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Excision of a *Ds*-like maize transposable element (*AcΔ*) in a transient assay in *Petunia* is enhanced by a truncated coding region of the transposable element *Ac*

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Summary. The excision of a *Ds*-like transposable element (*AcΔ*) is mediated *in trans* by the transposable element *Ac* or its derivatives in *Petunia* protoplasts cotransfected with two plasmid DNAs. Excision restores the activity of the β-glucuronidase (GUS) gene that is otherwise shut off by the presence of *AcΔ* in its leader sequence. A transient expression assay (histochemical test) is used to detect the β-glucuronidase activity at the protoplast level. The number of blue-stained protoplasts is a measure of the excision frequency. With *AcΔ* alone a near-zero background of GUS activity is detected, which is weakly enhanced by the presence, *in trans*, of either the wild-type *Ac* or the coding region (ORF_a) transcribed from the 2′ promoter of *Agrobacterium tumefaciens* TR-DNA. A strong enhancement is observed when a truncated *Ac* coding region, also under the control of the 2′ promoter, is supplied *in trans*. The truncated version has ATG_{10} at codon 103 in frame with ORF_a and is preceded by 7 out-of-frame ATGs. The assay is quick and well suited for detection of excision frequencies above the value obtained with the wild-type *Ac*. The presence of empty donor sites following excision can be demonstrated by PCR amplification and direct sequencing of the appropriate DNA fragment.

Key words: GUS transient expression – Mobile DNA – *Zea mays* – *Petunia hybrida* – Cotransfection

Introduction

The *Ac* transposable element of *Zea mays* is 4565 base pairs (bp) long (Pohlman et al. 1984; Müller-Neumann et al. 1984; English et al. 1987), and it is transcribed in maize kernels and seedlings into a 3.5 kb mRNA (Kunze et al. 1987; Finnegan et al. 1988). This mRNA has 2 open reading frames (ORF_a and ORF_b); the longer one (ORF_a) encodes a protein of 807 amino acids, which has been detected by immunoblotting techniques both in maize (Müller-Neumann et al. 1986) and in transgenic insect cells (Hauser et al. 1988).

The *Ac* transposable element has been shown to be capable of excision in transgenic tobacco plants (Baker et al. 1986). A phenotypic assay for the semi-quantitative estimation of excision frequencies has been reported (Baker et al. 1987). The assay is based on the use of an *NPT*II gene that is inactivated by insertion of a *Ds*-like transposable element into the leader sequence. Excision of the element restores the activity of the *NPT*II gene, which is under the control of a plant promoter. The restoration of activity is measured by the appearance of stably transformed kanamycin-resistant calli. This assay has been used to identify sequences necessary *in cis* for transposability as well as sequences necessary for allowing *Ac* to effect excision of an *AcΔ* element *in trans* (Baker et al. 1987; Coupland et al. 1988, 1989; Li and Starlinger 1990). A phenotypic assay has also been described by Masson and Fedoroff (1989), which measures the excision of the Spm (dSpm) transposable element at the callus stage, using the GUS gene as a reporter gene.

These assays are time-consuming. We report here on an analogous assay, which does not require stable integration of plasmids. *Petunia hybrida* protoplasts are transfected simultaneously with two plasmids: one plasmid carries the β-glucuronidase (GUS) gene under the control of a plant promoter, but with an *AcΔ* element inserted in the leader sequence, while the second carries *Ac* or a derivative thereof. The protoplasts are immobilized and stained for GUS activity, and the numbers of blue protoplasts are counted.

Materials and methods

Plasmid constructions. The plasmids pNT201 (6.1 kb) and pNT100 (9.1 kb) were obtained by cloning a *Pst*I fragment taken from pKU2 and pKU4 (Baker et al. 1987) in the *Pst*I site of the pGUS vector described be-

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low. The fragment originating from pKU2 contains the 1′ promoter of the octopine TR-DNA preceded by 752 bases from pBR322. In addition, the pKU4 fragment contains a deleted *Ac* (*AcΔ*) flanked by 60 bp of *waxy* sequence devoid of ATG triplets and located downstream of the 1′ promoter. The deletion (*Δ*) corresponds to the 1605 bp internal *Hin*dIII fragment of *Ac*. The pGUS plasmid DNA (4.8 kb) contains the coding region of the GUS gene and the nopaline synthase polyadenylation site taken from pBI101 (Jefferson et al. 1987) as a *Hin*dIII-*Eco*RI fragment and cloned in the corresponding sites of the pUC19 vector.

The plasmids pNT803, 804, 806, 900, 901 and 600gAc were constructed in the following steps. First, an *Eco*RI-*Hin*dIII fragment of pPCV701 (Koncz et al. 1987) containing the 1′ and 2′ promoters of *Agrobacterium tumefaciens* TR-DNA flanked by two polyadenylation regions (*ocspA* and *g7pA*, both from the TL-DNA) was cloned in the *Eco*RI and *Hin*dIII sites of pUC18. Then the *Sal*I site downstream of the 1′ promoter was filled-in with the Klenow enzyme; a *Bgl*II linker was ligated at this position. A *Bam*HI fragment from pDO432 (Ow et al. 1986) containing the coding region for firefly luciferase was cloned into the newly created *Bgl*II site to give plasmid pNT600. The *Bam*HI site located downstream of the 2′ promoter was filled-in with the Klenow enzyme and *Bss*HII linker was added. The complete *Ac* element, on a *Bss*HII fragment cleaved from plasmid pAc7B (Müller-Neumann et al. 1984) was ligated into the newly created *Bss*HII, resulting in pNT900 and pNT901. In pNT900, the *Ac* ORF is in the same orientation as the 2′ promoter while, in pNT901, the *Ac* ORF runs in the opposite orientation to the 2′ promoter. pNT804 (8.6 kb), contains the internal *Acc*I fragment of *Ac* (coordinates 1051–4194) taken from pNT901, filled in with the Klenow enzyme and cloned in the filled in *Bam*HI site of pNT600. This restores the *Bam*HI site at both ends. pNT803 contains the same *Acc*I fragment but in the opposite orientation. pNT806 (8.7 kb) contains the *Ban*II fragment of *Ac* (coordinates 944–4228) taken from pNT901; blunt ends were created by digestion with the T4 polymerase and the fragment was cloned in the filled-in *Bam*HI site of pNT600. This restores the *Bam*HI sites at both ends. pNT600gAc (8.7 kb) contains the *Nae*I-*Ban*II fragment of *Ac* (coordinates 966–4228) taken from plasmid pUAc, which contains *Ac* as a *Bss*HII fragment in pUC19, blunt-ended with the Klenow enzyme and, after adding *Bam*HI linkers (pGGGATCCC), ligated into the *Bam*HI site of pNT600. The structures of the various plasmids are shown schematically in Fig. 2.

Transient expression assays. Isolation of mesophyll protoplasts from sterile shoot cultures of *Petunia hybrida* RLO1 (Meyer et al. 1987) was performed as reported for *Nicotiana tabacum* cv. Petit Havana SR1 (Potrykus and Shillito 1986). $Ca(NO_3)_2$-polyethylene glycol-mediated DNA transfer was done as reported for *N. tabacum* by Negrutiu et al. (1987). Aliquots of 10^6 *Petunia* protoplasts were transformed with plasmid DNA which had been purified by CsCl gradient centrifugation. Sonicated calf thymus DNA (Aldrich) was used as carrier DNA. The transfected protoplasts were resuspended in 5 ml K3 medium (Nagy and Maliga 1976) and kept in the dark at 25° C. They were collected by centrifugation, after addition of 4 volumes of iso-osmotic seawater and used directly for transient assays. The fluorimetric test, using 4-methylumbelliferyl-*β*-D-glucuronide (MUG, Sigma) as substrate for measuring *β*-glucuronidase activity, was as described by Jefferson et al. (1987). A Perkin-Elmer LS-2B filter fluorimeter was used. Kinetics were measured and activity was calculated according to the protein content of the sample, which was determined by the method of Bradford (1976).

The histochemical assay was performed essentially as described by Jefferson et al. (1987) for tissue sections. The cells were collected on nitrocellulose filters (0.2 μm, Sartorius) by briefly applying a vacuum and fixed for 30 min at room temperature. After two washes in 50 mM phosphate buffer, pH 7.0, the filters were wetted with 1 ml of the same buffer containing the indigogenic substrate X-Gluc (5-bromo-4-chloro-3-indolyl-*β*-D-glucuronic acid; Clontech) at 2 mM, and incubated at 37° C, in sealed Petri dishes. The stained protoplasts were routinely counted after overnight incubation, with the help of a dissecting microscope. The activity of the firefly luciferase was measured as described by Ow et al. (1986). One milliliter of the protoplast suspension was collected and the cytoplasmic contents released by three cycles of freezing and thawing. The peak intensity of the light flash was measured with a luminometer (Berthold Biolumat LB 9500).

DNA analysis. DNA from protoplasts coinfected with the *AcΔ* plasmid and a plasmid carrying a truncated version of *Ac* was isolated 2 days after transfection by the method of Werr and Lörz (1986). This DNA was allowed to hybridize with the following two oligonucleotides (CTTACGTCACGTCTTGCGCA, TCCAGACT-GAATGCCCACAG) in a polymerase chain reaction (PCR, Saiki et al. 1985) in order to amplify the DNA segment containing the *AcΔ* excision site. The PCR cocktail (50 μl) contained 50 mM KCl, 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 2 μM of each primer, 0.12 μg of template DNA and 5 units of *Taq* DNA polymerase (Perkin-Elmer-Cetus) in 10 mM TRIS-HCl buffer, pH 8.3. The whole mixture was overlaid with 50 μl paraffin oil and placed in a Hybaid Thermal Reactor. After a 5 min initial denaturation step at 92° C, 35 cycles of amplification were carried out by using a step program (92° C, 1 min; 60° C, 1 min; 70° C, 3 min), followed by a final 15 min extension at 70° C. The amplified DNA fragments were purified by gel electrophoresis on a 1.2% agarose gel, in TAE buffer (Maniatis et al. 1982) and their sizes were estimated by comparison with a 123 bp ladder (Bethesda Research Labs). Agarose blocks containing the DNA fragments were cut out of the gel and sealed in a low melting agarose gel (SeaPlaque, FMC BioProducts). After electrophoresis in TAE buffer, the DNA fragments were excised and purified by using the Geneclean Kit (Bio 101). The fragments were used for DNA sequencing analysis (Sanger et al. 1977) without subcloning, using either of the above nucleotides as a primer.

Results

Description of the histochemical assay

In the presence of the substrate X-Gluc, protoplasts that display β-glucuronidase activity, above a certain threshold will appear as blue spots (Fig. 1). The conditions of the histochemical assay were first established using tobacco protoplasts stably transformed with GUS, under the control of the 1′ *A. tumefaciens* or the cauliflower mosaic virus (CaMV) 35S promoter. One day after protoplasting, about 70% of the expected number of GUS-expressing protoplasts were recorded. The addition of different amounts of untransformed protoplasts (10^2 to 10^5) did not interfere with the counts.

The histochemical assay was then tested with *P. hybrida* protoplasts that transiently express the GUS gene and compared to the fluorimetric assay. Protoplasts were transfected with the plasmid DNA carrying the GUS gene under the control of the 1′ promoter (plasmid pNT201, Fig. 2). We estimate that only 1%–2% of the protoplasts produce detectable levels of the enzyme activity. This estimate is based on the largest number of spots observed among three different batches of protoplasts, 1 day after transfection, i.e. when the protoplasts have not yet divided.

The GUS gene activity, measured histochemically and fluorimetrically, is proportional to the quantity of transfecting DNA (Fig. 3). The number of spots reaches a saturation level for high amounts of DNA (30 μg) suggesting that saturation is reached with respect to the number of cells competent for transfection. At the highest DNA concentration used, the saturation phenomenon is not observed with the fluorimetric measurements, indicating that the translational machinery has not yet been saturated. At low DNA concentrations (30, 100 ng), spots could be reliably counted (15 and 50 spots, respectively) 3 days after transfection while the fluorimetric measurements were not significantly different from the background. Based on these data, 10 μg of GUS DNA construct were used in the following experiments. The protoplasts were harvested after 3 days, in order to obtain reliable counts in the case of weak GUS expression.

AcΔ excision mediated by an Ac *element* in trans

If *AcΔ* is present in the leader sequence of the GUS gene (plasmid pNT100), GUS expression is decreased to a very low level and the number of blue-stained protoplasts is negligible compared to the positive control (plasmid pNT201). Thus, *AcΔ* inserted in the leader sequence can inhibit the expression of the 1′-GUS gene. In order to compare better the different cotransfections within each experiment, the expression of the luciferase gene carried on the *Ac* plasmids was measured and considered as an internal control for transfection.

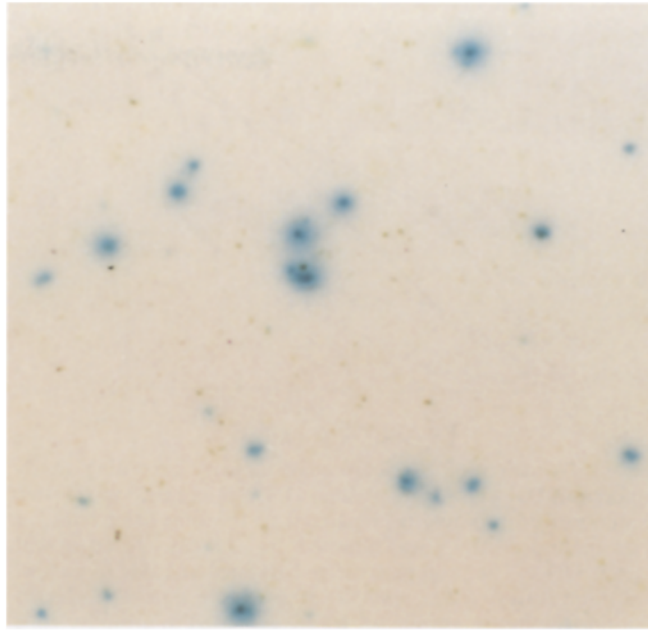

Fig. 1. β-Glucuronidase (GUS) activity in *Petunia hybrida* protoplasts, 3 days after transfection with the 1′ pro-GUS construct (pNT201 plasmid DNA). The fixed protoplasts have been incubated overnight with the indigogenic X-Gluc substrate (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid). Magnification ×12

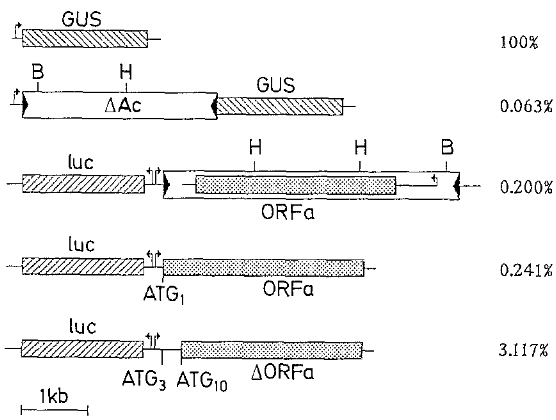

Fig. 2. Schematic representation of the relevant genes. The reporter genes are denoted luc for the firefly luciferase coding region and GUS for the β-glucuronidase coding region. The promoters (*Ac* promoter, 1′ and 2′ promoters of the octopine TR-DNA) are indicated by *arrows*. GUS and luc ORFs are transcribed from the 1′ promoter while the 2′ promoter is always upstream of *Ac* ORF_a. *Ac* or *AcΔ* are represented with *black arrows* at both ends corresponding to the terminal inverted repeats. The *numbers* listed at the right side are the average levels of the relative transient expression obtained with the different constructs: the number of GUS expressing cells with the positive control (pNT201) is normalized to 100%; the last three values are obtained if these constructs are cotransfected with the target plasmid pNT100. The position of some relevant restriction sites is given (B, *Bam* HI; H, *Hin*dIII)

Cotransfection of protoplasts with the target plasmid pNT100 and the plasmid carrying the wild-type *Ac* element, pNT901, produces a small, but significant, increase in the number of blue-stained protoplasts, compared with a protoplast suspension transfected with pNT100 alone (Table 1, Fig. 2). In addition, the results

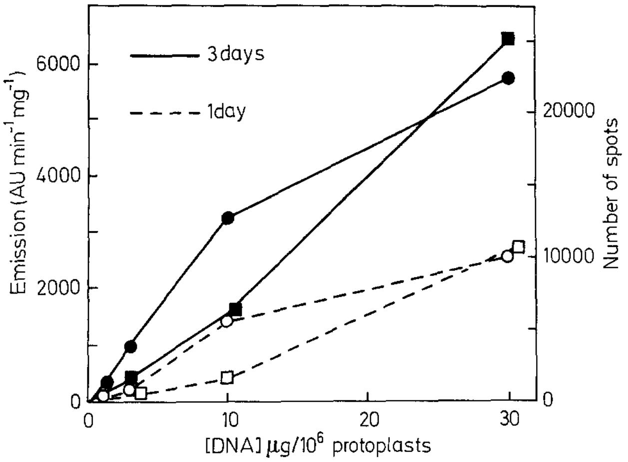

Fig. 3. Variation of β-glucuronidase activity according to the amount of DNA (pNT201 plasmid DNA, in µg) used to transfect aliquots of 10^6 protoplasts prepared from the same batch of leaves. The amount of DNA was adjusted, in each case, to 60 µg with carrier DNA. The protoplasts were harvested at 1 day (*open symbols*) and 3 days (*filled symbols*) after transfection. The spectrofluorimetric measurements (□, ■) are expressed in arbitrary emission units (AU) per min per milligram of protein. GUS expressing cells (○, ●) were counted as blue spots on the support filters, in duplicate, after incubation with X-gluc. Total spot numbers are calculated for 10^6 initial protoplasts (i.e. 5 ml suspension). The 3 day curves were confirmed with a second batch of protoplasts (2 samples per DNA concentration, 7 DNa concentrations)

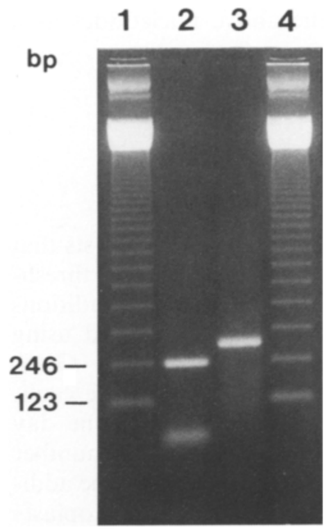

Fig. 4. Agarose gel electrophoresis of the DNA fragments amplified by PCR. One-fifth of the 50 µl reaction was loaded on a 1.2% agarose gel. Lanes 1 and 4, 123 bp ladder; lane 2, control plasmid DNA (2 pg of pNT201 digested with the *Hin*dIII enzyme); lane 3, DNA extracted from transfected protoplasts (5×10^5 protoplasts) and digested with the *Hin*dIII enzyme

obtained with plasmids pNT900 and pNT901, which carry *Ac* in opposite orientations are comparable (Table 1). This similarity indicates that transcription of *Ac* leading to the excision of *AcΔ* is governed by *Ac* itself and not by readthrough from an outside promoter.

Attempts to improve the excision rate of *AcΔ* by placing the coding region of *Ac* under the control of the 2′ promoter of *A. tumefaciens* TR-DNA (plasmids pNT806 and pNT600g*Ac*) were unsuccessful. The number of blue-stained protoplasts counted was similar to the number obtained with the wild-type *Ac* element (Table 1, Fig. 2).

Fortuitously, initial results had indicated that the *Acc*I fragment of *Ac* could provide a high excision efficiency. In order to confirm this observation, the *Acc*I fragment of *Ac* was cloned downstream of the 2′ promoter (plasmid pNT804). This fragment extends from $AccI_{1051}$ to beyond the stop codon of the *Ac* coding region. It lacks the first 22 codons of ORF_a including the first two ATGs. Its first ATG in frame with ORF_a is ATG_{10} which is preceded by 7 ATGs out of frame with ORF_a. A significantly larger number of blue-stained protoplasts is observed with this construct (Table 1, Fig. 2) compared to wild-type *Ac* (pNT901).

Ac *is excised correctly*

DNA was extracted from protoplasts cotransfected by pNT100 and pNT804. By PCR amplification, we screened for DNA carrying an empty excision site. Of the two primers used, one is located in the sequence of the 1′ promoter, while the other is derived from the GUS gene. With the control plasmid (pNT201, Fig. 4) a 245 bp long fragment is expected after amplification. Upon excision of *AcΔ* from the target plasmid pNT100, a 309 bp fragment is expected, due to the presence of 60 bp of *waxy* sequence flanking the *AcΔ* element (Baker et al. 1987) which include the 8 bp direct repeats.

Figure 4 shows that the expected bands can be detected after PCR amplification. The sequence of both strands is readable up to the insertion site and becomes blurred thereafter (data not shown), as expected when the DNA is a mixture carrying different excision events and consequently different transposon footprints (Peacock et al. 1984; Nevers et al. 1985).

Discussion

A histochemical assay was adapted to detect transient GUS expression in mesophyll protoplasts of *P. hybrida*. This assay allows the detection of the excision of *AcΔ* from the leader sequence of the GUS gene in a target plasmid, mediated by the trans-action of *Ac* or a derivative thereof. The assay presents a non-zero background of blue-stained protoplasts, estimated at 0.06% of the counts obtained in the control transfection with the plasmid carrying 1′-GUS. We have not investigated the structure of the GUS genes active in this background. We note, however, that a few blue-stained protoplasts among unstained ones indicate heterogeneity between the protoplasts which is more easily explained by assuming an alteration in single DNA molecules than by the presence of weak cryptic promoters allowing low-level transcription from all molecules still carrying *AcΔ*.

Table 1. Number of protoplasts transiently expressing GUS after transfection with the different plasmids

Experiment	I		II		III		IV		V		VI		VII		VIII		IX		X		XI	
	LUC[a]	GUS[b]	LUC	GUS	LUC	GUS	LUC	GUS	LUC	GUS	LUC	GUS	LUC	GUS	LUC	GUS	LUC	GUS	LUC	GUS	LUC	GUS
AcΔ		1; 1		2; 1		1; 2		4; 2		4; 0		1; 7		4; 1		2; 2		0; 0		0; 0		
		4; 1		2; 1		0; 1		1; 7		0; 1				5; 4		4; 0						
AcΔ+*Ac*													178	19; 12	114	7; 8						
													173	39; 31	84	8; 6						
AcΔ+rev*Ac*	272	11	58	2; 0	135	7; 6	417	8	417	7			160	56; 34	529	22; 17	60	0				
		10	85	3; 5	248	13; 9			332	5			300	37; 32	261	28; 33						
													273	38; 27*	223	20; 24*						
													357	27; 35	156	11; 15*						
AcΔ+*Acc*I							401	89	128	48	230	88*							412	40	155	47
							381	80	79	116	257	97**							266	8	76	36
							403	109			341	54										
AcΔ+rev*Acc*I									402	6												
									409	7												
AcΔ+*Ban*II											248	12									42	0
											184	6										
AcΔ+*Nae*I/*Ban*II																	56	2	218	2	74	3
																	35	1	231	1	53	2
1′-GUS		2472		1485		2940		2915		5360		6050		2760		2618		410		508		369
		2724		1230		4275				7015				2775		1875		550				1283

Eleven independent experiments are listed. In evaluating the data, it is important to compare the values in the same column. In each column, the numbers refer to blue protoplasts observed after transfection of different aliquots of 10^6 protoplasts. Ten micrograms of each plasmid DNA was used for transfection, except in experiments VII and VIII where 25 μg of *Ac* and rev*Ac* were used. The total DNA input was always adjusted to 60 μg by the addition of carrier DNA. *AcΔ*, pNT100; *Ac*, pNT900; rev*Ac*, pNT901; *AccI*, pNT804; rev *AccI*, pNT803; *Ban*II, pNT806; *Nae*I/*Ban*II, pNT600g*Ac*; 1′-GUS, pNT201; * DNA purified from another independent bacterial clone; ** DNA purified from a third independent bacterial clone

[a] LUC, Luciferase activity recorded as light units, measured on two different aliquots, calculated for the same aliquot as that used for the GUS test and divided by a factor 1000

[b] GUS, Number of GUS-expressing cells counted as blue spots after spreading an aliquot equivalent to 0.5 ml of the initial protoplast suspension on the filters and incubating the filters in the presence of X-Gluc. Each number derives from one filter. In experiments I, II and III, 0.8, 0.75 and 0.75 ml aliquots respectively were applied to the filters. Assays of the 1′-GUS transfection were carried out on suitable dilutions in order to distinguish individual blue spots. In this respect, the numbers presented for 1′-GUS were extrapolated from these counts and may represent overestimates

The number of blue spots increases by a factor of 3.2 over background to 2.0×10^{-3} of the control, when the wild-type *Ac* element is used *in trans* (Fig. 2). This is much lower than the excision frequency determined in the callus assay by Baker et al. (1987), where stably transformed protoplasts were used. The difference between integrated and non-integrated DNA may be responsible as the latter does not replicate. In maize, excision occurs preferentially at the time of DNA replication (Greenblatt 1984). In the callus assay, the protoplasts are allowed to divide for 10–12 days before application of the selecting agent (kanamycin).

The number of excision events detected does not increase when the *Ac* coding sequence (ORF_a) is placed downstream of the 2′ promoter. As we did not perform measurements of transcription activity, we cannot be sure that transcription of the *Ac*-coding region has been enhanced. If the 2′ promoter is inducing higher transcription levels, as observed for calli of different origins (Harpster et al. 1988), the low excision frequency cannot be explained at present. It may be related to the delay and decrease of the number of transposition events with increasing copy number of *Ac* in maize (McClintock 1951), where *Ac* transcription increases with *Ac* dose (Kunze et al. 1987).

It came as a surprise that a significant increase in the number of blue-stained protoplasts was observed, when a truncated version of the coding region of *Ac* was placed under the control of the 2′ promoter (about 16 times higher than with wild-type *Ac*, Fig. 2). Li and Starlinger (1990) have shown that the N-terminus encoded by the 5′ end of the message is dispensable for transposition, and in this case translation probably starts at codon 103 (ATG_{10}). In some of their experiments, a slight increase in excision frequency was detected using a truncated coding region. Due to the nature of the callus test, however, larger increases in excision could not be detected.

Thus, it is conceivable, but by no means proven, that the N-terminal region of the *Ac* transposase reduces the transposition-promoting activity of the intact *Ac* protein. An increase in the biological activity of proteins has been observed in other instances of removal of regulatory domains. Removal of the N-terminal repressor domain activates proto-oncogenes like *c-jun* (Bohmann and Tjian 1989) or *c-raf-1* (Stanton et al. 1989).

We do not understand the activity of the *Acc*I fragment in view of the fact that ATG_{10} is preceded by 7 ATGs out-of-frame with ORF_a. While it has been demonstrated that out-of-frame ATGs do not always decrease translation (Müller and Hinnebusch 1986), they very often show such an effect (Kozak 1987a, b). In addition, Coupland et al. (1988) and Li and Starlinger (1990) have observed a strong decrease in excision frequency when ATG_{10} was preceded by some (but not all) combinations of out-of-frame ATGs. However, their experiments differ from those described here in several respects: (i) stable transformation was studied rather than transient expression, (ii) the *Ac* transcription start was often used instead of the 2′ promoter, (iii) the experiments were performed in tobacco rather than in petunia and (iv) the combinations of ATGs preceding ATG_{10} differed in the experiments done in tobacco from those described here. Since neither of these differences can clearly explain why different results were obtained in the two studies, more experiments are clearly needed.

The transient test described was devised to facilitate the comparison of many mutated derivatives of *Ac*. In this respect, it may still be useful for a number of experiments. However, the observation by Li and Starlinger (1990) that a truncated version of *Ac* differs in its mode of action from the intact *Ac*, not only quantitatively but also in its requirement for a *cis*-acting site, will make it necessary to complement this test with others, which compare the action of a truncated ORF_a protein with that of the complete product.

Whether an increased excision rate is associated with truncated versions of *Ac* in transgenic plants is currently being tested. If a similar effect could be shown, the use of truncated *Ac* elements might be helpful in transposon tagging experiments. In addition, it is tempting to speculate that such a cotransfection system might be a useful tool to introduce genes carried by *Ds*-like elements into the plant genome, in a way similar to P element-mediated transformation in *Drosophila* sp. (Rubin and Spradling 1982).

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