Transposition pattern of a modified Ds element in tomato

Rommens, Caius M.T.; Munyikwa, Tichafa R.I.; Overduin, Bert; Nijkamp, H. John J.; Hille, Jacob

Published in:
Plant Molecular Biology

DOI:
10.1007/BF00023607

Publication date:
1993

Plant Molecular Biology **21**: 1109–1119, 1993.

Transposition pattern of a modified *Ds* element in tomato

Caius M.T. Rommens [1], Tichafa R.I. Munyikwa, Bert Overduin, H. John J. Nijkamp and Jacques Hille*

*Department of Genetics, Free University, De Boelelaan 1087, 1081 HV Amsterdam, Netherlands (*author for correspondence); [1] current address: Department of Plant Pathology, University of California, Berkeley, CA 94720, USA*

Received 21 October 1992; accepted 10 January 1993

Key words: transposition, *Ac/Ds*, transgenic tomato plants, RFLP mapping, plasmid rescue

Abstract

Several aspects of transposition of an *in vitro* modified *Ds* element are described. This *Ds* element, designated *Ds-r*, is equipped with bacterial plasmid sequences and can, therefore, be rescued from the plant genome. Our results indicate that the *Ds-r* element has a 'late' timing of transposition from T-DNAs. This feature of the element might be advantageous for tagging experiments because it leads to independently transposed germinally transmitted elements. Furthermore, it is shown that *Ds-r* transposition generates clusters of insertions, indicating that 'genes to be tagged' should be located in genomic regions covered by insertions.

Introduction

The maize transposable elements *Activator* (*Ac*) and *Dissociation* (*Ds*) were first genetically characterized by McClintock [38]. She showed that *Ac* transposes autonomously and that *Ds* transposition requires the presence of *Ac*. Both *Ac* and *Ds* have now been cloned and subsequently used as 'tags' to isolate several maize genes [5]. The frequency with which a particular gene in maize can be 'tagged' varies from 10^{-5} to 10^{-6} [23]. These frequencies can be increased up to 100-fold by linking the transposable element to a desired gene [15]. This increase is due to the preference of *Ac/Ds* elements to transpose to linked sites. In maize, 61% and 55% of transpositions from the *p-vv* gene and the *bz-m2* gene were in a 20 cM region proximal or distal to the donor site [19, 29].

Studies performed in tobacco showed that the *Ac* element transposed with high frequency (72%) to linked sites in the genome of this plant as well [20]. Transposition from four out of six loci analysed resulted in a pronounced clustering of new *Ac* insertions within 5 cM from the donor site, whereas *Ac* elements transposed from two other loci had integrated more dispersedly. The finding that *Ac* transposes in tobacco in a similar way as in maize indicates that the use of this element for gene isolation may be extended to other plant species as well.

It is our aim to study *Ac/Ds* transposition in tomato. This crop species contains many agronomically important genes that could be targets for transposon tagging experiments. Initial experiments have shown that *Ac* maintains its capacity to transpose and transactivate *Ds* in this host in a similar way as described for maize [37, 42,

47, 48]. The availability of a tomato RFLP map [7] made it possible to map transposed *Ac*'s at any location in the tomato genome. Two recent studies on the *Ac* transposition pattern in tomato showed that like in maize and tobacco, clusters of insertions were observed around the donor site [6, 42]. A new and unexpected finding was that clusters, comprising two to five analysed insertions, were also generated at unlinked positions. Possibly, the unlinked clusters originated from independent transposition events from the T-DNA, implying a preference for integration in certain regions. Alternatively, some early transposition events from the T-DNAs to unlinked sites may have been followed by later secondary transposition events to linked sites.

Recently, we reported the construction of a *Ds* element containing bacterial plasmid sequences between the ends of *Ac* [46]. This element has the origin of replication of plasmid pACYC184 and the chloramphenicol resistance gene (functional in *Escherichia coli*) inserted between 0.5 kb *Ac* terminal sequences. The presence of these bacterial plasmid sequences allow the *Ds* element, designated *Ds-r*, to be rescued from the plant genome [47]. Since the size of *Ds-r* is 7.8 kb, it is larger than *Ac* itself (4.6 kb) or any well characterized *Ds* element (0.4–4.4 kb) [21]. Furthermore, almost 90% of the element is non-homologous to the *Ac* element. These properties of *Ds-r* may influence its behaviour. To evaluate the applicability of *Ds-r* for transposon tagging in tomato, we studied several aspects of *Ds-r* behaviour like mobility, transposition pattern, integration specificity and integrity upon transposition, and compared these with reported data on *Ac* transposition.

Materials and methods

Plant material

The four F1 tomato plants used for our studies (designated AAT6515-02, -30, -33 and -64) were derived from a cross between primary transformants, harbouring a single and mapped *Ds-r*-containing T-DNA (AAT6514-02, -30, -33 and -64; [47] and B. Overduin, unpublished results), and a transgenic plant homozygous for two SLJ1515 T-DNAs, containing a modified *Ac* element [34]. The *Ds-r* element contains both the GUS gene driven by a 0.8 kb fragment of the CaMV 35S promoter and parts of plasmid pACYC184 (origin of replication and chloramphenicol resistance gene) between the 586 bp 5′ *Ac* terminal sequence and the 448 bp 3′ *Ac* terminal sequence [46]. The construction of the modified *Ac* element present between the borders of the T-DNA of SLJ1515 was initiated from a clone of the maize *bronze* allele *bz-m2* (*Ac*) [17]. A *Cla* I site was inserted by oligonucleotide mutagenesis, 82 bp 3′ to the mapped polyadenylation site [34].

Southern blot analysis

The structure of the pTT283 T-DNA before and after transposition of *Ds-r* is presented in Fig. 1. To examine whether *Ds-r* was either still present between the borders of this T-DNA or had transposed from the T-DNA to positions in the plant DNA, hybridizations were performed with the 35S promoter of cauliflower mosaic virus as a probe. DNA isolation, restriction digests, Southern blotting, hybridizations and autoradiography were performed as described previously [46]. Fragments indicative of an untransposed *Ds-r* have sizes of 8.2 kb and 1.9 kb. Fragments referring to excision and integration of *Ds-r* elsewhere have sizes of 2.4 kb and >6.7 kb respectively.

To place transposed *Ds-r* elements on the RFLP map of tomato, *Ds-r*-flanking plant DNA fragments were used as probes on filters containing DNA of 38 F2 plants (laboratory of S. Tanksley) from a self-pollinated *L. esculentum* × *L. pennellii* F1 hybrid [7]. The F2 plants segregate for 64 RFLP markers (S.D. Tanksley *et al.*, in preparation). The segregation data for the plant DNA segments isolated from rescued clones were translated into map positions using the interactive computer package Mapmaker [36]. Details are described in Rommens *et al.* [47].

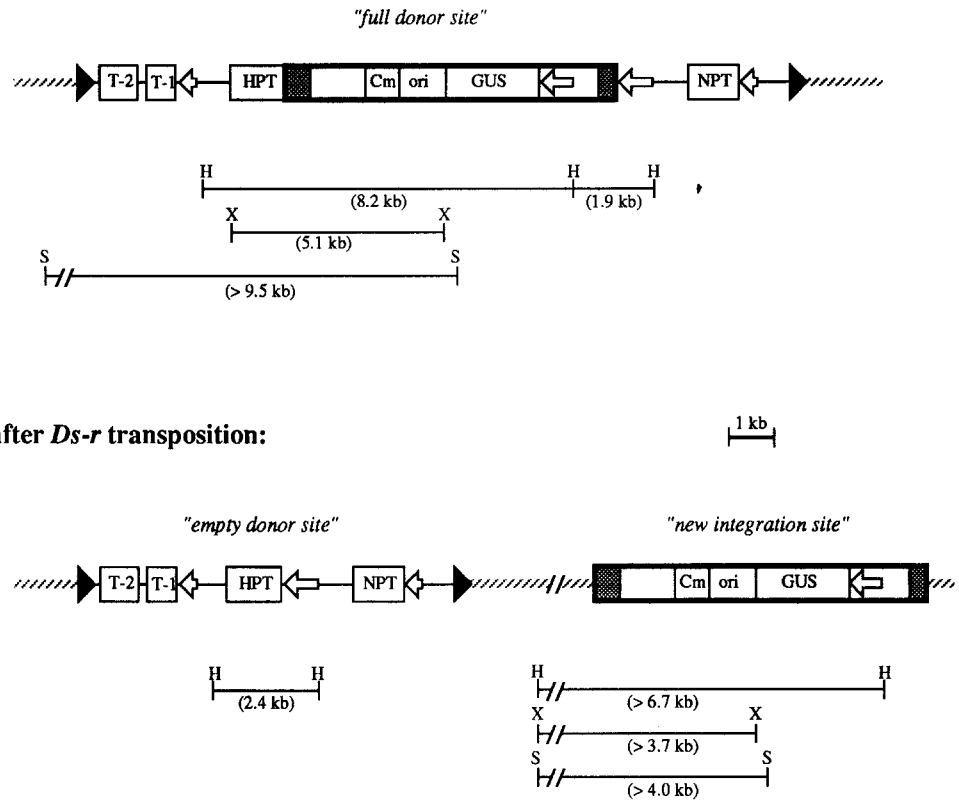

Fig. 1. Schematic structure of *Ds-r* before and after transposition from the T-DNA. The *Ds-r* element is represented with a thick-lined box; parts of *Ds-r* homologous to *Ac* are dashed. *Hin*d III fragments indicative for 'full donor site' fragments, 'empty donor site' fragments and 'new integration site' fragments are represented with lines under the diagrams. The probe used to visualize these fragments on Southern blots is the 35S promoter of cauliflower mosaic virus (indicated with a large white arrow). The nopaline synthase promoter is indicated with a small white arrow. The borders of the T-DNA are represented with black triangles. HPT, hygromycin phosphotransferase gene; NPT, neomycin phosphotransferase gene; GUS, β-glucuronidase gene; T-2, *tms-2* gene; T-1, *tms-1* gene; Cm, chloramphenicol resistance gene; ori, origin of replication of plasmid pACYC184.

Plasmid rescue

Transposed *Ds-r* elements were rescued from the genomes of transgenic plants as described previously [47].

Sequence analysis

Ds-r plant DNA junctions were sequenced using the Taq Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems). To sequence junctions at the 5′ side of *Ds-r*, a 23-mer primer was used with a sequence inverse to the *Ds-r* sequence from base pairs 70 to 92 (same coordinates as for *Ac* [35]). To sequence junctions at the 3′ side of *Ds-r*, a 21-mer primer was used homologous to an *Ac* sequence from base pairs 4490 to 4510.

To sequence 'footprints' generated by excision of *Ds-r*, empty donor site fragments were amplified using a primer for the 35S promoter of the cauliflower mosaic virus and a primer for the hygromycin phosphotransferase gene as described previously [31]. Amplified products were cloned

into pTZ18 and 'footprints' were sequenced using the Taq Dye Primer Cycle Sequencing Kit (Applied Biosystems).

Results

Delayed Ds-r *transposition from T-DNAs*

Indications for the mobility of *Ds-r* elements were obtained by rescuing these elements with flanking DNA segments. The rescue of these '*Ds-r* border fragments' was based on the presence of bacterial plasmid sequences within *Ds-r*. Since the efficiency of plasmid rescue is dependent on the size of plasmids, four different restriction enzymes (*Hin*d III, *Pst* I, *Sac* I or *Xba* I) were used to generate '*Ds-r* border fragments'. In this way, rescued plasmids represented a more or less random selection of *Ds-r* elements present at different positions in the genome of tomato. The structures of rescued plasmids referred either to the situation before transposition, i.e. *Ds-r* flanked by T-DNA sequences, or implied transposition of *Ds-r* to new positions in the plant genome.

Four F1 plants harbouring single *Ds*-containing T-DNAs on chromosomes 1, 3, 6 and 12 (designated AAT6515-02, -30, -33 and -64) were used to study somatic transposition of *Ds-r* from T-DNAs. Restriction analysis of border fragments rescued from these plants showed a low ratio between transposed and untransposed elements (0–0.02; Table 1).

The F1 plant with *Ds* originally located on chromosome 6 (plant AAT6515-02) was subsequently self-pollinated to determine the frequency of germinally transmitted transposition events in the progeny. For this purpose, a filter prepared from *Hin*d III digests of 32 F2 plants was hybridized to the CaMV35S promoter as a probe. We used the complete absence of a 'full donor site' band, the presence of a strongly hybridizing 'empty donor site' band and the presence of a 'new integration site' band as indications for the occurrence of transposition in F1 plants, followed by transmission of the transposed *Ds-r* elements to the next generation. Based on these criteria, we found that 3 out of 32 F2 plants had inherited a transposed *Ds-r* (data not shown). The absence of a 'new integration site' band in DNA of a fourth F2 plant, furthermore, implied either segregation out or loss of a pre-meiotically excised element. Our data suggest a relatively high frequency, ca. 10%, of germinally transmitted transposed *Ds-r* elements. Similar results were found by analysing the progeny of an F1 plant containing *Ds-r* on chromosome 3 (plant AAT6515-30): analysis of 100 F2 plants implied a frequency of germinally transmitted transposed elements of 7% (B. Overduin, unpublished results).

The three F2 plants derived from plant AAT6515-02 and harbouring a germinally trans-

Table 1. *Ds-r* elements rescued from F1 plants.[1]

F1 plant	Untransposed	Transposed		Ratio transposed
AAT6514-02 × *Ac*	442	10[2]		0.02
AAT6514-30 × *Ac*	67	0		0
AAT6514-33 × *Ac*	59	1		0.02
AAT6514-64 × *Ac*	94	0		0
F2 plant		Primary event	Secondary event	
AAT6516-01	0	66	10	0.13
AAT6516-14	0	61	12	0.16
AAT6516-30	0	14	47	0.77

[1] Untransposed: *Ds-r* elements still present at their original position between the borders of the T-DNA; transposed: *Ds-r* elements present at new genomic positions.

[2] Results have been presented previously in Rommens *et al.* [47].

mitted transposed *Ds-r* element (designated AAT6516-01, -14 and -30) contained at least one copy of *Ac*. The transposed elements could, thus, be mobilized again. To identify and characterize both the germinally transmitted (primary) and the secondary transposition events, '*Ds-r* border fragments' were rescued from the genome of these plants. Restriction analysis of border fragments revealed a predominant pattern for the *Ds-r* flanking plant DNA for each plant. This pattern was found in 87% and 84% of the cases for plants AAT6516-01 and AAT6516-14, respectively, and in 23% of the cases for plant AAT6516-30 (Table 1). It most likely referred to the germinally transmitted transposition event. As expected, the size of the predominant border fragment isolated from a *Hin*d III digest of each of the three plants was identical to the size of the 'new integration site' band observed in the lane of a Southern blot containing the same DNA digest (data not shown).

Transposed *Ds-r* border fragments with restriction patterns other than that of the predominant fragments were different from each other and, probably, referred to secondary transposition events. As can be deduced from Table 1, the average ratio transposed versus untransposed *Ds-r* elements rescued from the F2 plants mentioned is 0.49 and, thus, strikingly higher than this ratio for *Ds-r* elements isolated from F1 plants.

Based on the low transposition frequency in leaves of F1 plants and the relatively high frequency of germinally transmitted transposition events, it is concluded that the *Ds-r* element had a 'late' timing of transposition from T-DNAs. Elements, transposed to new positions in plant DNA, did not show this phenomenon and displayed a higher mobility during vegetative plant development.

Ds-r *transposition-generated clusters of insertions*

Nine plant DNA fragments flanking transposed *Ds-r* elements, isolated from the F1 plant with *Ds-r* originally located as part of the T-DNA on chromosome 6 (plant AAT6515-02), were hybridized to DNA of non transformed *L. esculentum* and *L. pennellii*. Seven fragments hybridized to single-copy DNA sequences and revealed unique RFLPs, whereas two fragments hybridized to repetitive plant DNA sequences. The first seven fragments were used to place transposed *Ds-r* elements on the tomato RFLP map. It appeared that two of these elements had integrated on the chromosomes 3 and 12 respectively [47]. Five other elements were mapped at linked sites on chromosome 10 (trDs-202, -204, -228, -239 and -243; Fig. 2).

This phenomenon of integration at linked sites on another chromosome was also found for the germinally transmitted transposed *Ds-r* elements. Two of these elements (designated trDs-013 and trDs-021 and isolated from the F2 plants AAT6516-01 and -30 respectively) were mapped on chromosome 1 at a distance of 5cM from each other. A third germinally transmitted transposed element (trDs-019, isolated from plant AAT6516-14) had entered a repetitive plant DNA sequence and could not be mapped.

Twenty-five plant DNA fragments flanking secondary transposed *Ds-r* elements, isolated from either plant AAT6516-01 or plant AAT6516-30 all hybridized to a single-copy plant DNA sequence and most of the fragments visualized RFLPs between cultivated and wild tomato. These RFLPs were in all cases different. Four fragments, which had been isolated from plant AAT6516-01, and six fragments from plant AAT6516-30 were subsequently used to map the positions of the corresponding transposed *Ds-r* elements. One secondary transposed *Ds-r* element of plant AAT6516-01 (trDs-209) and two elements of plant AAT6516-30 (trDs-059 and -061) were in this way mapped on the same position, at a distance closely linked to the germinally transmitted transposed elements of these plants (Fig. 2). Five other elements had also integrated at linked positions, while two elements had transposed interchromosomally to chromosome 2 (trDs-248) and chromosome 6 (trDs-057).

It can be concluded that the *Ds-r* element, present between the borders of the T-DNA on chromosome 6, has a tendency to transpose in-

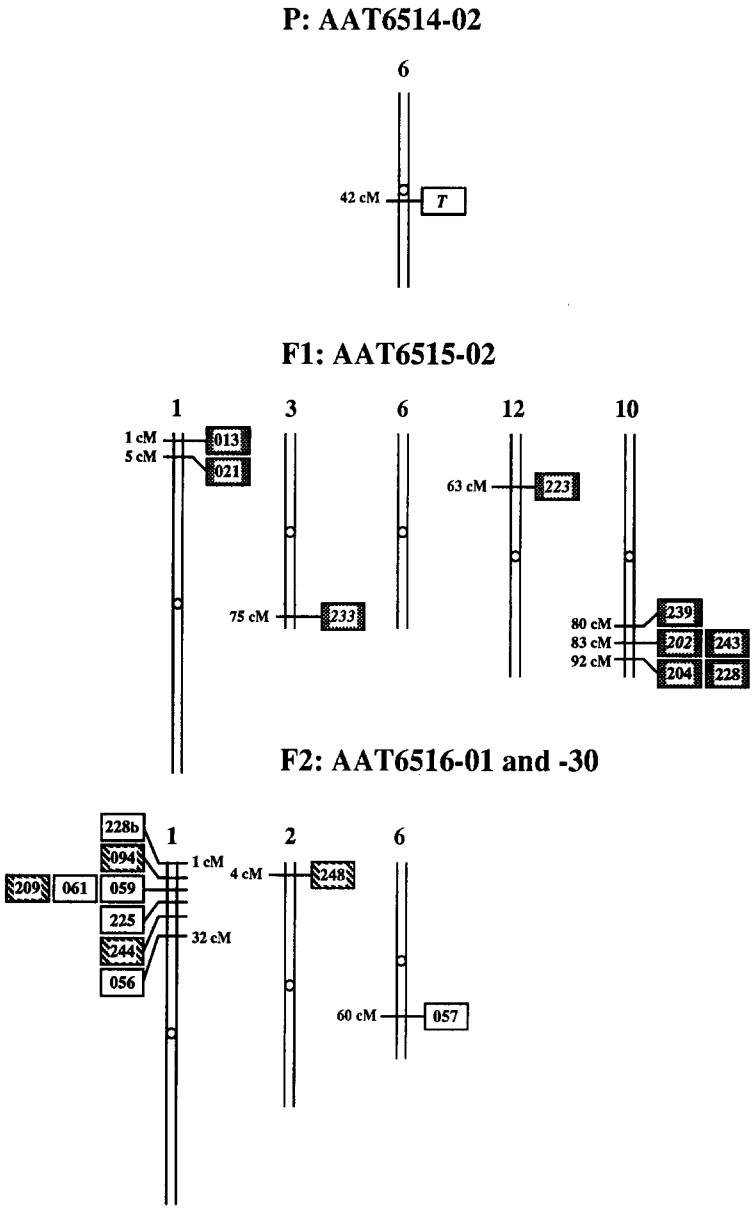

Fig. 2. Assignment of *Ds-r* elements to the tomato RFLP map. The original position of *Ds-r*, as part of the T-DNA in plant AAT6514-02, is indicated with a box designated 'T'. New positions of *Ds-r*, transposed during vegetative development of a progeny plant of a AAT6514-02 × Ac cross, are indicated with shaded boxes. Two of these positions had been rescued from the F2 plants AAT6516-01 ('013') and AAT6516-30 ('021') and were considered as germinally transmitted transposed elements (see text). Striped and white boxes represent secondary transposed *Ds-r* elements rescued from plants AAT6516-01 and AAT6516-30 respectively. Only the systematic numbers of clones are shown in the boxes; numbers in italics ('233', '223' and '202') refer to clones previously mentioned in Rommens *et al.* [47].

terchromosomally, and can create clusters of insertions. Transposition of two *Ds-r* elements inserted into plant DNA on chromosome 1 also generated clusters of insertions although these clusters were around the donor site.

Characterization of plant DNAs flanking transposed Ds-r

Fifty-one plant DNAs flanking transposed *Ds-r* elements at the 5′ side, isolated from both F1 and

F2 plants, were sequenced to examine the character of target DNA. A comparison between the sequenced plant DNAs did not show any general similarities for either the 8 bp sequences flanking *Ds-r* or more extended stretches (100–300 bp) of *Ds-r* flanking DNA. The average CG content of these sequences was 39%, which is in line with the average CG content of tomato [39].

Homology was, however, found between plant DNAs flanking *Ds-r* elements clusterly integrated on chromosome 10. Analysis of plant DNAs flanking trDs-202 and trDs-243 (ca. 300 bp) showed an almost complete sequence homology: only the base pairs immediately flanking the *Ds-r* elements were different (5′-TA-3′ for trDs-202 and 5′-TTAA-3′ for trDs-243). Possibly, the two elements refer to the same integration event. One of the elements should then have undergone a second transactivation event resulting in only a small mutation of flanking base pairs. Alternatively, *Ds-r* may have integrated twice independently at almost the same site. Similar events have been described for intragenic transposition of the *Ac* element in maize [3, 40]. The *Ds-r* elements on position 92 cM (trDs-204 and -228) are flanked by identical plant DNA sequences. Nevertheless, the elements can be distinguished from each other because the structure of trDs-204 is completely intact (as deduced from restriction and sequence analysis), while trDs-228 suffers an internal 882 bp deletion destroying part of the β-glucuronidase gene (see further on).

Assuming that integration preferentially occurred in hypomethylated DNA [11], i.e. in DNA sequences possibly associated with genes [2], *Ds-r* flanking plant DNAs were used to screen databases for homology [1]. In three cases homology was found with the regulatory or structural parts of plant genes (see Discussion). In a fourth case, a secondary transposed *Ds-r* element rescued from plant AAT6516-14 had integrated into the 5′ side of the truncated *Tms-1* gene present between the borders of the T-DNA.

The sequence comparisons described here support our genetic data implying non-random *Ds-r* integration. There is, however, no general sequence specificity for integration that might complicate tagging experiments.

Consequences of Ds-r *transposition on the structure of both the element and flanking DNA*

To examine the consequences of *Ds-r* excision/integration, both an excision site in the T-DNA of plant AAT6515-02 and junctions between *Ds-r* and plant DNA in a 'full donor site' of the progeny plant AAT6516-30 were sequenced (see Materials and methods). It was shown that excision resulted in C-G transversions of the two central base pairs of the *waxy* 8 base pairs sequence repeat, generating a 'footprint' of 9 bp. Similar events have been reported to be associated with excision of *Ac* in tobacco [4] and *Dsl* excision in maize [16]. Furthermore, it was shown that the transposed *Ds-r* element analysed was flanked by an 8 bp direct repeat (ATAATGGT), indicating that *Ds-r* integration can, like *Ac*/*Ds* integration, lead to target site duplications.

The junction between the 5′ end of *Ds-r* and plant DNA had been sequenced for 51 independently transposed *Ds-r* elements (see above). In none of the cases deletions or point mutations at the 5′ end of *Ds-r* were observed. This is in contrast to results presented for transposed *Ac* elements in tomato: three out of eleven analyzed elements contained a modified 5′ terminus [42].

Restriction analysis of a total of 89 independently transposed *Ds-r* elements showed that the structure of these elements was mostly identical to the structure of the untransposed element. In two cases, however, rescued transposed *Ds-r* elements suffered an internal deletion. The sequence at the breakpoints of one of these deletion derivatives (trDs-228) is presented in Fig. 3. Restriction analysis showed that the deletion of another *Ds-r* element (trDs-299) comprised at least 1.5 kb (Fig. 2). The frequency of transposed *Ds-r* elements with an internal deletion is thus ca. 2%. This frequency is not significantly different from the frequency in which *Ac* elements can be involved in internal deletions [47].

It is concluded that the consequences of *Ds-r*

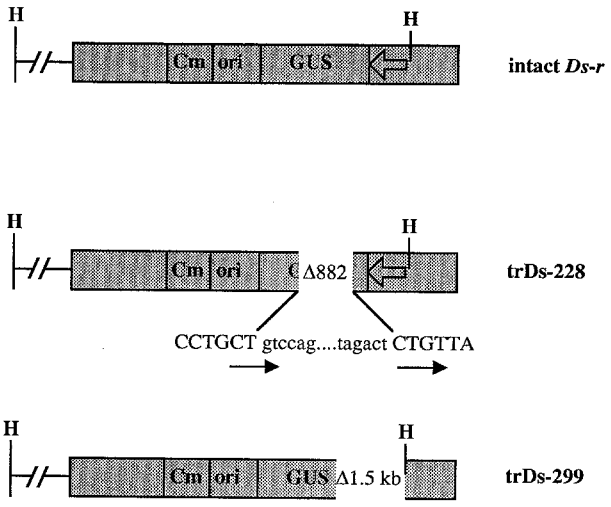

Fig. 3. Schematic structure of *Ds-r* deletion derivatives. The deleted elements were identified among elements rescued from self-ligated *Hind* III digests. Deleted regions are spared out. Deletion breakpoints of trDs-228 are indicated with non-deleted sequences in upper case and deleted sequences in lower case. Arrows indicate a 4 bp direct repeat at the breakpoints.

transposition can be similar to those described for *Ac/Ds*. Elements generally maintain their integrity upon transposition and the excision/integration process is carried out in a comparable way in tomato as in maize.

Discussion

Differences in Ds-r *transposition from either T-DNAs or plant DNAs*

It is shown that *Ds-r* elements have a 'late' timing of transposition in F1 plants. Since our data are based on the analysis of transposition in four F1 plants with the *Ds-r*-containing T-DNA on different chromosomes, the observed phenomenon is not merely a reflection of the variable somatic transposition activities reported for independent transgenic plants [27, 31]. Upon *Ds-r* transposition from T-DNAs, no delayed timing of (secondary) transposition was observed anymore and transposed elements displayed a higher somatic mobility. This phenomenon suggests that the 'late' timing of transposition from T-DNAs might be ascribed to the structure or specific position of the T-DNAs. The increased somatic mobility of *Ds-r* may also (partially) have resulted from increased *Ac* copy numbers. Although the number of *Ac* elements has not been determined in the F2 plants studied here, it has been reported that *Ac* transposition can result in multiplication of *Ac* in either maize [13] or tomato [49]. In tobacco, an increase of *Ac* elements was shown to double transposition frequencies [34].

Of all transposed *Ds-r* elements which were rescued in the F1 generation, i.e. elements possibly transposed from the T-DNA, 25% was integrated in repetitive plant DNA. Out of 30 elements transposed from new positions in the plant DNA, none was integrated in repetitive plant DNA. This finding again indicates a difference in the behaviour of *Ds-r* elements present in the T-DNA and *Ds-r* elements flanked by plant DNA sequences. Assuming that associations between 'donor' and 'target' sites play a role in the transposition process [14, 20], it might be envisaged that *Ds-r* elements flanked by T-DNA sequences are 'targeted' to other sites as elements integrated into plant DNA.

None out of nine *Ds-r* elements which had transposed in the F1 plant AAT6515-02 were mapped on chromosome 6. This suggests that the *Ds-r* element as part of the T-DNA on chromosome 6 has a tendency to transpose interchromosomally. The occurrence of interchromosomal primary transpositions is, however, not a general phenomenon for *Ds-r* present between the borders of the T-DNA of pTT283. A *Ds-r* element integrated as part of the T-DNA on chromosome 1 (plant AAT6514-33 [47]), transposed in the presence of *Ac* to a linked position (C.M.T. Rommens, unpublished results).

Transposition of Ds-r

We show that transposition of *Ds-r* can generate clusters of both unlinked and linked insertions in the tomato genome. Unlinked clusters of insertions are generated upon transactivation of *Ds-r*,

integrated as part of the T-DNA on chromosome 6. Transactivation resulted in five insertions in a 10 cM region on chromosome 10. Also, two insertions were on chromosome 1, at a distance of only 5 cM from each other. Since the *Ds-r* transposition frequency in F1 plants is low, the mapped transposed *Ds-r* elements could refer to independent transposition events from the T-DNA, suggesting that *Ds-r* elements have a preference to integrate into certain genomic regions. However, the restoration of high *Ds-r* mobilities after transposition from the T-DNA may have resulted in the clustering of insertions as well. Transposition of *Ds-r* from plant DNAs in F2 plants also generated clusters of insertions, but these clusters were around the donor sites, implying that secondary transpositions were to linked sites.

Sequence analysis of plant DNAs flanking *Ds-r* elements revealed a weak homology of target sites for clusterly integrated elements. Some target site specificity had been observed previously for clusterly integrated *Ac* elements in maize: nine out of nineteen insertions in the *P* gene were flanked by the GCNAG motif, with a maximum of one mismatch [3]. Another study on *Ac* transposition in the maize *P* gene showed that *Ac* might have inserted twice into a TACAAC sequence [30].

Our results imply that the transposition pattern of *Ds-r* in tomato can correspond to that described for *Ac* in tomato [6, 42]. The tendency of *Ds-r* transposition to generate clusters of insertions, combined with the absence of a strong sequence specificity for integration of *Ds-r*, suggests the usefulness of *Ds-r* for targeted transposon tagging: genes located in genomic regions which are frequently visited by *Ds-r* are potential 'targets' for tagging experiments. Indications for the ability of *Ds-r* to actually integrate into DNAs associated with genes, as suggested by Dooner *et al.* [20], have been obtained by screening target DNAs for homology with characterized genes. DNA segments (> 100 bp) flanking trDs-035, -057 and -201 showed 70–80% homology with the first exon of the carrot extensin gene [11], the second intron of the tomato ATP-dependent protease gene [28] and the promoter of the tobacco gene for pathogenesis-related protein 1c [43] respectively. No hybridization of tomato mRNA with trDs-035 was, however, performed to confirm the integration of the corresponding *Ds-r* element into a transcribed gene. A fourth DNA segment flanking trDs-220 was 100% homologous to a truncated gene (the *Tms-1* gene), which is part of the T-DNA. Previously, Hehl and Baker [32] reported interruption of a transcribed region by a transposed *Ac* in tobacco.

The generation of Ds-r *deletion derivatives*

The involvement of transposable elements in the generation of internal deletions has been described for *Ac* [17, 26, 47] and for several other transposable elements of the so-called '*Ac* family' [10] such as the *Drosophila P* [41] and *hobo* [8] elements. Here we show that sequences not homologous to *Ac* but inserted between *Ac* terminal sequences can also be involved in deletions. Sequence analysis of the *Ds-r* deletion derivative trDs-228 showed the breakpoints of the deletion to be at small direct repeats. Also, *Ac*-derived *Ds* elements like *Ds2* [18], *Ds6* [22], *Ds9* [44] and *Ac*Δ [47] and deletion derivatives of the *Drosophila P* element [41] have small direct repeats at breakpoints. The mechanism which was used to explain the *P* deletion derivatives is 'double-strand gap repair' [24]. When a sister chromatid is used as a template to repair a double-stranded gap generated by *P* excision, a copy of *P* can be created at the donor site. If the repair is, however, interrupted, two partially extended 3′ ends might pair with one another at points of weak homology to complete the synthesis. Therefore, short direct duplications would be a preferred site for deletion breakpoints. The similar structure of *Ac* or *Ds* deletion derivatives suggests that double-strand gap repair might be involved in these infrequently occurring events.

Acknowledgements

The authors thank Dr George N. Rudenko for fruitful discussions and M. Joana Guimaraes and

M. Teresa Domingues for excellent practical assistance.

References

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman J: Basic local alignment search tool. J Mol Biol 215: 403–410 (1990).
2. Antequera F, Bird AB: Unmethylated CpG islands associated with genes in higher plant DNA. EMBO J 7: 2295–2299 (1988).
3. Athma P, Grotewold E, Peterson T: Insertional mutagenesis of the maize *P* gene by intragenic transposition of *Ac*. Genetics 131: 199–209 (1992).
4. Baker B, Schell J, Lörz H, Fedoroff NV: Transposition of the maize controlling element *Activator* in tobacco. Proc Natl Acad Sci USA 83: 4844–4848 (1986).
5. Ballcells L, Swinburne J, Coupland G: Transposons as tools for the isolation of plant genes. TIBTECH 9: 31–37 (1991).
6. Belzile F, Yoder JI: Pattern of somatic transposition in a high copy *Ac* tomato line. Plant J 2: 173–179 (1992).
7. Bernatzky R, Tanksley SD: Towards a saturated linkage map of tomato based on isozymes and random cDNA sequences. Genetics 112: 887–898 (1986).
8. Blackman RK, Gelbart WM: The transposable element *hobo* of *Drosophila melanogaster*. In: Berg DE, Howe MM (eds) Mobile DNA, pp. 523–529. American Society for Microbiology, Washington DC (1989).
9. Brink RA, Williams E: Mutable R-navajo alleles of cyclic origin in maize. Genetics 73: 273–296 (1973).
10. Calvi BR, Hong TJ, Findley SD, Gelbart WM: Evidence of a common evolutionary origin of inverted repeat transposons in *Drosophila* and plants: *hobo*, *Activator* and *Tam3*. Cell 66: 465–471 (1991).
11. Chen J, Varner JE: An extracellular matrix protein in plants: Characterization of a genomic clone for carrot extensin. EMBO J 4: 2145–2151 (1985).
12. Chen J, Greenblatt IM, Dellaporta SL: Transposition of *Ac* from the *P* locus of maize into unreplicated chromosomal sites. Genetics 117: 109–116 (1987).
13. Chen J, Greenblatt IM, Dellaporta SL: Molecular analysis of *Ac* transposition and DNA replication. Genetics 130: 665–676 (1992).
14. Coen ES, Robbins TP, Almeida J, Hudson A, Carpenter R: Consequences and mechanisms of transposition in *Antirrhinum majus*. In: Berg DE, Howe MM (eds) Mobile DNA, pp. 413–436. American Society for Microbiology, Washington DC (1989).
15. Dash S, Peterson PA: Chromosome constructs for transposon tagging of desirable genes in different parts of the maize genome. Maydica 34: 247–261 (1989).
16. Dennis ES, Gerlach W, Peacock WJ: Excision of the *Ds* controlling element from the *Adh1* gene of maize. Maydica 31: 47–57 (1986).
17. Dooner HK, Weck E, Adams S, Ralston E, Favreau M, English J: A molecular genetic analysis of insertions in the *bronze* locus in maize. Mol Gen Genet 200: 240–246 (1985).
18. Dooner HK, English J, Ralston E, Weck E: A single genetic unit specifies two transposition functions in the maize element *Activator*. Science 234: 210–211 (1986).
19. Dooner HK, Belachew A: Transposition pattern of the maize element *Ac* from the bz-m2(*Ac*) allele. Genetics 122: 447–457 (1989).
20. Dooner HK, Keller J, Harper E, Ralston E: Variable patterns of transposition of the maize element *Activator* in tobacco. Plant Cell 3: 473–482 (1991).
21. Döring HP, Starlinger P: Barbara McClintock's controlling elements: now at the DNA level. Cell 39: 253–259 (1984).
22. Döring HP, Tillmann E, Starlinger P: DNA sequence of the maize transposable element *Dissociation*. Nature 307: 127–130 (1984).
23. Döring HP: Tagging genes with maize transposable elements. An overview. Maydica 34: 73–88 (1989).
24. Engels WR, Johnson-Schlitz DM, Eggleston WB, Sved J: High frequency *P* element loss in *Drosophila* is homolog dependent. Cell 62: 515–526 (1990).
25. Fedoroff N, Wessler S, Shure M: Isolation of the transposable maize controlling elements *Ac* and *Ds*. Cell 35: 243–251 (1983).
26. Fedoroff NV: Maize transposable elements. In: Berg DE, Howe MM (eds) Mobile DNA, pp. 375–411. American Society for Microbiology, Washington DC (1989).
27. Fitzmaurice WP, Lehman LJ, Nguyen LV, Thompson WF, Wernsman EA, Conkling MA: Development and characterization of a generalized gene tagging system for higher plants using an engineered maize transposon *Ac*. Plant Mol Biol 20: 177–198 (1992).
28. Gottesman S, Squires C, Pichersky E, Carrington M, Hobbs M, Mattick JS, Dalrymple B, Kuramitsu H, Shiroza T, Foster T: Conservation of the regulatory subunit for the Clp APT-dependent protease in prokaryotes and eukaryotes. Proc Natl Acad Sci USA 87: 3513–3517 (1990).
29. Greenblatt IM: A chromosome replication pattern deduced from pericarp phenotypes resulting from movements of the transposable element, *Modulator*, in maize. Genetics 108: 471–485 (1984).
30. Grotewold E, Athma P, Peterson T: A possible hot spot for *Ac* insertion in the maize *P* gene. Mol Gen Genet 230: 329–331 (1991).
31. Haring MA, Rommens CMT, Nijkamp HJJ, Hille J: The use of transgenic plants to understand transposition mechanisms and to develop transposon tagging strategies. Plant Mol Biol 16: 449–461 (1991).
32. Hehl R, Baker B: Properties of the maize transposable element *Activator* in transgenic tobacco plants: a versatile inter-species genetic tool. Plant Cell 2: 709–721 (1990).
33. Hille J, Koornneef M, Ramanna MS, Zabel P: Tomato:

a crop species amenable to improvement by cellular and molecular methods. Euphytica 42: 1–23 (1989).
34. Jones JDG, Carland FM, Maliga P, Dooner HK: Visual detection of transposition of the maize element *Activator* (*Ac*) in tobacco seedlings. Science 244: 204–207 (1989).
35. Kunze R, Stochaj U, Laufs J, Starlinger P: Transcription of transposable element *Activator* (*Ac*) of *Zea mays* L. EMBO J 6: 1555–1563 (1987).
36. Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L: MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181 (1987).
37. Lassner M, Palys JM, Yoder JI: Genetic transactivation of *Dissociation* elements in transgenic tomato plants. Mol Gen Genet 218: 25–32 (1989).
38. McClintock B: Chromosome organization and genic expression. Cold Spring Harbor Symp Quant Biol 16: 13–47 (1951).
39. Messeguer R, Ganal MW, Steffens JC, Tanksley SD: Characterization of the level, target sites and inheritance of cytosine methylation in tomato nuclear DNA. Plant Mol Biol 16: 753–770 (1991).
40. Moreno MA, Chen J, Greenblatt I, Dellaporta SL: Reconstitutional mutagenesis of the maize *P* gene by short-range *Ac* transpositions. Genetics 131: 939–956 (1992).
41. O'Hare K, Rubin GM: Structures of *P* transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell 34: 25–35 (1983).
42. Osborne BI, Corr CA, Prince JP, Hehl R, Tanksley SD, McCormick S, Baker B: *Ac* transposition from a T-DNA can generate linked and unlinked clusters of insertions in the tomato genome. Genetics 129: 833–844 (1991).
43. Oshima M, Harada N, Matsuoka M, Ohashi Y: The nucleotide sequence of pathogenesis-related (PR) 1b protein gene of tobacco. Nucl Acids Res 18: 182 (1990).
44. Pohlman RF, Fedoroff NV, Messing J: The nucleotide sequence of the maize controlling element *Activator*. Cell 37: 635–643 (1984).
45. Robbins TP, Carpenter R, Coen ES: A chromosome rearrangement suggests that donor and recipient sites are associated during *Tam3* transposition in *Antirrhinum majus*. EMBO J 8: 5–13 (1989).
46. Rommens CMT, van Haaren MJJ, Buchel AS, Mol JNM, van Tunen AJ, Nijkamp HJJ, Hille J: Transactivation of *Ds* by *Ac*-transposase gene fusions in tobacco. Mol Gen Genet 231: 433–441 (1992).
47. Rommens CMT, Rudenko GN, Dijkwel PPD, van Haaren MJJ, Ouwerkerk PBF, Blok KM, Nijkamp HJJ, Hille J: Characterization of the *Ac*/*Ds* behaviour in transgenic tomato plants using plasmid rescue. Plant Mol Biol 20: 61–70 (1992).
48. Yoder JI, Palys J, Alpert K, Lassner M: *Ac* transposition in transgenic tomato plants. Mol Gen Genet 213: 291–296 (1988).
49. Yoder JI: Rapid proliferation of the maize transposable element *Activator* in transgenic tomato. Plant Cell 2: 723–730 (1990).