSV DNA–plant genomic fusions

Previously, we analyzed several *D. caryophyllus* L. cultivars for the presence of CarSV RNA and its corresponding

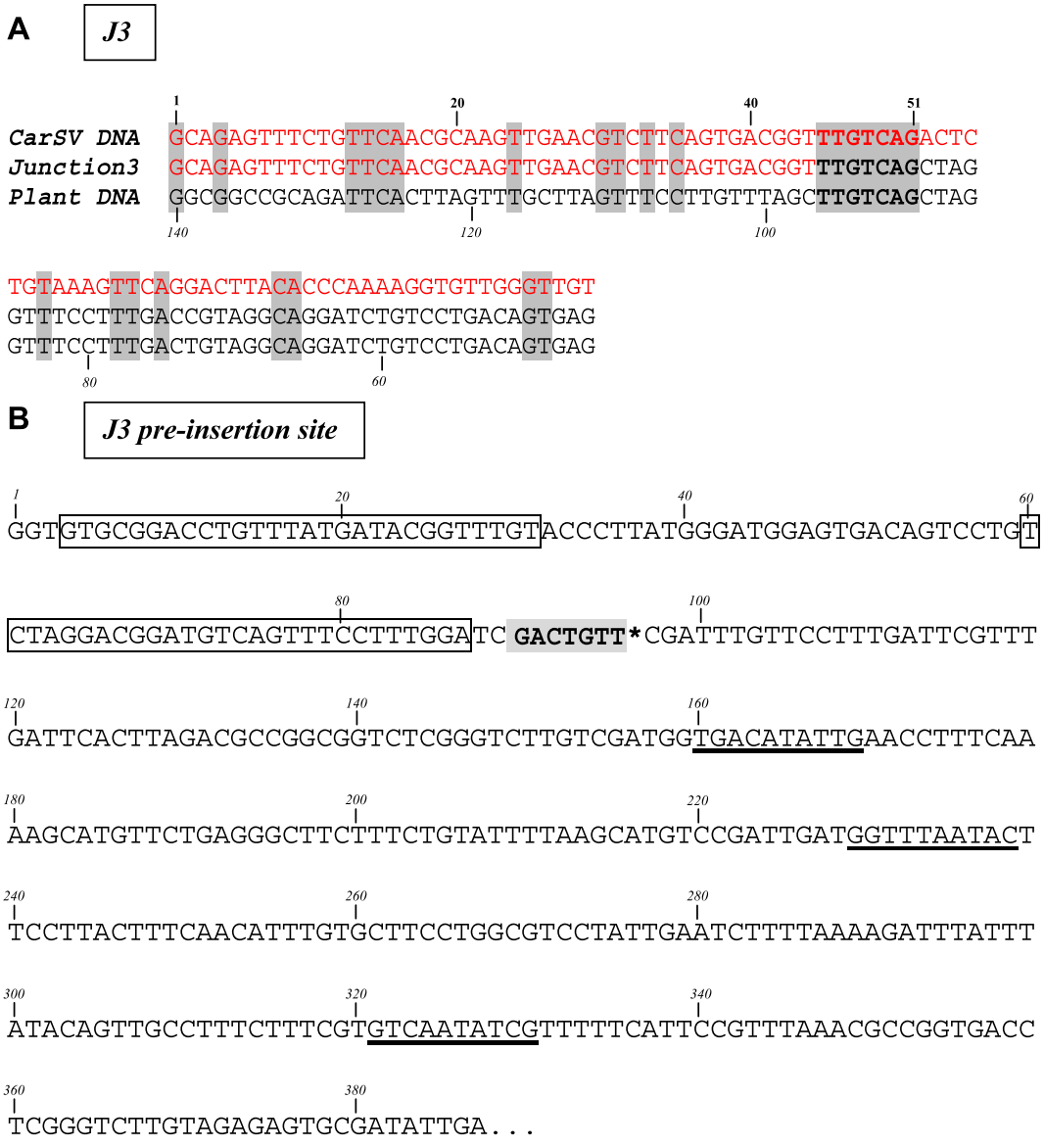


Fig. 3. Characteristics of CarSV–plant junction sequence J3 (A) and its corresponding preinsertion site (B). Symbols are the same as in Fig. 2.

DNA form. Although CarSV RNA could be detected by Northern blot analysis, a more sensitive approach was necessary for CarSV DNA. Southern hybridization using 10 pg of cloned CarSV DNA as positive control gave no signal with a CarSV-specific probe (Daròs and Flores, 1995b). As a more sensitive approach, a PCR-based method was necessary in the localization of CarSV DNA, which was previously detected within the plant cell nucleus (Hegedűs et al., 2001). The detection of fusion sequences was carried out using a nested PCR amplification strategy and a GenomeWalker Kit (Clontech). Each of the resulting DNA fragments begins with a known sequence at the 5′ end of the second viroid-specific primer and extends into the genomic fragment (Fig. 1A). The first library was designed using the genomic DNA of a

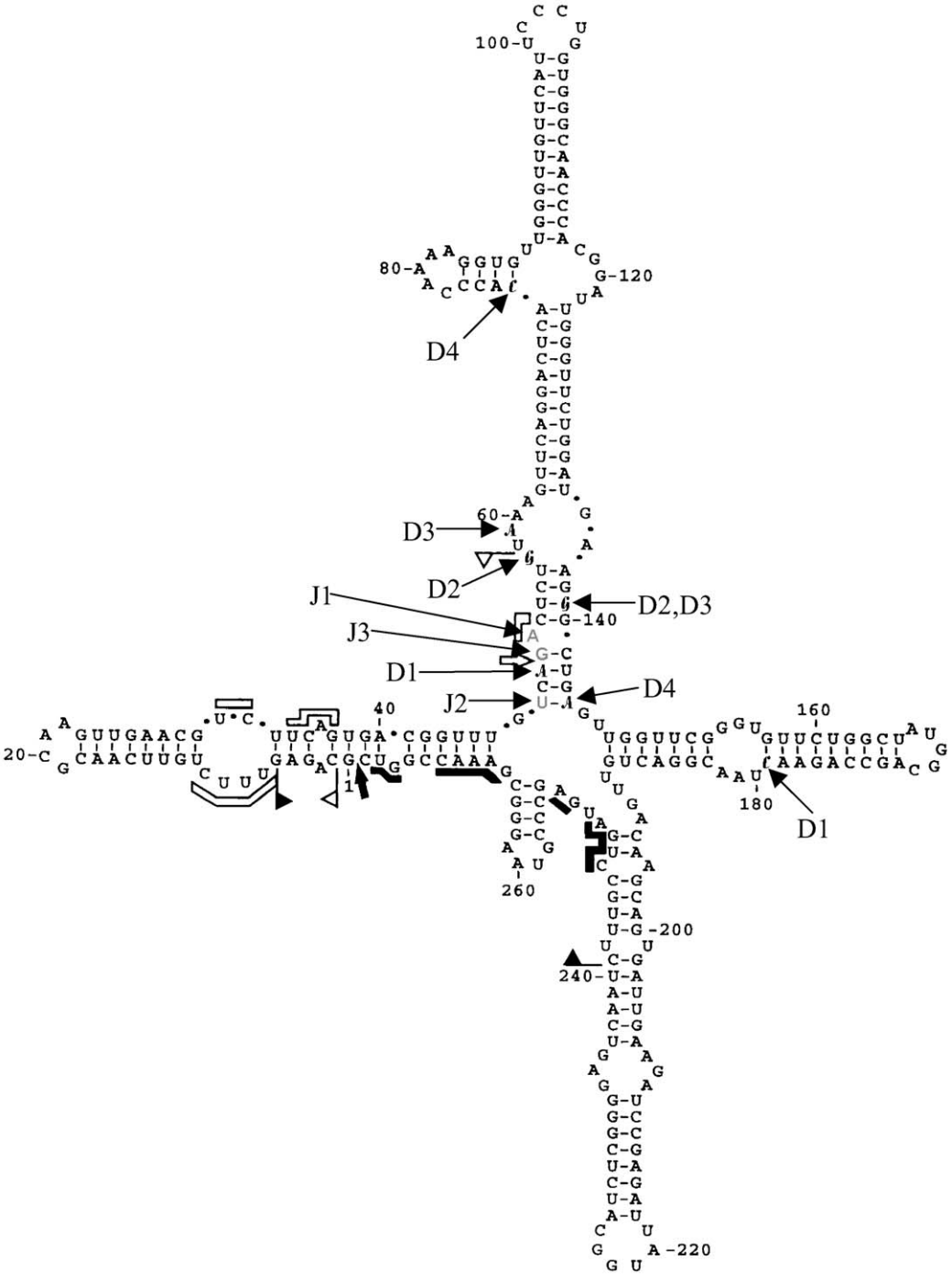


Fig. 4. Recombination sites of CarSV DNA represented on the secondary structure proposed for CarSV RNA. The self-cleavage domains of both polarities are delimited by flags, the 13 residues conserved in most natural hammerhead structures are indicated by bars, and the self-cleavage sites are indicated by arrows. Solid and open symbols refer to the plus and minus polarities, respectively, as previously depicted by Hernández et al. (1992). Arrows denote nucleotide borders for junctions (J) and deletions (D).

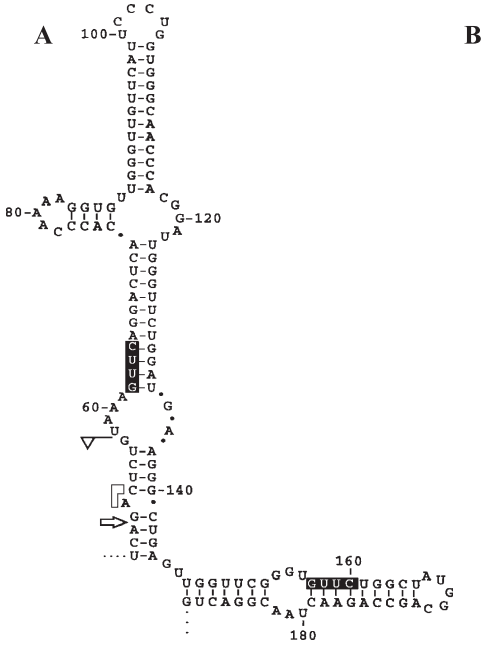
CarSV-infected *D. caryophyllus* plant of cultivar (cv.) Praline. Then, to test whether integration was a unique event or not, we searched for similar fusion sequences in a second (also CarSV-infected) *D. caryophyllus* cv. Tanga library. The same CarSV–plant genomic fusion sequences were present in the second library. The fusions named J (junction) 1, J2, and J3 were used to design a nested pair of primers that was used in combination with adapter-specific primers in PCR amplifications of a CarSV-free library to clone and sequence the possible integration target sites (Fig. 1B).

Sequence and structure of junctions

Our approach to study plant genomic loci with and without CarSV DNA-related sequences revealed the existence of a six (J1) and a seven (J3) nucleotide-length microsimilarity between junction sites of CarSV DNA and the plant genome. Several other regions of microsimilarity were detected around the junction sites of J1 and J3, probably stabilizing the recombination intermediates (Figs. 2A and 3A). In J1, near the border, a perfect homology of five nucleotides was also observed forming part of a larger inverted repeat flanking the junction site (Fig. 2A). Such short homologous regions did not appear in J2. However, a 10-nt long palindromic sequence (5′-TTTGTACAAA-3′) at the junction site might have enhanced base pair formation, and the junction region was potentially capable of adapting a hairpin-like structure thus promoting intermolecular polymerase switching (Fig. 2B). The plant genomic regions of J1 and J2 might be part of the same locus that is the target of two different integration events in a distance of only 367 nt (Fig. 2C). Junction regions of the viroid-like element (Fig. 4) are part of the CarSV RNA minus hammerhead structure (Hernández et al., 1992), which is a stable secondary structure formed during the replication cycle of the element.

Characteristics of preinsertion sites

Several studies have previously underlined the importance of not only short homologous sequences in the genomic integration of transgenes through illegitimate recombination, but also of the topological genome structure in the region of preinsertion sites (Kohli et al., 1999; Sawasaki et al., 1998; Takano et al., 1997). According to these studies, a high AT content at or near to the junction site seems to be a common characteristic. The sequences of the plant DNA junction sites of J1 (Fig. 2C, nt 1–119) and J2 (Fig. 2C, nt 1–487) indeed presented high AT contents of 74% and 68%, respectively. These border sequences have other common characteristics: runs of As and Ts (also in J3), and repeated patterns similar to widespread microsatellite sequences. In the genomic border of J1, 101 out of the 113 nt were similar (71%) to the genomic scaffold region of *Drosophila* (GenBank accession no. AE002607). Similar AT-rich sequences carrying scaffold attachment region (SAR)-like sequences were previously suggested to be common integration targets (Sawasaki et al., 1998; Takano et al., 1997). Genomic adjacent sequences bear TATA-like similar patterns (Figs. 2C and 3B) that might be indicative of possible chromatin accessibility in these regions.



B

(1-65)
GCAGAGTTTCTGTTCAACGCAAGTTGAAC
GTCTTCAGTGACGGTTTGTCAAACTCTGT
AAA**GTTC**

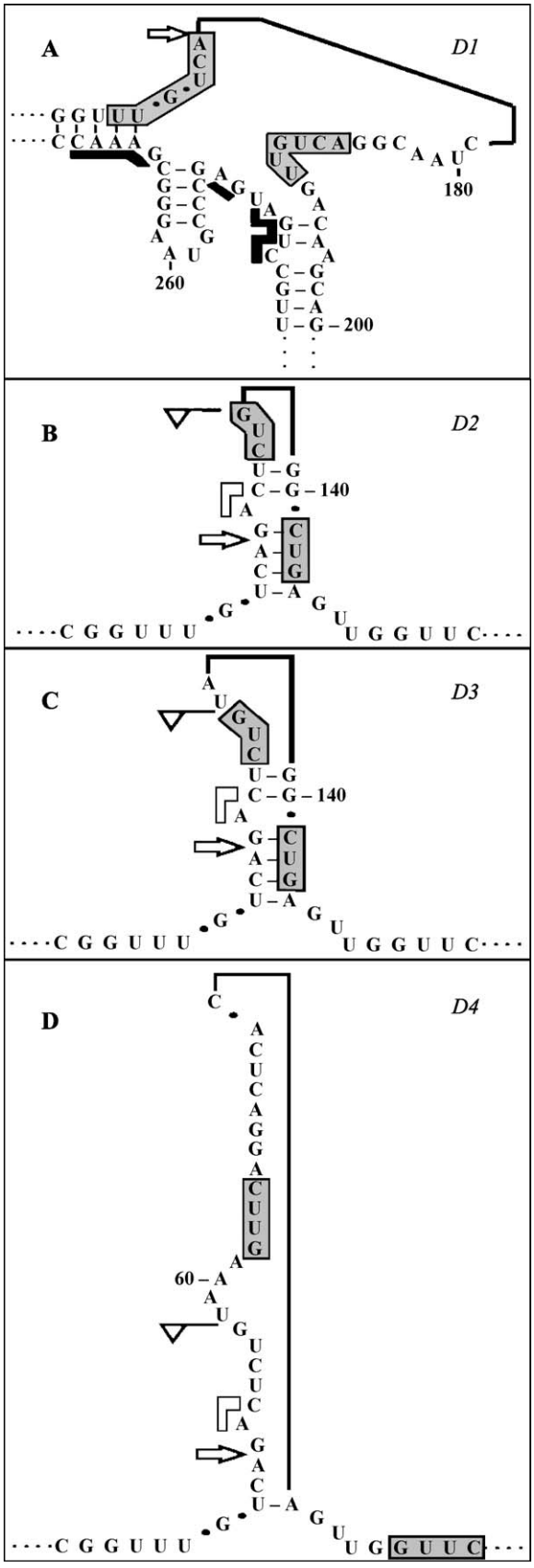
(66-156)
AGGACTCACACCCAAAAGGTGTTGGGATG
TTCATACCCTGATGGGCATCCCACGGATT
GGGTTCTGGATGAAGGGCTGAGTTGGTTC
GAGT

(157-160) or (62-65)
GTTC

(66-156)
AGGACTCACACCCAAAAGGTGTTGGGATG
TTCATACCCTGATGGGCATCCCACGGATT
GGGTTCTGGATGAAGGGCTGAGTTGGTTC
GAGT

(157-275)
GTTCTGGTTATGGCAACCAGAACTAACGG
ACTGTTGGCAGACAGTGATTGAAGATCCG
AGATTATGGCATCTCGGGGAGTCAATCTT
TGCCTGATGAGCCCGTAAGGGCGAAACCG
GTC

Fig. 5. CarSV DNA form with a sequence repetition. (A) Location on the secondary structure proposed for CarSV RNA of the homologous nucleotides (in white against a black background) involved in the formation of the duplicated region. Broken lines denote the remaining parts of the CarSV RNA structure. For reference on the secondary structure and other symbols used, see previous figure legend. (B) Nucleotide sequence of the CarSV DNA duplicated form with the repeated region boxed. Numbers in brackets refer to nucleotide positions relative to the unit-length (275 nt) CarSV RNA sequence.



The structure of a CarSV DNA with an intramolecular rearrangement

Sequencing of a longer-than-unit variant of CarSV DNA revealed that a 4-nt sequence (5′-GTTC-3′), corresponding to nucleotides 62–65 and 157–160 of the CarSV DNA, may have been involved in the formation of this enlarged variant through internal recombination at the DNA or RNA level (Fig. 5B). The duplicated sequence spans most of the upper arm and part of the right arm of the cruciform structure proposed for CarSV RNA (Hernández et al., 1992) (Fig. 5A). In the observed variant of CEVd, a 4-nt series precedes the start and end of the repeated region, resulting in the production of a 92-nt enlarged variant (CEV D-92) (Semancik et al., 1994).

CarSV DNA forms with sequence deletions

As previously reported, nested PCR amplifications of CarSV DNA yielded a variant with a deletion between nt 59 and 139 (Hegedűs et al., 2001) later named as D3 deletion type. Further analyses revealed the existence of three other types, D1, D2 and D4, with deletions of variable length in CarSV DNA (Fig. 6). The 5′ boundaries of deletions in D1, D2, and D3 occurred near the self-cleavage site of the proposed minus hammerhead structure (Hernández et al., 1992), which is the same region involved in plant–CarSV fusions (Fig. 4). It was suggested that transcription pausing, which could be a requisite for the generation of fusions and deletions, can be caused by strong secondary structure elements (Daròs and Flores, 1995a; Vera et al., 2000). One of our deleted CarSV DNA variants (Fig. 6C) had exactly the same primary structure as that of a previously described CarSV RNA (Daròs and Flores, 1995a) and its corresponding DNA form (Vera et al., 2000). The reason because we refer to CarSV RNA for junction-forming regions (Fig. 4) and rearrangements (Figs. 5 and 6) is the possible relevance of secondary structure elements in their formation.

Discussion

An approach to study chromosomal integration mechanisms is the comparison of the structure of the plant genomic loci with and without inserts. We found that short stretches of common nucleotides between the plant genome and CarSV DNA might be responsible for the genomic integration

Fig. 6. Schematic representation of CarSV DNA forms with sequence deletions. (A to D) Deletions found in CarSV DNA represented on the secondary structure proposed for CarSV RNA. Black connecting lines represent deleted regions. Nucleotides forming part of direct (B, C, and D) and inverse repeats (A) found close to the deletion boundaries are boxed on a grey background. For reference on the secondary structure and other symbols used, see previous figure legends. Panel A contains a deletion between nt 50 and 179; (B) between nt 57 and 139; (D) between nt 74 and 144. Panel C has been previously described (Hegedűs et al., 2001).

process of the viroid-like element (Figs. 2 and 3). The presence of these nucleotides seems to be one of the hallmarks of illegitimate recombination between nonhomologous molecules. The predominant mechanism of illegitimate recombination in higher eucaryotes is double-strand break repair (DSBR) that can be associated with DNA insertions (Salomon and Puchta, 1998). Parts or the whole CarSV, which is present in DNA form in the nucleus, might be captured by a similar mechanism. In the proposed model for DSBR, the joining reaction is initiated by base pairing between complementary sequences of the two DNA ends followed by DNA synthesis and ligation (Tsukamoto and Ikeda, 1998). The interaction can be further stabilized by regions of weak homology surrounding the junction site (Kohli et al., 1999). In agreement with this observation, our data reveal the existence of homologous regions around the junction sites (Figs. 2A and 3A) that might have extended the stability of the initial fusions. Supposing the involvement of DSBR, reverse transcription generating full- and partial-length molecules would be followed by integration with the aid of a DNA-dependent polymerase.

Alternatively, an RNA-based mechanism could be assumed. There are examples of nonhomologous recombination between viral RNAs (Hu and Temin, 1990; Nagy and Simon, 1997). The copy-choice model assumes that recombinants are formed as a result of template-switching, which could also be breakage-induced. Not only short homologous sequences between the recombining RNAs but also secondary structure elements are of considerable importance (Figlerowicz, 2000; Nagy and Simon, 1997). Near the junction regions of our inter- and intramolecularly recombining molecules elements of secondary structure were found (Fig. 4) which might be recombinogenic sites at the RNA level.

A replicase-driven template-switching model was advanced for the origin of the observed CarSV–CERV junctions (Vera et al., 2000). The virus-encoded reverse transcriptase has been proposed as being involved in replicase-driven recombination in caulimoviruses to which CERV belongs (Vaden and Melcher, 1990). The observed CarSV–plant junctions could have also been generated by the involvement of an endogenous reverse transcriptase activity forced to accept a DNA template, with the participation of CarSV RNA molecules and the plant chromosomal DNA in the recombination mechanism.

The intramolecularly rearranged partially duplicated CarSV DNA form (Fig. 5) might have originated at the RNA level through illegitimate recombination, although the participation of a reverse transcriptase in the generation of the duplication cannot be excluded. A recombination model involving similar nucleotide patches was previously suggested for the occurrence of terminal repeats of CCCVd (Keese and Symons, 1985) and CEVd (Semancik et al., 1994). Crossover sites in the deleted CarSV DNA forms do not present the shared nucleotides characteristic of CarSV–CERV and CarSV–plant fusions and of the intramolecular duplication. However, short nucleotide repeats around the deletions probably influencing the generation of some shorter-than-unit CarSV RNA and DNA forms (Daròs and Flores, 1995a; Vera et al., 2000) were also present in our deleted molecules (Fig. 6). A 6-nt inverse repeat found in CarSV D1 near the deletion boundary (Fig. 6A) was particularly close in space and might have contributed to the generation of a crossover site. Similarly, template switches in D2 and D3 around position 60 and 140 (Figs. 6B and C) probably occurred because their spatial proximity in the secondary structure and because they flank a helix that might cause polymerase pausing, as was also proposed previously (Daròs and Flores, 1995a). Polymerase jumps of this kind might be promoted by template regions with a stable folding (Daròs and Flores, 1995a), underlying the importance of secondary and probably tertiary elements rather than just nucleotide sequence similarity. However, the importance of both structural and sequence elements was demonstrated by the CarSV–plant junctions. Several junction sites also affect the left and lower arm of the CarSV RNA secondary structure (Daròs and Flores, 1995a; Vera et al., 2000) that we could not possibly detect because they were incompatible with the primers used (see Materials and methods).

Our observations of several events indicative of illegitimate recombination accompanying intermolecular jumps and intramolecular rearrangements suggest that the CarSV RNA–DNA system has a high propensity for this form of recombination with the likely involvement of different mechanisms. The relatively high number of CarSV recombinant forms observed in different carnation cultivars of different origin suggests that a polymerase or polymerases with particularly low processivity might have a role in their generation. Integration into plant genomic DNA have no precedent until now among viroids, although they lack a corresponding DNA form. Strikingly, sequences of various DNA plant viruses have been recently found integrated into the host genome, indicating that this kind of events might have occurred frequently throughout the evolution of both plants and viruses (Harper et al., 2002). The presence of CarSV DNA in the plant genome appears to be the first representative of a similar situation among subviral agents, and further analyses of the integrated sequences could contribute to our understanding of not only the integration mechanisms but also of the plant genome and viroid evolution.

Materials and methods

Plant materials and DNA isolation

Carnation plants (*D. caryophyllus* L.), cultivars Praline and Tanga, provided by the Óbuda Horticulture Co., were serologically tested and found to be free of the usually occurring pathogens in carnation (including CERV). The plants were maintained under normal greenhouse conditions. Total leaf DNA from CarSV-infected (contains CarSV DNA

when analyzed by PCR using CarSV-specific primers) and CarSV-free plant material was purified as previously described (Shure et al., 1983).

Identification of the junction-containing DNA clones and preinsertion sites

DNA isolated as described above was used for the construction of GenomeWalker "libraries" with the Universal GenomeWalker Kit from Clontech according to the manufacturer's recommendations (Fig. 1). Aliquots of DNA from CarSV-infected cultivars were completely digested with different restriction endonucleases that leave blunt ends, and each batch of digested genomic DNA was then ligated separately to the adaptor. After the libraries have been constructed, two rounds of PCR amplifications using adapter-specific and gene-specific primers were performed. An outer (AP1) and a nested (AP2) adaptor primer (provided by the supplier) were used in combination with CarSV DNA-specific primers: CarSVSP1 (sense, nt 195–223) and CarSVSP2 (sense, nt 233–261). PCR amplifications were carried out with the Advantage Genomic Polymerase Mix (Clontech) with the cycling parameters recommended by the supplier. The annealing temperature was optimized for each pair of primers. PCR products were examined by electrophoresis in a 1.5% agarose gel and the fragments of interest were excised, purified using the QIAEX II Gel Extraction Kit (QIAGEN), and ligated in *Eco*RV-digested pBluescript II KS+ plasmid (Stratagene) as previously described (Sambrook et al., 1989). Inserts were sequenced using an Applied Biosystems sequencing apparatus and a reaction kit according to the manufacturer's instructions. To analyze the genomic sequences around the integration target sites, nested primers designed for the plant genomic regions of the junction sequences (for primer sites see Figs. 2C and 3B) were used in combination with the adapter primers. The genomic library of a CarSV-free cv. Tanga plant was used in this experiment.

Isolation of CarSV DNA clones

Detection of CarSV DNA forms with sequence deletions and duplications with respect to the reference sequence (Hernández et al., 1992) was performed by PCR amplification using two pairs of CarSV-specific nested primers: PI (sense, nt 1–23) and PII (antisense, nt 236–258), and PIII (sense, nt 23–47) and PIV (antisense, nt 216–240), using the same cycling profile described previously (Hegedűs et al., 2001) and *Taq* DNA polymerase (Promega).

Sequence analysis

Motifs in the genomic junction sequences were searched using the Hamming Clustering (HC) method for TATA signal prediction in eukaryotic genes (http://125itba.mi.cnr.it/cgi-bin/wwwHC_tata.html) (Milanesi et al., 1996).

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References

Brunaud, V., Balzergue, S., Dubreucq, B., Aubourg, S., Samson, F., Chauvin, S., Bechtold, N., Cruaud, C., DeRose, R., Pelletier, G., Lepiniec, L., Caboche, M., Lecharny, A., 2002. T-DNA integration into the *Arabidopsis* genome depends on sequences of pre-insertion sites. EMBO Rep. 3, 1152–1157.

Daròs, J.A., Flores, R., 1995a. Characterization of multiple circular RNAs derived from a plant viroid-like RNA by sequence deletions and duplications. RNA 1, 734–744.

Daròs, J.A., Flores, R., 1995b. Identification of a retroviroid-like element from plants. Proc. Natl. Acad. Sci. U.S.A. 92, 6856–6860.

Diener, T.O., 2001. The viroid: biological oddity or evolutionary fossil? Adv. Virus Res. 57, 137–184.

Epstein, L.M., Gall, J.G., 1987. Self-cleaving transcripts of satellite DNA from the newt. Cell 48, 535–543.

Fadda, Z., Daròs, J.A., Flores, R., Duran-Vila, N., 2003. Identification in eggplant of a variant of citrus exocortis viroid (CEVd) with a 96 nucleotide duplication in the right terminal region of the rod-like secondary structure. Virus Res. 97, 145–149.

Ferbeyre, G., Smith, J.M., Cedergren, R., 1998. Schistosome satellite DNA encodes active hammerhead ribozymes. Mol. Cell. Biol. 18, 3880–3888.

Figlerowicz, M., 2000. Role of RNA structure in non-homologous recombination between genomic molecules of brome mosaic virus. Nucleic Acids Res. 28, 1714–1723.

Green, B., Pabón-Peña, L.M., Graham, T.A., Peach, S.E., Coats, S.R., Epstein, L.M., 1993. Conserved sequence and functional domains in satellite 2 from three families of salamanders. Mol. Biol. Evol. 10, 732–750.

Harper, G., Hull, R., Lockhart, B., Olszewski, N., 2002. Viral sequences integrated into plant genomes. Annu. Rev. Phytopathol. 40, 119–136.

Haseloff, J., Mohamed, N.A., Symons, R.H., 1982. Viroid RNAs of cadang–cadang disease of coconuts. Nature 299, 316–321.

Hegedűs, K., Palkovics, L., Tóth, E.K., Dallmann, G., Balázs, E., 2001. The DNA form of a retroviroid-like element characterized in cultivated carnation species. J. Gen. Virol. 82, 687–691.

Hernández, C., Daròs, J.A., Elena, S.F., Moya, A., Flores, R., 1992. The strands of both polarities of a small circular RNA from carnation self-cleave in vitro through alternative double- and single-hammerhead structures. Nucleic Acids Res. 20, 6323–6329.

Hu, W., Temin, H.M., 1990. Retroviral recombination and reverse transcription. Science 250, 1227–1233.

Keese, P., Symons, R.H., 1985. Domains in viroids: evidence of intermolecular RNA rearrangements and their contribution to viroid evolution. Proc. Natl. Acad. Sci. U.S.A. 82, 4582–4586.

Kennell, J.C., Saville, B.J., Mohr, S., Kuiper, M.T.R., Sabourin, J.R., Collins, R.A., Lambowitz, A.M., 1995. The VS catalytic RNA replicates by reverse transcription as a satellite of a retroplasmid. Genes Dev. 9, 294–303.

Kohli, A., Griffiths, S., Palacios, N., Twyman, R.M., Vain, P., Laurie, D.A., Christou, P., 1999. Molecular characterization of transforming plasmid rearrangements in transgenic rice reveals a recombination hotspot in the CaMV 35S promoter and confirms the predominance of microhomology mediated recombination. Plant J. 17, 591–601.

Kumar, S., Fladung, M., 2002. Transgene integration in aspen: structures of integration sites and mechanism of T-DNA integration. Plant J. 31, 543–551.

Milanesi, L., Muselli, M., Arrigo, P., 1996. Hamming clustering method for signals prediction in 5′ and 3′ regions of eucaryotic genes. Comput. Appl. Biosci. 12, 399–404.

Nagy, P.D., Simon, A.E., 1997. New insights into the mechanism of RNA recombination. Virology 235, 1–9.

Rojas, A.A., Vazquez-Tello, A., Ferbeyre, G., Venanzetti, F., Bachmann, L., Paquin, B., Sbordoni, V., Cedergren, R., 2000. Hammerhead-mediated processing of satellite pDo500 family transcripts from Dolichopoda cave crickets. Nucleic Acids Res. 28, 4037–4043.

Roth, D.B., Wilson, J.H., 1986. Nonhomologous recombination in mammalian cells: role for short sequence homologies in the joining reaction. Mol. Cell. Biol. 6, 4295–4304.

Salomon, S., Puchta, H., 1998. Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. EMBO J. 17, 6086–6095.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York.

Sawasaki, T., Takahashi, M., Goshima, N., Morikawa, H., 1998. Structures of transgene loci in transgenic *Arabidopsis* plants obtained by particle bombardment: junction regions can bind to nuclear matrices. Gene 218, 27–35.

Semancik, J.S., Szychowski, J.A., Rakowski, A.G., Symons, R.H., 1994. A stable 463 nucleotide variant of citrus exocortis viroid produced by terminal repeats. J. Gen. Virol. 75, 727–732.

Shure, M., Wessler, S., Fedoroff, N., 1983. Molecular identification and isolation of the waxy locus in maize. Cell 35, 225–233.

Takano, M., Egawa, H., Ikeda, J., Wakasa, K., 1997. The structures of integration sites in transgenic rice. Plant J. 11, 353–361.

Tsukamoto, Y., Ikeda, H., 1998. Double-strand break repair mediated by DNA end-joining. Genes Cells 3, 135–144.

Vaden, V.R., Melcher, U., 1990. Recombination sites in cauliflower mosaic virus DNAs: implications for mechanisms of recombination. Virology 177, 717–726.

Vera, A., Daròs, J.A., Flores, R., Hernández, C., 2000. The DNA of a plant retroviroid-like element is fused to different sites in the genome of a plant pararetrovirus and shows multiple forms with sequence deletions. J. Virol. 74, 10390–10400.