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Fusion of the transposase with a classical nuclear localization signal to increase the transposition efficiency of *Ac* transposon

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Abstract. A new strategy was applied to improve the transposition efficiency of the maize transposon *Activator* (*Ac*) in heterologous plants. The *Ac* transposase was fused with a classical nuclear localization signal (NLS) of SV40 to promote the transport of transposase into a nucleus. Base on this, two NLS-TPase constructs were yielded, one containing the full length transposase gene (termed as SV40TPase), the other containing the truncated transposase gene (lacking its first NLS-like signal, termed as SV39TPase). These two NLS-TPase genes were expressed in transgenic tobacco plants under the control of PR-1a promoter. Excision of non-autonomous transposable element (*Ds*) from luciferase (LUC) reporter gene constructs was employed to analyze the induction of *Ac* transposase containing NLS. Applying the LUC assay and PCR analysis, these new NLS-TPase sources triggered higher *Ds* excision efficiencies then the native transposase. Furthermore, the SV40TPase showed more ability then the SV39TPase to trigger the *Ds* element. The usage of this new inducible transposon for plant functional genomics is discussed.

Keywords: *Ac* transposase; Inducible transposon; Luciferase reporter gene; Nuclear localization signal.

Abbreviations: HPT, hygromycin phosphotransferase; **NLS,** Nuclear localization signal; **NPT,** neomycin phosphotransferase; **LUC,** luciferase; **SA,** salicylic acid; **TPase,** transposase.

Introduction

The maize transposon *Activator* (*Ac*) is an autonomous transposable element with a size of 4565 bp. It codes for a single gene product, the transposase (TPase), which together with the inverted repeats and about 300 bp from each end of the transposon and putative host factors is the only prerequisite for transposition of the *Ac* element in plants. The *Ac* element is active in a wide range of plant species, including several members of the Solanaceae, and in rice, carrot and Arabidopsis (Becker et al., 1986; Van Sluys et al., 1987; Knapp et al., 1988; Yoder et al., 1988; Houba-Herin et al., 1990; Izawa et al., 1991), and has proven to be a powerful genetic tool for yielding knockout mutants for plant functional genomic studies (for a review, see Haring et al., 1991). Recently, the International Rice Genome Sequencing Project (IRGSP) completed its sequencing of the entire rice (*Oryza sativa*) genome. Various strategies, including transposon tagging, have been used to produce a large population of mutant plants adequately assigning function to the abundant sequence information (for review see Jeon and An, 2001). However, using transposon as a tool to create knockout mutants in plants with large genomes, seems to require increased transposition efficiency. To this end, Scofield et al. have fused the *Ac* TPase with the cauliflower mosaic virus (CaMV) 35S RNA promoter and found, in tobacco, no direct proportionality between the amounts of TPase mRNA and *Ac*/*Ds* transposition activity (Scofield et al., 1992). Furthermore, transpositions occur only at TPase transcription level, below a critical threshold (Scofield et al., 1993). One possible explanation is that above this threshold the TPase starts to aggregate and transpositions cease (Heinlein et al., 1994), forcing us to consider another approach to improving transposition efficiency.

In principle, in order to perform the transposition events, the TPase can be transported into the nuclei, a process mediated by specific signals called nuclear localization sequences (NLSs) (Stochaj and Silver, 1992a). The TPase has three NLSs near its amino-terminal end, NLS (44-62), NLS (159-178), and NLS (174-206), each of which is sufficient to direct GUS to the nucleus (Boehm et al., 1995). However, NLS (44-62) and NLS (159-178) are bipartite NLSs while the structure of NLS (174-206) is not in one of the three major NLS categories. Interestingly, all three sequences were determined to be "weak" NLSs or NLS-like signals (Heinlein et al., 1994; Wang et al., 1998 and personal communication). We predict then that fusing a classical-NLS sequence (e. g. a source from SV40) to the TPase protein could promote the transport of the *Ac* TPase and consequently increase transposition efficiency.

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To date, the nuclear targeting signals of more than 70 mammalian and yeast proteins have been characterized (Dingwall and Laskey, 1991; Garcia-Bustos et al., 1991). Studies indicate that the nuclear transport machinery is highly conserved between animals, yeast and plants (Nelson and Silver, 1989; van der Krol and Chua, 1991; Lassner et al., 1991; Stochaj and Silver, 1992b; Hicks and Raikhel, 1993; Wagner and Hall, 1993). Three different categories of NLSs were identified. The SV40 large-T antigen NLS (ppKKKRKv) is the prototype NLS category, characterized by a short uninterrupted stretch of basic amino acids (Kalderon et al., 1984; Lanford and Butel, 1984). The second most common category is the bipartite NLSs, consisting of two clusters of basic residues separated by a spacer peptide. The paradigm for the bipartite class is the *Xenopus laevis* nucleoplasmin NLS (KRpaatkkagqa KKKKI) (Dingwall and Laskey, 1991). The third category is represented by the yeast MAT alpha-2 NLS (KipiK) and contains only one or two basic residues contiguous to hydrophobic amino acids (Hall et al., 1984).

Accordingly, we decided to use the SV40 large-T antigen NLS (ppKKKRKv) sequence to fuse with the TPase gene. Based on the inducible transposon system, the prototype NLS of SV40 containing TPase was used as the source to trigger the transposition of the non-autonomous *Ds* element. We found that the NLS-containing TPase sources triggered higher *Ds* transposition efficiencies than did the native TPase source. Furthermore, it has been reported that the N-terminally truncated TPase derivative is inefficiently transported into the nucleus and aggregates predominantly in the cytoplasm (Heinlein et al., 1994). We constructed a similarly truncated TPase lacking the first NLS-like sequence of the native TPase. This construct was then fused with the prototype NLS of SV40. We found that this truncated TPase derivative triggered slightly lower *Ds* transposition efficiency than did the NLS-containing full length TPase. The role of the NLSs for the transport of the TPase and the subsequent transposition was discussed.

Materials and Methods

DNA Manipulation

Recombinant DNA technology was performed according to Sambrook et al. (1989). The materials and methods required for the construction of plasmids pBC SK+ and pBinHygTs have been previously reported (Charng et al., 1995).

In order to generate modified NLSs constructs, which consist of the NLS of the SV40, two DNA fragments were yielded by polymerase chain reactions. To this end, the following synthetic oligonucleotide primers were used: primer SV39 (harboring 18 nucleotides coding for NLS and the remaining nucleotide identical to the *Ac* sequence from position 1404 to 1416, 5'-GGATCCATGAAGAAGAAGCG-CAAAGCTATTGTTCATG-3'); primer SV40 (harboring 18 nucleotides coding for NLS and the remaining nucleotide identical to the *Ac* sequence from position 990 to 1104, 5'-GGATCCATGAAGAAGAAGCGCAAGACGCC TCCGGT-TGG-3'), and primer CSV (complementary to the *Ac* sequence from position 1845 to 1823, 5'-AGTACTCATGTTC TACAATATTG-3'). Each polymerase chain reaction contained approximately 0.1µg template DNA (plasmid pBinHygTs), 0.25 µg of each primer, 0.2 mM dNTPs, 1.5 m $MgCl_2$, 1 unit of Taq DNA polymerase, and 10X buffer. The samples were subjected to 40 cycles of amplification with each cycle consisting of 1 min at 94°C, 30 s at 50°C, and 30 at 72°C. After reactions an agarose gel electrophosis was performed to recover the DNA fragments. Each DNA fragment was then ligated with a 3.4 kb *Sma*I fragment of plasmid pBC SK+. Then a *Nsi* I excised 1.7 kb TPase fragment from the pPCV720ORF was ligated to the *Nsi* I treated pBCSV40 and pBCSV39 vector, yielding the plasmids pLc40TP and pLc39TP. These two plasmids were digested with *Bam H*I, yielding the 2.5 kb fragment. This fragment was then ligated to the *Bam H*I treated pBinHyg binary vector (Charng et al., 1995), resulting in two plasmids designated pBH40Ts and pBH39Ts.

Plant Transformation

All transformations were performed with tobacco plants containing the *Ds*::reporter gene construct, and the transgenic tobacco plants were regenerated as described by Charng et al. (1997).

In Vivo and In Vitro Assays for Luciferase Gene Activities

Luciferase enzyme activity was determined as described by Howell et al. (1989) using a Lumat LB 9501 luminometer from Berthold, München, Germany.

For in vivo assaying, the plant material was sprayed with 0.15 mg/l of luciferin aqueous solution, placed in a dark room and then measured by the luminometer immediately. The luminometer consisted of an intensified CCD camera (Hamamatsu, Japan), with a Nikon 35 mm lens, connected to a personal computer. The live plant material image and the luminescent image were taken separately, and the latter revealed callus with luciferase activity.

Genomic DNA Isolation

Genomic DNA was isolated from transformed plants with the use of a kit (BIO101, Vista, AC). Half gram calli were collected, frozen with liquid nitrogen in a mortar, and ground with a pestle. The nuclei were collected and lysed by protease treatment according to the manufacturer's instructions. Genomic DNA was precipitated by adding ethanol and dissolved in 100 µl TE (10 mM Tris-HCl, 1 mM EDTA pH 8.0).

Analysis of Ds Excision Events by Polymerase Chain Reaction

For the analysis of *Ds* transposition from the *Ds*::LUC construct in transgenic tobacco plants, three synthetic oligonucleotide primers were used: ACP (complementary to the *Ac* sequence from nucleotide 480-462, 5'-

CTGGGAGACAGGGAGAGTC-3'), primer LUC (complementary to the luciferase coding sequence from position 577 to 556 as numbered by De Wet et al. (1987), 5'-CGGGAGGTAGATGAGATGTGAC-3'), and primer 35S (identical to the CaMV 35SRNA promoter sequence, 5'-TCCTTCGCAAGACCCTTCCT-3'). Each reaction mixture contained ca. 0.1 µg template DNA, 0.25 µg of each primer, 0.2 mM deoxynucleoside triphosphate, 1 U of Taq DNA polymerase, 1.5 mM $MgCl_2$ and 10x buffer. The amplification protocol comprised 40 cycles of 1 min at 94°C, 30 s at 50°C, and 30 s at 72°C.

Results

Construction of the PR-1a::SVTPase Chimeric Gene and its Introduction into Tobacco

In order to improve the usage of the inducible transposon system, we had previously transformed a two-component system into *Nicotiana tabacum* cvs. Samsun nn; i.e. the first component for detection of *Ds* excision efficiency and the second component for providing the TPase source (Figure 1). In this study, various second components were constructed based on the previous two-component system (Charng et al., 1997).

For the first component, a *Ds* element was inserted between the CaMV 35S promoter and the coding region of the luciferase (LUC) reporter gene from firefly (*Ds*::LUC; Figure 1A). This leads to the inactivation of the LUC gene, which is restored upon removal of the *Ds* element from the chimeric gene by transposon excision. This gene construct has been inserted in the binary vector Bin19 (Bevan, 1984) and used to transform tobacco plants. Transformants were selected by maintaining the leaf disks and the regenerating plantlets on kanamycin-containing medium.

For the second component, as a source for the NLS containing TPase, plasmid pBinHygTs was used (Charng et al., 1995). This plasmid contains the PR-1a promoter fusion with a full length cDNA clone of the *Ac* TPase transcript and was used to provide the native TPase source in this report (as a control). Based on this construct, we then generated two additional second components (Figure 1B). First, the full length *Ac* TPase gene was fused to the typical NLS of SV40 (referred as PR-1a::SV40TPase). The second construct, designated PR-1a::SV39TPase, was a deleted derivative of the PR-1a::SV40TPase. It lacks the first NLS and retains only the second NLS of the putative TPase, but harbors the prototype NLS of SV40.

The kanamycin-resistant progeny of self-pollinated transformants containing the first component (*Ds*::LUC construct) were transformed with the second component, and the transformants were selected on hygromycin B- and kanamycin-containing medium. All transformed tobacco plants contained the same *Ds*::LUC construct and a second component, according to the TPase source. We termed the transformed tobacco plants N- (containing the native TPase), 39- (containing the SV39TPase construct), or 40- (containing the SV40TPase construct). The *Ds* excision

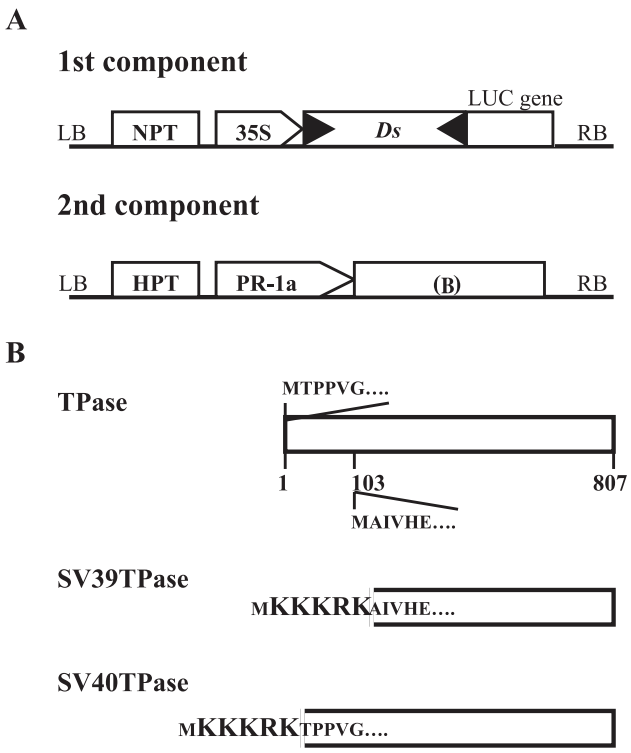

Figure 1. Schematic representation of two-component system used to access the activity of the *Ac* transposase under the influence of NLS of SV40. A, The two-component system. The *Ds*::LUC gene construct (first component) was used to analyze *Ac* transposase activity in transgenic tobacco plants via excision of non-autonomous *Ds* element. For the selection of transformed plants, the first component contains the neomycin phosphotransferase (NPT) gene, and the second component contains the hygromycin phosphotransferase (HTP) gene. "35S" indicates the promoter region for expression of the LUC reporter gene. RB and LB indicate the left and right border sequences of the T-DNA. B, The NLS derived TPase sequences used to support the transposase source under the control of the PR-1a promoter in transformed tobacco plants. Shown are the transposase local amino acids 1-6 and 103-108 of the putative transposase (TPase), inserted by the prototype of NLS (highlighted) to yield SV40TPase and SV39TPase, respectively.

events were monitored by analyzing the transformants for reporter gene activities.

Spontaneous Transposition of Ds Element in Shoot Derived from Primary Transformed Calli

To determine whether *Ds* undergoes spontaneous transposition, we assayed luciferase activity in the primary regenerated shoots of tobacco transformants. Twenty independent transformed lines for each construct were extracted for luciferase activity assay. For the transformed lines harboring the native TPase source (PR-1a::TPase), 5 out of 20 independent transformed tobacco shoots (N3, N10, N12, N16 and N20) exhibited luciferase activity. For the transformed plants containing PR-1a::SV39TPase

construct, 8 out of 20 independent transformed shoots (39-2, 39-4, 39-5, 39-7, 39-10, 39-11, 39-15 and 39-17) exhibited luciferase activity, and of the transformed shoots containing PR-1a::SV40TPase construct, 11 out of 20 independent transformed shoots (40-1, 40-4, 40-6, 40-8, 40-9, 40-11, 40-12, 40-15, 40-16, 40-18 and 40-19) exhibited luciferase activity (Table 1). These results indicate that *Ds* can be triggered spontaneously, and the transposition efficiencies were 25%, 40% and 55% for PR-1a::TPase, PR-1a::SV39TPase and PR-1a::SV40TPase construct, respectively.

To verify that the observed reporter gene activities were due to excision of the *Ds* element, we analyzed genomic DNA from plants by multiplex PCR with primers LUC (complementary to the luciferase coding sequence), ACP (complementary to the *Ac* sequence), and 35S (identical to a region of the 35S promoter) (Figure 2A). With primers 35S and ACP, a 580 bp PCR product was obtained with DNA from LUC^- tissue of either transformed tobacco plants N1, 39-1 or 40-2 (Figure 2B; lane 1, 3 and 5). In the presence of all three primers, no 670 bp product was generated from DNA of those transformants with 35S and LUC. The distance between these two primers is about 4 kb in the intact *Ds*::reporter gene construct. Together, these results indicate that *Ds* element had not undergone transposition in LUC^- tissue. In contrast, genomic DNA from LUC^+ tissue of either transformed tobacco plants N16, 39-10 or 40-1 yielded PCR products of 580 and 670 bp (Figure 2B), indicating that the LUC^+ tissue contained both cells in which *Ds* had undergone transposition and cells in which it had not.

A NLS of SV40 Fusion with TPase Triggers Highest Ds Excision Efficiency after Induction

The fact that *Ds* transposition occurred spontaneously in transformed tobacco harboring SV39/40::TPase indicates the NLS-containing TPase is active in tobacco plants. To determine whether the NLS-TPase fusion construct could improve the inducible transposon system, we studied the induction of *Ds* transposition using salicylic acid (SA) as the inducer in transgenic tobacco calli and plants. All primary transformed LUC^- tobacco plants (Table 1) were allowed to self-pollinate, and the progeny were collected for induction experiments. The T1 tobacco seeds of each transformed line were incubated on MS medium containing kanamycin and hygromycin to ensure the presence of the two components. Five independent transformants for each NLS-TPase construct and at least 50 induced leaf discs for each transformed line were assayed. To induce the expression of the *Ac* TPase, tobacco plants harboring the two components were treated with SA according to the previous report (Charng et al., 1997). The leaf discs from each transformed line were divided into several portions and were induced by SA or incubated directly on callus regeneration selection medium. For induction, the leaf discs were incubated on callus regeneration medium containing 1 or 5 mM SA for 24 h and then transferred to callus regeneration selection medium without SA. Reporter gene activities were analyzed 2 weeks after induction. The *Ds* excision efficiency was analyzed by recording the presence of leaf discs yielding LUC reporter gene activities, either in vivo or in vitro.

Table 1. Spontaneous transposition of the *Ds* element in primary transformed tobacco plants. Luciferase activity was measured in shoots of transformed tobacco plants harboring the native transposase (TPase), SV39TPase (39TPase) or SV40TPase (40TPase) construct. Activity is expressed in relative light units (emission was measured for 2 s) per microgram of total protein; values of <50 RLU mg^{-1} produced no typical luciferase-luciferin activities corresponding to the absence of luciferase activity, referred to as LUC^-. RLU, relative light units.

Tobacco/(TPase)	LUC RLU	Tobacco/(39TPase)	LUC RLU	Tobacco/(40TPase)	LUC RLU
ΔAc-1	1.5				
N1	3.3	39-1	3.3	40-1	1389
N2	2.7	39-2	128	40-2	8.5
N3	58	39-3	29.5	40-3	20
N4	11.4	39-4	714	40-4	352
N5	9.5	39-5	903	40-5	36
N6	10.8	39-6	30	40-6	89
N7	20.1	39-7	400	40-7	5.9
N8	8.4	39-8	25	40-8	325
N9	12.8	39-9	11	40-9	128
N10	114	39-10	1231	40-10	9.8
N11	6.4	39-11	143	40-11	1066
N12	78	39-12	44	40-12	252
N13	8.6	39-13	8.5	40-13	33
N14	3.2	39-14	12.1	40-14	10.5
N15	8.8	39-15	208	40-15	556
N16	117	39-16	10.5	40-16	302
N17	9.5	39-17	601	40-17	21.5
N18	8.2	39-18	30	40-18	899
N19	13	39-19	7.5	40-19	636
N20	70	39-20	12.1	40-20	25

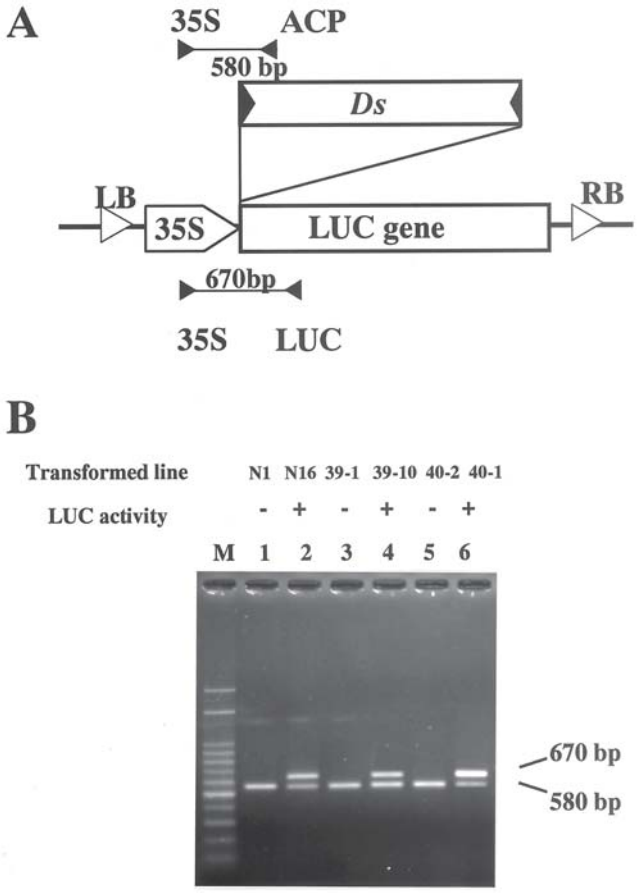

Figure 2. PCR analysis of *Ds* excision from the *Ds*::LUC chimeric gene. A, Structure of the *Ds*::LUC chimeric gene and the location of primers (shown as solid triangles) used for PCR analysis. The sizes of expected PCR products (580 and 670 bp before and after excision of *Ds*, respectively) are indicated. B, Ethidium bromide-stained agarose gel on which PCR products were separated. PCR was performed with genomic DNA from the indicated transformants. Lane M, 100 bp DNA ladder.

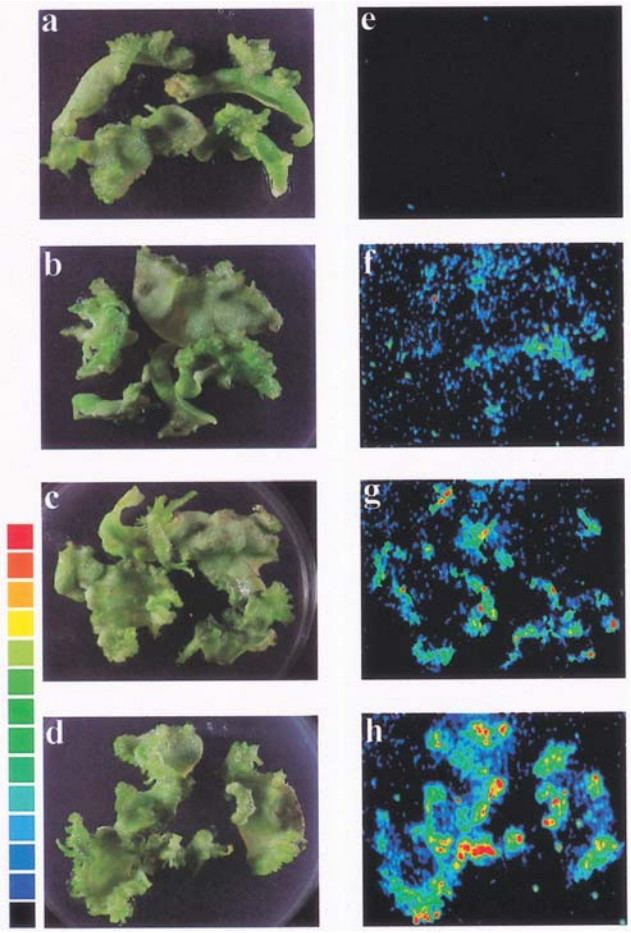

Figure 3. In vivo assay of LUC enzyme activity as revealed by *Ds* excision events in transgenic tobacco leaf discs and in response to NLS-TPase fusion. Induced *Ds* excision patterns are revealed as light images produced by leaf discs after 14 days cultivation on medium. Reflected-light reference images (a to d) show leaf discs sprayed with luciferase substrate solution. The luminescent images (e to h) detected in dark. (a) and (e): tobacco plants containing only the first element; (b) and (f): TPase as the second element to trigger the *Ds* element; (c) and (g): 39TPase as the second element to trigger the *Ds* element; (d) and (h): 40TPase as the second element to trigger the *Ds* element.

For in vivo assay of luciferase activities, *Ds* excision events are revealed as light images produced by the tissue. We observed that reporter gene activities were always restricted to the calli (Figure 3). This observation indicated that the *Ds* excision events were triggered mainly during the development of calli, mostly regenerated from the edge of excised leaf discs (Charng et al., 1997). Furthermore, when the light intensities of LUC^+ leaf discs for each construct were used as a record, a similar *Ds* excision efficiency was observed for each NLS-TPase construct (see below).

For in vitro assay, the regeneration calli for each transformed line were collected and extracted for luciferase activity assay. Figure 4 shows the mean luciferase activity yielded by each NLS-TPase transformed line with various SA-treatments. With in vitro assay, light is emitted as a peak because the luciferin-luciferase reaction is rapidly feedback-inhibited by a reaction product. All un-induced leaf discs yielded no typical luciferase activity kinetics but background values (RLU<50). These findings suggest that

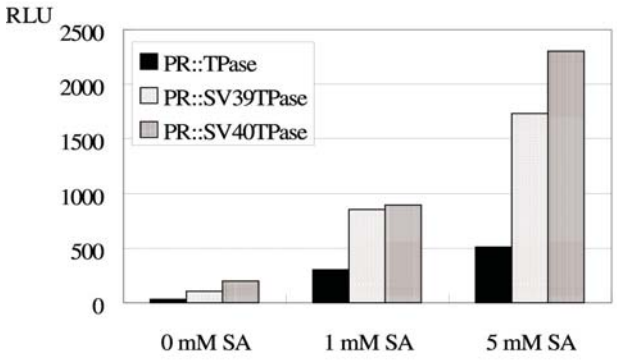

Figure 4. Schematic representation of the LUC activities of SA induced transgenic tobacco containing various NLS-TPase fusions.

no spontaneous transposition event occurred. When the leaf discs of these transformed lines were induced with 1 mM SA, the transformed lines harboring the native PR-1a::TPase yielded typical peaks of luciferase light emission, but the LUC activities were low. For the same SA-treatment, transformed plants containing PR-1a::SV40TPase or PR-1a::SV39TPase construct yielded about twofold as much as the transformed plants containing the native PR-1a::TPase construct. On the other hand, when the leaf discs of these transformed lines were induced with 5 mM SA, the transformed lines harboring the native PR-1a::TPase yielded a threefold increase in LUC activities over 1 mM SA-treated tobacco. Furthermore, the 5 mM SA-treated transformed tobacco containing PR-1a::SV40TPase and PR-1a::SV39TPase construct yielded about fourfold and threefold, respectively, what the tobacco containing native PR-1a::TPase construct with the same treatment yielded. Taken together, these results show that the transposition efficiency of *Ds* was induced by SA in a dose-dependent manner. Furthermore, the NLS-containing TPase can trigger higher *Ds* transposition more efficiency than the native TPase.

DNA Blot Analysis of Somatic Excisions Arising from the NLS TPase Fusions

To determine whether the somatic excisions triggered by Tpase-derived constructs were bona fide transposition events, DNA was extracted from SA-induced transgenic tobacco plants with two components, which yielded various *Ds* excision efficiencies after 5 mM SA treatment as described above. As probe, the 1.2 kb *Bam* HI/*Eco* RV fragment comprising the LUC reporter gene was used. Bands of 4.5 kb (if *Ds* is not excised) and 1.5 kb (if *Ds* is transposed from its donor site) can be expected. As shown in Figure 5a, plants with the highest luciferase activity (40-3) showed only the 1.5 kb band but no detectable 4.5 kb band. This indicates an early excision event during regeneration in this plant. In tobacco plants, which contained both the 4.5 kb band and the 1.5 kb fragments, transposition of *Ds* was incomplete or occurred at a later stage of development. In these plants the luciferase activities are proportional to the relative intensity of the 1.5 kb band (Figure 5a). In plant N1, only a 4.5 kb band was observed. This plant showed no enzyme activity and, as such, suggested an inactive transposon. The same filter was also probed with a 2.9 kb *Ds* fragment. Bands corresponding to the non-transposed donor site can hybridize to both the LUC probe and the *Ds* probe, yielding a band of 4.5 kb. Additional bands of various sizes (all larger than 2.9 kb), which hybridized to *Ds* probe but not to LUC probe, indicated the reinsertion of *Ds*. As shown in Figure 5b, plant 39-6 yielded three and plant 40-3 yielded four *Ds*-specific bands.

Discussion

Transposable elements have proven to be a powerful genetic tool for functional genomics studies. Several strategies have been applied to maximize the transposition efficiency the *Ac*/*Ds* system in heterologous plants: (1) fusion of a constitutive or inducible promoter to control the expression level of TPase (Scofield et al., 1992; Charng et al., 1995), and (2) application of a demethylating agent, 5AzaC, to regulate the transposition mechanism (Scortecci et al., 1997; Charng et al., 2000). Interestingly, by fusing the TPase with the CaMV 35S promoter, Scofield et al. (1992) found that, in tobacco, the accumulation of high levels of the *Ac* TPase may inhibit subsequent transposon

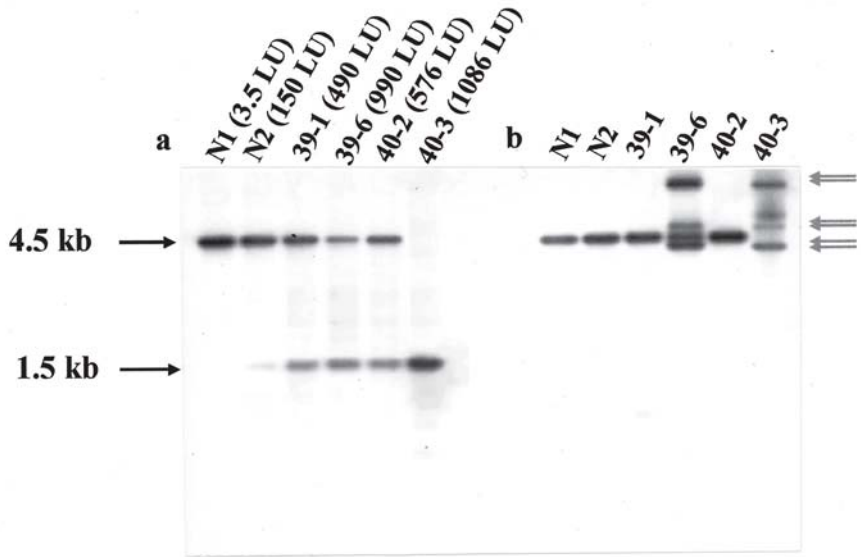

Figure 5. Southern blot analysis of *Ds* transposition events. DNA gel blot hybridization of endonuclease *Eco* RV digested genomic DNAs isolated from different transgenic tobacco lines with the luciferase gene probe (a) or with the *Ds* probe (b). Expected LUC-specific bands of the 4.5 kb fragment (inactive luciferase) and the 1.5 kb (active luciferase after *Ds* transposition) are indicated as black arrows. Reintegrated *Ds* elements are indicated as hatched arrows. On the top, the name of each sample and the luciferase activities are given.

excision. This compels us to develop another strategy to improve transposition efficiency. The native *Ac* TPase has three NLSs near its amino-terminal end, NLS (44-62), NLS (159-178) and NLS (174-206). However, all three were determined to be "weak" NLSs or NLS-like signals (Wang, 1998) though each is sufficient to direct GUS to the nucleus (Boehm et al., 1995). Here, we fused a classical nuclear localization signal (NLS) of SV40 with the TPase gene and tried to increase the nuclear import efficiency of TPase and consequentially raise its transposition frequency. Indeed, after SA induction, the fusion TPase harboring NLS triggered a transposition efficiency that was fourfold the native TPase triggered in tobacco.

A curious aspect of the *Ac* TPase has been reported. A truncated TPase lacking 102 amino acids from the amino-terminus is still functional in transgenic tobacco and Arabidopsis (Li and Starlinger, 1990; Grevelding et al., 1992). In this work, in additional to the full length NLS-TPase construct, we have constructed a similarly truncated NLS-fused TPase (SV39TPase). Our results indicated that when these two kinds of NLS-fused TPase genes were expressed under the control of the PR-1a promoter, the tobacco plants harboring SV40TPase construct always yielded higher transposition efficiency then the plants harboring SV39TPase did. Previously, Heinlein et al. (1994) suggested that a combination of several NLSs of the *Ac* TPase is required for efficient nuclear transport. On the other hand, these authors also suggested that *Ac* TPase that forms large aggregates in nuclear and the N-terminally truncated TPase derivative is inefficiently transported into the nucleus and aggregates predominantly in the cytoplasm (Heinlein et al., 1994). The fusion of NLS of SV40 may interfere with the formation of aggregates and leave more free and active TPase to perform the *Ds* excision. Alternatively, owing a higher number of NLSs, nuclear uptake of SV40TPase may proceed more quickly and lead to higher transposition frequency.

The *Ac*/*Ds* transposon system has been widely used to create knockout mutants in many heterologous plants. In order to maximize the *Ac* transposition efficiencies in many species, we have developed several inducible transposon systems (Charng et al., 1995; 1997; 2000). These systems have demonstrated functionality in tobacco, tomato and rice plants. Here, a new strategy, of fusing the TPase with a putative NLS, was introduced for expanding the usage of the inducible transposon systems. All these efforts will allow us to develop more efficient transposon systems for future plant functional genomic studies.

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轉位酶與典型之核定位信號融合後增加 *Ac* 轉位子之轉位效率

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Ac/*Ds* 轉位子為研究植物功能基因體學之重要工具。在機制上，轉位酶合成後必須靠核定位信號（NLS)進入細胞核才能執行轉位。轉位酶雖含有內生NLS，但並非典型有效。本研究建構兩種含 SV40病毒之 NLS 之轉位酶之構築，配合先前建立之「可誘導轉位子」系統，期望增強轉位酶穿入細胞核之能力，也因而提高轉位子之轉位效率。兩構築分別為：全長之轉位酶與 SV40 病毒之 NLS 融合（稱SV40TPase 構築）及原轉位酶缺失一段（含內生 NLS）再與 SV40 之 NLS 融合（稱 SV39TPase 構築）。檢測轉位效率方法為：將一被動轉位子 *Ds* 建構於冷光報導基因與啟動子之間，*Ds* 受轉位酶驅動後轉位而使報導基因表現而發冷光。結果發現含 NLS 之轉位酶之兩構築均可驅動 *Ds* 轉位，且轉位效率較原轉位酶之轉位效率高。此外，SV40TPase 構築之驅動能力高於 SV39TPase 構築。本文最後討論轉位酶與典型NLS 融合後增加轉位效率之策略，配合已建立之「可誘導轉位子」系統，對於研究植物功能基因體學之影響。

關鍵詞：*Ac* 轉位酶；「可誘導轉位子」；冷光報導基因；核定位信號。