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 Benbow 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 *HindIII* 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...
and stability of the SAR-containing HindIII 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 EcoRI 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 BamHI and the HindIII 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 (Tthl-EaeI) 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 polyethylene glycol (PEG) fusion technique (Hein et al. 1983) as described by Engels and Meyer (1992).

Isolation of nuclei and preparation of nuclear scaffolds

About $2 \times 10^7$ 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),

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**Fig. 1a-d** Structure of the constructs used. **a** Map of the digestion products of the transposon booster sequence (TBS) EcoRI fragment used for the DNA binding assay. Digestion of the plasmid with HindIII and EcoRI leads to the formation of five fragments. Abbreviations: E, EcoRI; H, HindIII; 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.