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ORIGINAL PAPER

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The transformation booster sequence from *Petunia hybrida* is a retrotransposon derivative that binds to the nuclear scaffold

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Abstract The transformation booster sequence (TBS) from *Petunia hybrida* enhances transformation frequencies in *P. hybrida, Nicotiana tabacum* and *Zea mays.* TBS also stimulates homologous inter- and intramolecular recombination in *P. hybrida*, the molecular basis for this stimulation is not known. We investigated whether TBS contains sequence elements that might contribute to the stimulation of recombination and whether its recombinogenic potential reflects a biological function of TBS. We identified a scaffold attachment region (SAR) within TBS and analysed its distribution in the genome and its homologies to other genomic sequences. A 516 bp subfragment of TBS binds to the nuclear scaffold. The sequence of the TBS-SAR fragment shows strong homologies to retroviral elements from plants, suggesting that TBS is an inactive derivative of a retrovirus that still promotes DNA recombination.

Key words Transformation booster sequence
Recombination · Scaffold attachment region
Retrotransposon · Petunia

Introduction

The transformation booster sequence (TBS) from *Petunia hybrida* enhances polyethylene glycol-mediated transformation of protoplasts about 20-fold in in *P. hybrida* and *Nicotiana tabacum* (Meyer et al. 1988). It has recently been shown that transformation frequencies are also increased in *N. tabacum* (7.8- to 16-fold) and in *Zea mays* (1.7- to 2.4-fold) when a plasmid containing TBS is introduced by microprojectile bombardment (Buising and Benhow 1994). TBS also shows a stimulatory effect on homologous recombination between two recombination substrates transferred into *P. hybrida* (Engels and Meyer 1992); the molecular basis of this stimulatory effect is not known. Computer sequence analysis of the TBS segment (Kartzke et al. 1990; Buising and Benbow 1994) revealed the presence of numerous modular elements, such as DNA unwinding elements (Umek and Kowalski 1988), topoisomerase II binding sites (Sander and Hsieh 1985) and pyrimidine tracts (Benbow et al. 1992). These elements are likely to be associated with eukaryotic replication origins (Buising and Benbow 1994) or scaffold-associated regions (Mirkovitch et al. 1984).

In previous experiments (Meyer et al. 1988) we were unable to detect any replication activity of TBS. We therefore focused on the examination of a potential role of TBS as a scaffold-associated region (SAR). SARs are AT-rich sequences, several hundred base pairs in length, containing topoisomerase II binding sites. They are known to bind specifically to nuclear scaffolds and are proposed to form the bases of chromatin loops (Mirkovitch et al. 1984; Gasser and Laemmli 1986). SAR elements are often located close to promoters, together with upstream regulatory sequences (Gasser and Laemmli 1987), and have been proposed to be involved in gene regulation, protecting genes against position effects (Stief et al. 1989; Phi Van et al. 1990). To test the activity of TBS as a SAR element, we examined a 516 bp *Hin*dIII fragment of TBS that carries a consensus sequence for a scaffold-associated region. We tested whether this fragment binds to the nuclear scaffold and whether it is still able to enhance homologous recombination. We further analysed the distribution

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and stability of the SAR-containing *Hind*III fragment of TBS in the genomes of *P. hybrida* and other species and searched for sequence similarities with other genomic sequences. Our data suggest that TBS binds to the nuclear matrix and that its sequence is derived from a retroviral element. We detected a high degree of polymorphism in TBS-specific sequences even among individual varieties of *P. hybrida*, which argues for significant recombinogenic activity of TBS.

Materials and methods

Construction of plasmids

For the DNA binding experiment, the 2 kb TBS fragment (Meyer et al. 1988; Fig. 1a) was inserted as an *Eco*RI fragment into the plasmid pBluescript KS^{+} (pB-TBS; stratagene). For analysis of homologous recombination, two different vectors were constructed on the basis of the plasmid pINS3, which contains a 96 bp synthetic intron based on a dicot-specific consensus sequence (Goodall and Filipowicz 1991). The artificial intron sequence was inserted into the coding region of the NPTII gene (Fig. 1b; R. Hörold, personal communication). Two copies of a 352 bp fragment of phage lambda, [positions − 252 to + 100, relative to the *attP* recombination site (Landy 1989)] were inserted in the same orientation into the *Bam*HI and the *Hind*III sites respectively of the intron. In order to test its influence on intrachromosomal recombination, a 516 bp subfragment of the TBS was inserted between the two *attP* regions (Fig. 1c). In the control plasmid, a 531 bp lambda fragment (*Tth*I-*Eae*I) was inserted at the same position (Fig. 1d).

Protoplast isolation and transformation

Protoplasts of *P. hybrida* var. RLOI (Stotz et al. 1985) were isolated from leaves of sterile shoot cultures as described by Engels and Meyer (1992). Direct gene transfer was performed by a modification of a polyethyleneglycol (PEG) fusion technique (Hein et al. 1983) as described by Engels and Meyer (1992).

Isolation of nuclei and preparation of nuclear scaffolds

About 2×10^7 protoplasts were lysed in 40 ml of Honda buffer (2.5% Ficoll, 5% Dextran-T-40, 25 mM TRIS, pH 8.5, 5 mM $MgCl_2$, 0.5% Triton X-100, 0.44 mM sucrose) supplemented with 2.5 mM dithiothreitol, 1 mM phenylmethylsulphonyl fluoride (PMSF),

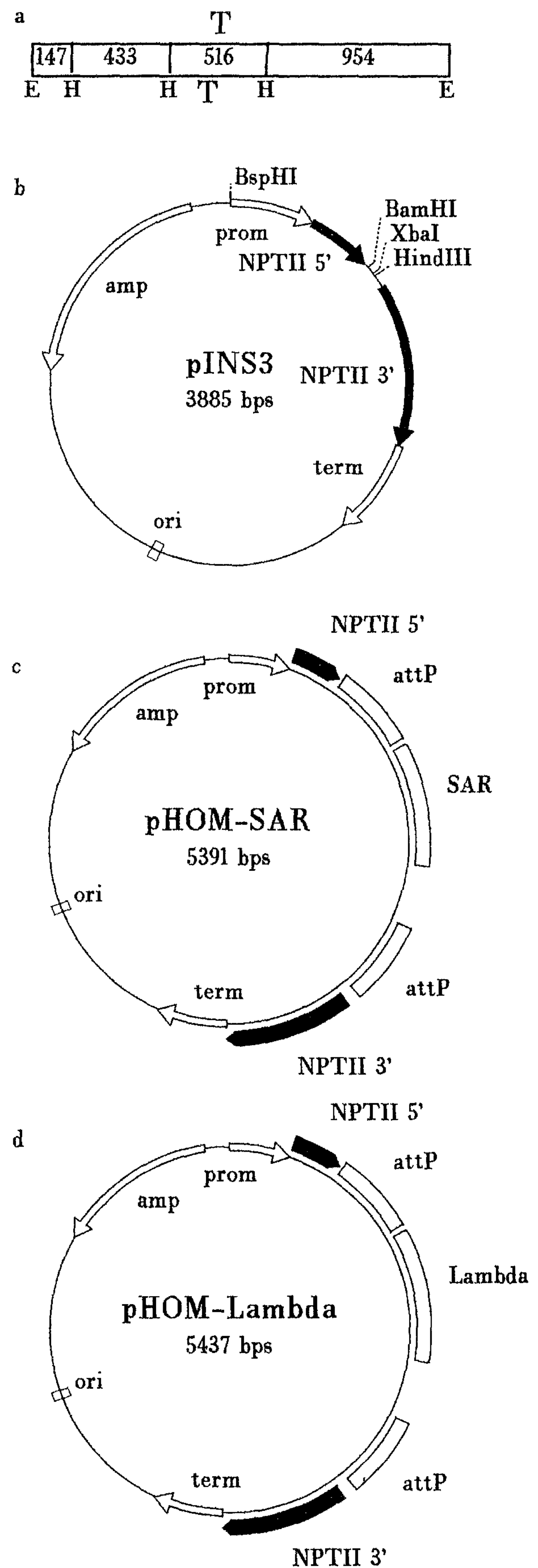

Fig. 1a–d Structure of the constructs used. **a** Map of the digestion products of the transposon booster sequence (TBS) *Eco*RI fragment used for the DNA binding assay. Digestion of the plasmid with *Hind*III and *Eco*RI leads to the formation of five fragments. Abbreviations: E, *Eco*RI; H, *Hind*III; T, topoisomerase binding site. **b** Plasmid pINS3; the NPTII gene is interrupted by a synthetic intron that carries three unique restriction sites. **c**, **d** Plasmids used for transformation experiments containing two attP regions as recombination regions separated by: **c** the 516 bp TBS-scaffold attachment region (SAR) fragment for pHOM-SAR, and **d** a 531 bp lambda region for pHOM-Lambda as control. The arrows indicate the transcriptional orientation of the NPTII and amp (ampicillin resistance) genes

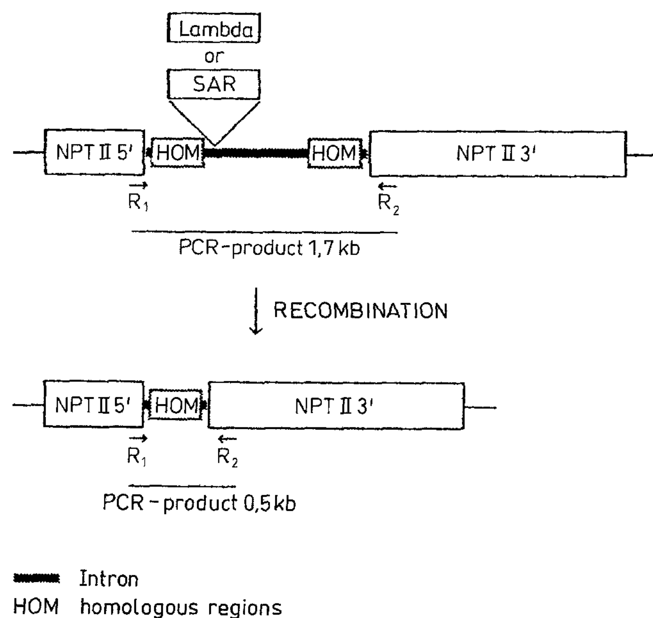

Fig. 2 Schematic representation of the principle of the polymerase chain reaction (PCR) test. The efficiency of the intramolecular recombination is compared for plasmids containing either the TBS-SAR fragment or the lambda control region. The amplification of both recombination substrate and produce is primed by the same oligonucleotides $R_1 + R_2$. The amplified recombinant and non-recombinant fragments can be distinguished by the size because recombination leads to loss of the region between the repeats (HOM) and one copy of HOM

10 µg/ml leupeptin and 2 mM spermine (Breyne et al. 1992). After cell lysis for 30 min on ice, the nuclei were pelleted by centrifugation at $3000 \times g$ for 5 min and washed three times in the same buffer. Nuclear scaffolds were extracted as described by Breyne et al. (1992) except that they were stabilized for 20 min at 37° C prior to extraction. The nuclear pellets were digested overnight at 37° C in the presence of 300 units of *Eco*RI and 300 Units of *Hin*dIII. The solubilized DNA was separated from the nuclear scaffolds by centrifugation at $2000 \times g$ for 10 min at room temperature.

In vitro binding of DNA to scaffolds

The plasmid pB-TBS containing the 2 kb *Eco*RI fragment of TBS (Fig. 1a) was digested with *Eco*RI and *Hin*dIII and the five restriction fragments were end-labelled with Klenow enzyme using [α-P^{32}]dCTP (Sambrook et al. 1989). The nuclear scaffold preparations were incubated for 4 h at 37° C with 50 ng of end-labelled DNA fragment and 5 µg of poly-dIdC as competitor in 100 µl of binding buffer (Schöffl et al. 1993). After centrifugation for 10 min at $5000 \times g$, the DNA was extracted from both the pellet and the supernatant fraction. Samples with equal counts of radioactivity (5000–8000 cpm) were loaded onto an 1.5% agarose gel and, after separation of the fragments, the gel was dried and autoradiographed.

DNA reisolation and polymerase chain reaction (PCR) analysis

Three days after transformation, protoplasts were lysed and DNA was isolated as described previously (Werr and Lörz 1986). Quantification of the recombination products obtained was performed by a PCR test as presented schematically in Fig. 2. PCR was performed in a total volume of 50 µl containing 5 µl DNA, 0.125 µl of a 10 mM primer mixtre (R_1 and R_2), 6.25 µl of a 2 mM dNTP solution, 5 µl of $10 \times$ *Taq* polymerase buffer (Promega), 2 mM $MgCl_2$, 4% (v/v) dimethylsulphoxide (DMSO) and 1 Unit *Taq* polymerase (Promega). The two primers used were R_1 (5'-TTCTTTTTGT-CAAGACCGACC-3') and R_2(5'-CATAAAACCATGGAAAATG-TTAGCC-3'). For determination of the DNA concentration in tests involving linear plasmids, we used two primers located in the ampicillin resistance region of the vector: A_1 (5'-CGAACTGGATC-TCAACAGCGG-3') and A_2 (5'-GGCACCTATCTCAG-CGATCTG-3'). In both assays, 30 amplification cycles were performed each comprising 1 min at 95° C, 2 min at 63° C and 2 min at 72° C. For standardisation of the PCR analysis, we used CsCl purified DNA of known concentration. After amplification, recombination substrates and products were separated on agarose gels and visualized by ethidium bromide starving.

Southern blot analysis

DNA samples were run in TBE buffer (Sambrook et al. 1989) and transferred to Hybond nylon membrane by the Southern blotting technique (Southern 1975). Filters were hybridized to ^{32}P-labelled DNA fragments according to Feinberg and Vogelstein (1983).

Results

In vitro binding of TBS DNA fragments to petunia nuclear scaffolds

In order to localize possible scaffold-binding regions (SARs), five *Eco*RI-*Hin*dIII fragments of the TBS plasmid (four TBS subfragments and one vector fragment; Fig. 1a) were tested for their scaffold-binding activity by incubation with the nuclear scaffold preparation. The pellet and supernatant fractions were recovered by centrifugation, separated by electrophoresis and autoradiographed. The results obtained showed that only the 516 bp *Hin*dIII fragment bound to the nuclear scaffolds leading to its selective enrichment in the pellet fraction (Fig. 3). This fragment, containing the topoisomerase II binding site, was termed the TBS-SAR fragment and used for further analysis.

Effect of the TBS-SAR subfragment on intramolecular homologous recombination

To determine the effect of the TBS-SAR fragment on intramolecular recombination, this fragment was cloned into a plasmid between two copies of the *attP* region (see the Materials and methods, Fig. 1c) and its capacity to promote recombination between them was measured. Recombination between the *attP* regions should lead to loss of the TBS-SAR fragment and one copy of the *attP* segment and can be detected by PCR analysis.

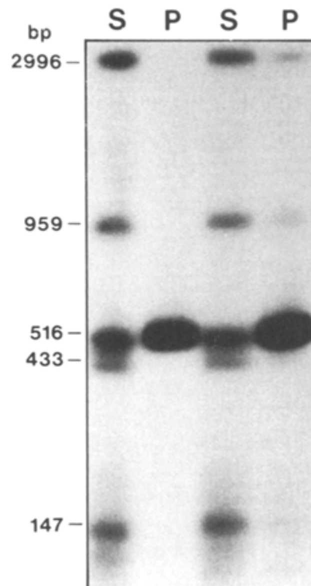

Fig. 3 Autoradiograph of the in vitro scaffold binding test. After incubation of the four TBS subfragments + plasmid with the nuclear scaffolds, pellet (P) and supernatant (S) fractions were recovered by centrifugation, separated by electrophoresis and autoradiographed. The results of two independent experiments are shown. The band sizes are indicated

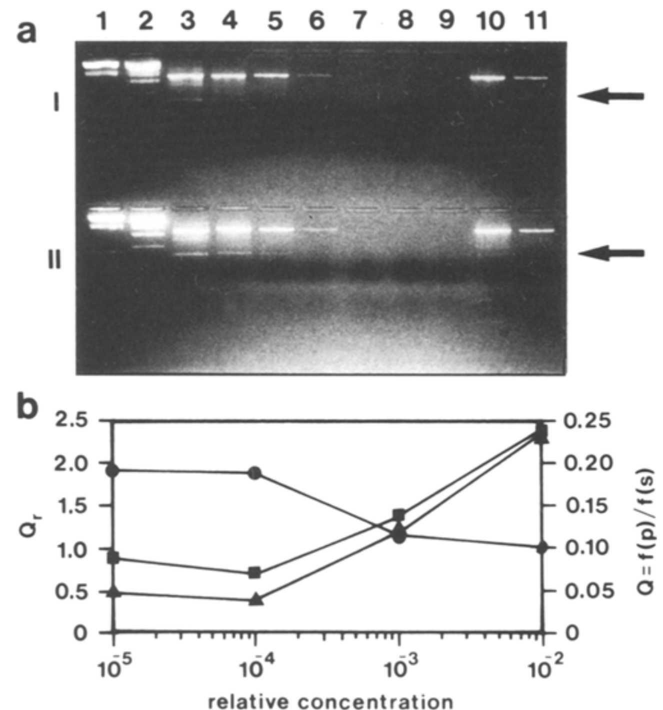

Fig. 4a, b Recombination of circular transformation vectors. **a** PCR amplification of transformed sequences, isolated from protoplasts 3 days after transformation with pHOM-Lambda (I) or pHOM-SAR (II). Lane 1, λ/*Hin*dIII (1.5 μg); lane 2, λ/*Bst*EII (1.5 μg); lanes 3–9, protoplast DNA (dilutions 10^{-1} to 10^{-7})/PCR ($R_1 + R_2$); lanes 10–11, positive control CsCl-purified DNA (1 pg/μl, 100 fg/μl)/PCR ($R_1 + R_2$). Recombination products are shown by *arrows*. **b** Q, the ratio of recombination product (P) to recombination substrate (S) was determined by scintillation counting of radioactive bands following hybridization with an *attP* probe. The SAR effect (Q_r) is the ratio $Q_{pHOM\text{-}SAR}/Q_{pHOM\text{-}Lambda}$. ■, pHOM-SAR; ▲, pHOM-Lambda; ●, the SAR effect

Recombination of circular transformation vectors

Forty micrograms of supercoiled plasmid pHOM-SAR and control plasmid pHOM-Lambda, carrying a lambda fragment for which no significant scaffold binding activity was observed, were introduced into petunia protoplasts in two parallel experiments. After 3 days, the DNA was isolated, and various dilutions were analysed by PCR. The results obtained after separation of the fragments by electrophoresis are presented in Fig. 4a. In both cases, two DNA bands are visible, a 1.7 kb band representing the recombination substrate and a 472 bp band representing the recombination product. In the thousand-fold dilution equivalent quantities of the substrate band are seen, but the product band appears to be more prominent in the case of pHOM-SAR (Fig. 4, lane 5). To provide further evidence of this, the gel was blotted and hybridized with a probe specific for *attP*. The hybridizing bands were cut out of the membrane and the amounts of radioactivity were determined by scintillation counting (see Fig. 4b). At input dilutions of 10^{-5}, the ratio between the two bands indicates that the frequency of recombination was $\sim 5 \times 10^{-2}$ for pHOM-Lambda and $\sim 1 \times 10^{-1}$ for pHOM-SAR. Hence, for the SAR-containing plasmid, the frequency of intramolecular homologous recombination is increased about twofold.

Recombination of linearized transformation vectors

In order to determine the influence of linearization of the vectors on intrachromosomal recombination, the two plasmids were digested with *Cla*I prior to transformation. As linearization occurs between the two homologous sequences located between the two primer fragments (Fig. 2), only the band corresponding to the PCR product is visible on the gel (Fig. 5a). The quantity of plasmid DNA reisolated was determined using two primers that amplify a fragment which was not interrupted by the *Cla*I digestion. The results, presented in the Fig. 5b, showed that similar quantities of DNA had been reisolated from protoplasts transformed with the two plasmids pHOM-Lambda and pHOM-SAR. The relative concentrations of the PCR products were determined by scintillation counting. As for the circular plasmids, the frequency of homologous recombination of the linearized transformation vectors was again increased about twofold in the presence of the TBS-SAR subfragment (Fig. 5c).

Distribution of the TBS fragment in the genome

To analyse the recombinogenic capacity of TBS, we examined whether the stimulatory effect of TBS on homologous recombination was correlated with unusually high instability of TBS regions within the

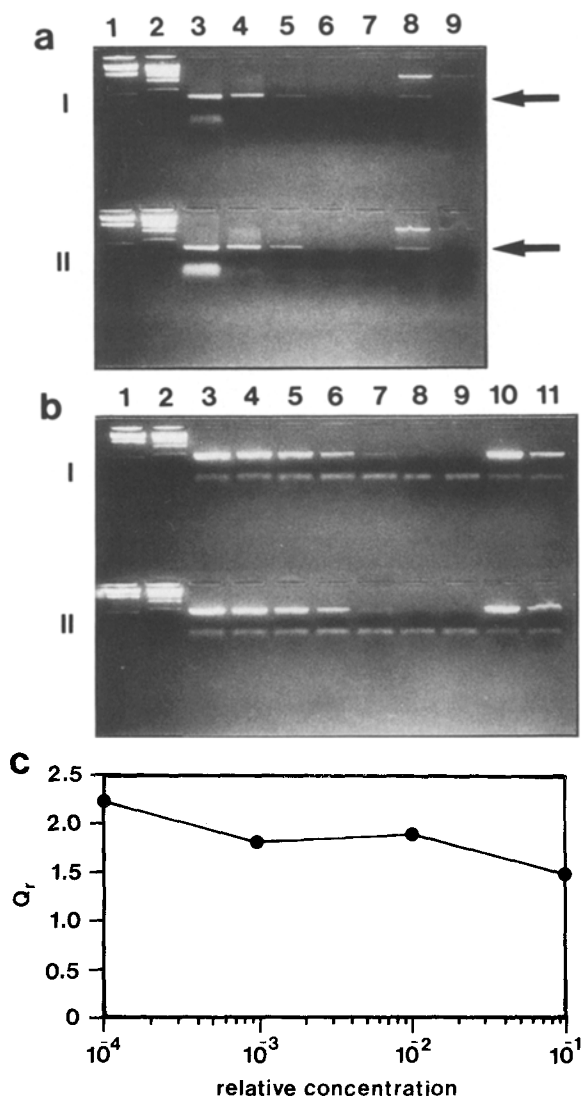

Fig. 5a–c Recombination of linearized transformation vectors. **a** PCR amplification of reisolated transformed sequences isolated from protoplasts 3 days after transformation with pHOM-Lambda (I) or pHOM-SAR (II). Recombination products are shown by *arrows*. Lane 1, λ/*Hin*dIII (1.5 μg); lane 2, λ/*Bst*EII (1.5 μg); lanes 3–7, protoplast DNA (dilutions 10^0 to 10^{-4})/PCR ($R_1 + R_2$); lanes 8–9, positive control CsCl-purified DNA/*Cla*I (1 pg/μl, 100 fg/μl)/PCR ($A_1 + A_2$). **b** Control PCR for comparison of the quantities of DNA isolated. Lane 1, λ/*Hin*dIII (1.5 μg); lane 2, λ/*Bst*EII (1.5 μg); lanes 3–9, protoplast DNA (dilutions 10^{-1} to 10^{-7}); lanes 10–11, positive control CsCl-purified DNA/*Cla*I (1 pg/μl, 10 fg/μl)/PCR ($A_1 + A_2$). **c** The magnitude of the SAR effect Q_r was determined as described in the legend to Fig. 4

genome. After extraction, the DNA was digested with restriction enzymes, separated by electrophoresis, then blotted and hybridized with the 516 bp TBS fragment. In a first experiment, the DNA was extracted from three varieties of *P. hybrida* (RL01, Red titan and Picasso blue) and digested with *Asn*I and *Eco*RV, respectively.

The autoradiograph presented in Fig. 6a shows that TBS-SAR specific fragments differ in size among the three petunia varieties. As confirmed by hybridization of the blot with chloroplast DNA, these differences are not due to incomplete digestion of the DNA (Fig. 6b). Moreover, we have isolated other genomic sequences that show the same degree of repetitiveness as TBS Comparative analysis of variability (data not shown) indicated that the variability of the TBS fragment was particularly high and sequence-specific. To detect possible differences in C methylation, the DNA was digested with the two methylation-sensitive enzymes *Bst*UI and *Msp*I. After hybridization with the TBS-SAR fragment, the only signals obtained were at the top of the autoradiograph (Fig. 7) and indicate that the TBS-(SAR-) elements are located in hypermethylated genomic regions.

Computer sequence analysis

We examined whether the TBS fragment shows similarities to other genomic sequences in order to obtain insight into the origin of this fragment and/or its function in recombination mechanisms. The TBS sequence was compared with the complete sequence databank (Genbank + EMBL) using the BLAST algorithm (Altschul et al. 1990). Several sequences of plant retrotransposons were found with significant homology to the complementary strand of the TBS sequence. In particular, the complementary strand of TBS shows 63% identity over a 179 bp stretch will the *copia*-like transposable element Tst 1 from potato (Camirand et al. 1990) and 55% identity over a 171 bp stretch from the retroviral-like transposon Tnt 1 from tobacco (Grandbastien et al. 1989). The homology is especially high in the domain of the reverse transcriptase, which corresponds to the 516 bp SAR fragment of TBS, but extends throughout the 2 kb TBS fragment (Fig. 8).

Additionally, when the complementary strand of TBS was conceptually translated and the sequence compared with the protein database, patches of high homology to reverse transcriptase proteins from a variety of genetic elements were found distributed throughout the TBS sequence. For example, there were 11 segments, comprising 445 amino acids in all of strong homology (58% conservative exchanges including 42% identities) to reverse transcriptase from the *Zea mays* copia-like retrotransposon (Voytas et al. 1992). These segments of homology were found in all open three reading frames (ORFs) of TBS, suggesting that this sequence has acquired numerous frameshift mutations.

Discussion

The TBS from *P. hybrida* enhances transformation in protoplasts of *P. hybrida* and *N. tabacum* and homologous

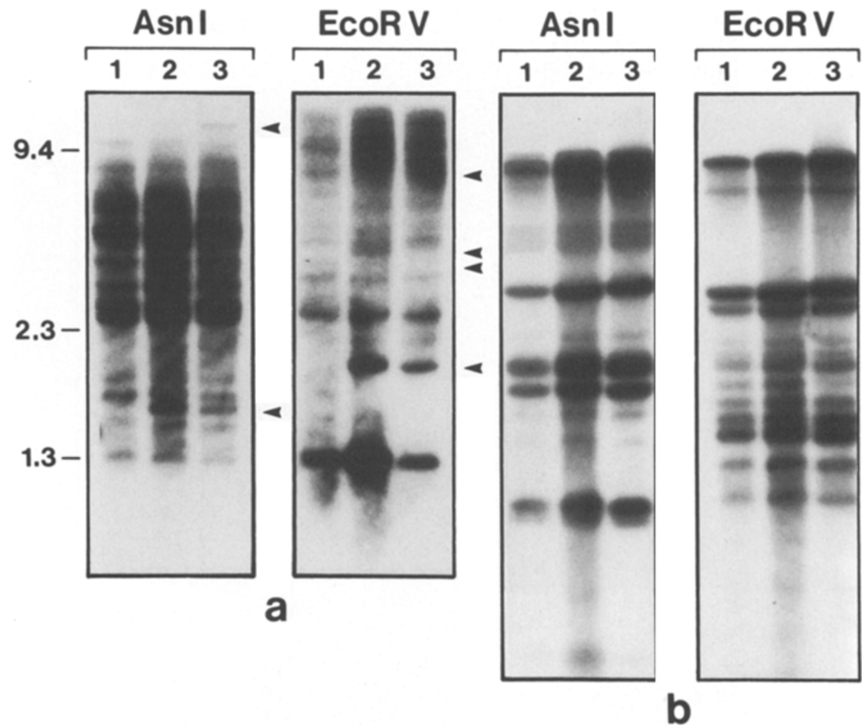

Fig. 6a, b Hybridization patterns of genomic DNA from petunia varieties RL01, Red titan and Picasso blue, after digestion with *Asn*I and *Eco*RV. **a** Hybridization with the 516 bp TBS-SAR fragment. **b** Control hybridization with chroloplast DNA. Lane 1, RLO1; lane 2, Red titan; lane 3, Picasso blue. The *arrows* shown at the right of gel **a** indicate hybridization bands that vary between the petunia varieties. Marker sizes are given in kb

recombination in *P. hybrida* (Meyer et al. 1988; Engels and Meyer 1992). In this work, we analysed molecular features that might be involved in these effects. The TBS fragment contains numerous elements that might participate in facilitating replication, recombination, nuclear retention, amplification or integration, respectively. In particular, TBS is enriched in DNA unwinding elements (DUEs), autonomously replicating sequences (ARSs), pyrimidine tracts and DNA topoisomerase II binding sites (Kartzke et al. 1990; Buising and Benbow 1994). As many of these elements are likely to be associated with scaffold attachment regions (Mirkovitch et al. 1984), we examined the scaffold binding activity of different TBS subfragments. SAR elements are likely to be involved in several aspects of chromosome structure such as organization of transcriptionally active domains or replication units (Gasser and Laemmli 1986; Benbow et al. 1992) and normalization of exogenous gene expression (Breyne et al. 1992; Allen et al. 1993). We found that a 516 bp subfragment of TBS binds effectively to nuclear scaffolds. This fragment contains, among others, two topoisonmerase II binding sites. This enzyme is known to be the major non-histone protein present in nuclear scaffolds (Gasser and Laemmli 1987) and has been proposed to be involved in homologous recombination (Dillehay et al. 1989; Miura-Masuda and Ikeda 1990). According to our results, it appears that this 516 bp subfragment contains all the elements which are necessary to increase homologous recombination to the same degree as the whole 2 kb TBS fragment (Engels and Meyer 1992). The frequency of intramolecular homologous recombination is increased about twofold in the plasmids containing the TBS-SAR subfragment. We propose that the SAR fragment of TBS has an indirect effect on recombination efficiencies; binding to the nuclear matrix facilitates and enhances retention of the SAR-containing plasmid in the nucleus. This could facilitate the action of enzymes which are involved in recombination mechanisms and are located in the nuclear matrix, such as topoisomerase II.

Although the petunia genome contains several TBS homologous sequences, we do not consider that the enhancement of transformation is due to homologous recombination. More probably it is a consequence of enhanced illegitimate integration. It is obvious that TBS does not enhance homologous recombination to the same degree as it stimulates integration into the genome via illegitimate recombination (Meyer et al. 1988; Buising and Benbow 1994). Also the magnitude of the TBS-specific effect on intramolecular homologous recombination is less than the two- to five-fold increase reported for intermolecular homologous recombination (Engels and Meyer 1992). As the intermolecular recombination assay required not only homologous recombination, but also integration into the genome via illegitimate recombination, we assume that TBS influences both homologous and illegitimate recombination with different efficiencies. Binding to the nuclear matrix may be the common facilitating step in both mechanisms, while the efficiency of TBS-specific stimulation of illegitimate recombination

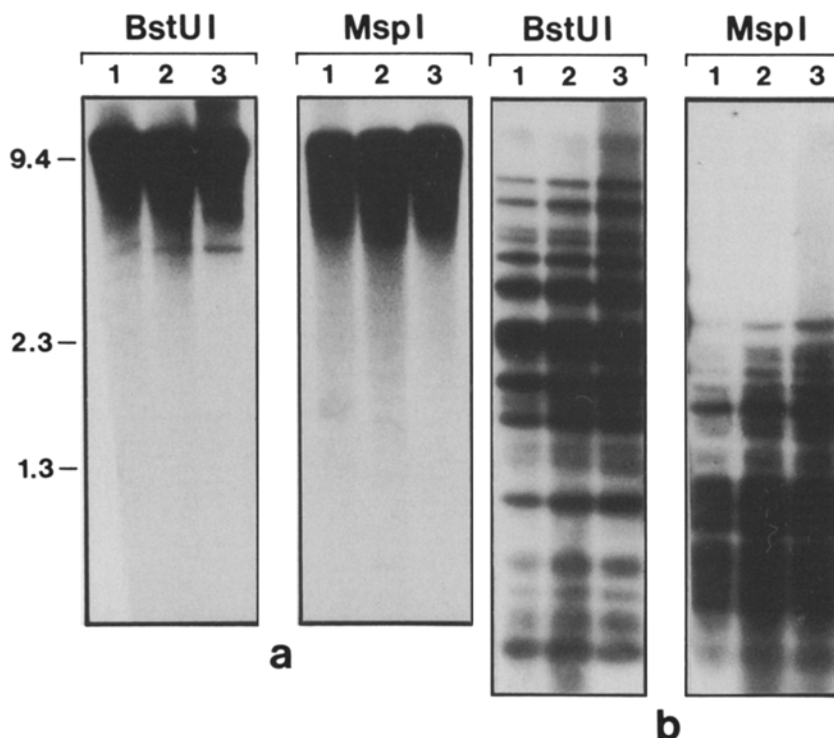

Fig. 7a, b Hybridization pattern of genomic DNA from the petunia varieties RL01, Red titan and Picasso blue after digestion with *Bst*UI and *Msp*I. **a** Hybridization with the 516 bp TBS-SAR fragment. **b** Control hybridization with chloroplast DNA. Lane 1, RLOI; lane 2, Red titan; lane 3, Picasso blue

is higher than the degree of enhancement of homologous recombination. Although our data show that the capacity of TBS to enhance homologous recombination is located on the SAR fragment, we cannot exclude the possibility that neighbouring fragments also contribute to the enhancement of illegitimate recombination.

The potential of TBS for promoting transformation and recombination mechanisms is accompanied by an instability of this region within the genome, as we observe differences between certain TBS-specific restriction fragments from three varieties of *P. hybrida*. Repetitive elements are known to participate in DNA rearrangement processes by ectopic recombination or transposition, but, compared to other repetitive sequences, the degree of TBS-specific variability appears unusually high. In order to gain insight into the origins of this variability, the TBS sequence was compared with the complete databank. Computer sequence analysis showed that the inverse sequence of the TBS fragment presents significant homology with the transcribed strand of plant retrotransposons. These transposable elements, representing the largest and best characterized class of mobile genetic elements, encode a reverse transcriptase and replicate through an RNA intermediate.

Retrotransposons can be divided into two principal categories. The retrotransposons of the "viral" family are flanked by long terminal repeats (LTRs) and encode proteins similar to those encoded by retroviruses (Voytas et al. 1992). Examples of this family are the Ty elements from *Saccharomyces cerevisiae* (Boeke 1989), the *copia*-elements from *Drosophila melanogaster* (Bingham and Zachar 1989) and, for plants, Wis-2 from *Triticum aestivum*, Tnt 1 from *N. tabacum* and Tst 1 from *Solanum tuberosum* (Grandbastien 1992). The second category or "non-viral" retrotransposons lack LTRs and encode proteins with significantly fewer similarities to those of the retroviruses. To this family belong, for example, the LINE sequences in mammals (in particular the L1 element, Hutchinson et al. 1989) and the Cin-4 element from *Z. mays* (Schwarz-Sommer et al. 1987). TBS presents homology with the retrotransposons of the first class, especially with the *copia*-like elements Tst 1 from potato and Tnt 1 from tobacco. The homologies involve mainly the domain of the reverse transcriptase, but also that of the viral integrase. The 2 kb TBS fragment does not extend to the region containing the LTR, but the degree of homology with Tst 1 strongly suggests that the TBS element belongs to, or is derived from, the family of viral retrotransposons. *Copia*-like retrotransposons are ubiquitous among plants (Voytas et al. 1992), and a common sequence encoding the peptide YGLKQ of the reverse transcriptase could be defined from a comparison of 33 Ty 1/*copia* retrotransposon sequences (Hirochika and Hirochika 1993). This sequence can also be found at positions 607–621 (reverse) in the TBS-SAR fragment (Meyer et al. 1988).

Cis-activity of retrotransposable elements in plants was first demonstrated for the Tnt 1 element from tobacco (Grandbastien et al. 1989). In recent studies, a PCR approach has been applied to identify actively transcribed reverse transcriptase domains by amplifica-

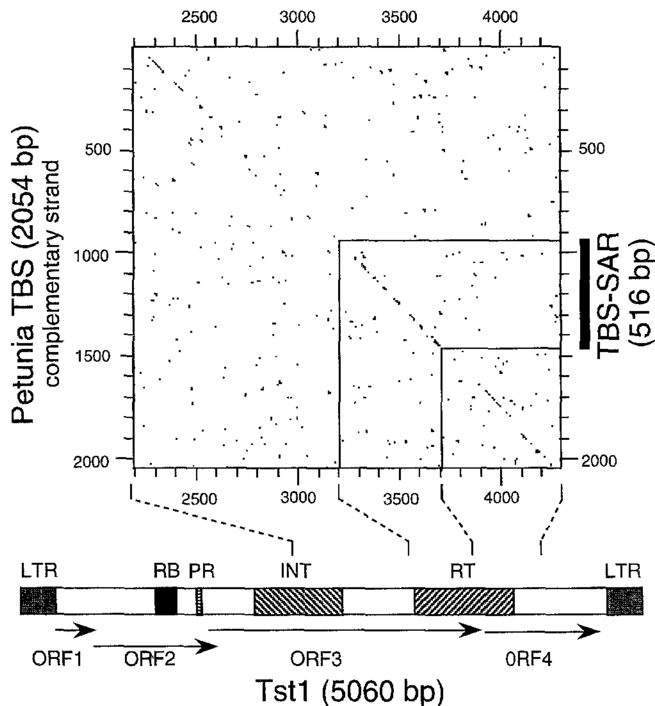

Fig. 8 Comparison of the nucleotide sequences of petunia TBS and the *copia*-like transposable element Tst 1 from potato. The complementary strand of the 2054 bp TBS and the 5060 bp sequence of the Tst 1 element were compared in a dot matrix. Each dot denotes at least 11 identities in a window of 15 nucleotides. The matrix is shown only for the part of Tst 1 (from 2.2 to 4.4 kb) that is homologous to the TBS. The genetic structure of Tst 1 is shown as described by Camirand et al. (1990). The positions of conserved functional domains are indicated: RB, RNA binding; PR, protease; INT, integrase; RT, reverse transcriptase. The position of the 516 bp TBS-SAR subfragment is marked by a *black bar*

tion of cDNA sequences (Grandbastien 1992; Hirochika 1993). Nevertheless, the majority of plant retrotransposons seems to be *cis*-inactive. While Tnt 1 is a functional element, Tst 1 cannot autonomously transpose because of a stop codon located in the region of the reverse transcriptase (Camirand et al. 1990). In the TBS region, no ORF of more than 550 bp in length could be detected, indicating that TBS is not a *cis*-functional retrotransposon. However, as in the case of transposable elements in general, retrotransposition events can be mediated in *trans* by the activity of enzymes encoded elsewhere in the genome. Thus, the Wis-2-1A element from *T. aestivum* exhibits a high degree of variability resulting in restriction fragment length polymorphisms (RFLPs) even between individuals (Moore et al. 1991). The absence of long continuous ORFs in this element (Harberd et al. 1987) indicates a possible role for *trans*-activation. The degree of activity of TBS, however, seems more limited. Considering that TBS is found in different species, such as petunia, tobacco, maize, snapdragon and sugarbeet (Engels 1991), and the variable distribution of the TBS-SAR fragments between the different petunia species, we can presume a common origin and an ancestral transposition activity of these elements.

The TBS-SAR subfragment is located in a TBS region that displays the highest homology with other retrotransposons, i.e. in the domain of the reverse transcriptase, the ORF of which is interrupted several times. With few exceptions, SAR elements are located in non-transcribed regions (Gasser and Laemmli 1987). The derivation of a SAR element from a coding region presumably involved reorganization and mutation events. The TBS-SAR subfragment is located in an hypermethylated region. The methylation of a DNA fragment favours mutations within CpG residues, due to deamination of ^{5Me}C residues, creating T residues (Kricker et al. 1992). The transition of CG to TG or CA dinucleotides would have increased the incidence of A/T nucleotides, resulting in the AT-rich character typical of SAR elements. The suggestion that a number of T residues in the TBS-SAR region are derived from deamination of ^{5Me}C residues is supported by the fact that, in the TBS-SAR subfragment, no CG dinucleotides are present (25 would be expected) and the content of TG dinucleotides is increased by 50% (with 36 TG dinucleotides, compared to 24 GT dinucleotides).

Transposition of retroelements results in an amplification of these elements in the genome. We suppose that the evolution of the retrotransposon into a scaffold associated region could have been drawn by constraints affecting the organization of the nucleus or the control of replication. The development of the SAR from a region coding for an enzyme, the reverse transcriptase, presents an interesting example of an evolutionary process in which a sequence-specific DNA function is converted into a structural function.

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