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Genetics

Mutational analysis of the N terminus of the protein of maize transposable element *Ac*

(site-directed mutagenesis/protein truncation/cis-acting sites)

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ABSTRACT **Mutations of transposable element *Ac* were tested for their capability to excise themselves from their location autonomously, to be excised by an active *Ac*, or to act in trans in the excision of an AcΔ element. Removal of 101 amino acids from the N terminus of the Ac protein does not decrease excision. A cis-acting site between base pairs 186 and 207 is important for excision by the wild-type protein but is not necessary for excision by the truncated protein. Improvement of the sequence context of the first AUG does not have a significant effect. Mutations in a small open reading frame of *Ac* encoding a 102-amino acid protein do not visibly alter excision frequency.**

In maize, transposable element *Ac* has the capability to excise itself from its location and to transpose itself to other sites (1, 2). In the presence of more than one copy of the element within a cell, transposition is shifted to later developmental stages, and in several cases the frequency of transposition decreases. This indicates that *Ac* is capable of sensing both its own copy number and the developmental stage of the cell in which transposition is observed. In transgenic tobacco, however, the number of transposition events is increased with copy number (3).

In spite of these various functions, only one *Ac*-specific transcript of 3.5 kilobases (kb) has been detected (4). Its amount increases with the copy number of *Ac*. In preliminary studies, an Ac protein with an apparent size of 110 kDa was detected on immunoblots (5). Its amount also increases with increasing *Ac* copy number (H. Fusswinkel and P.S., unpublished data). Analysis of the cDNA sequence shows the presence of a major open reading frame (ORF) of 807-amino acid length (ORFa) (4). A second, smaller, different ORF of 102-amino acid length (ORFb) is completely enclosed in the larger ORF. The first ATG (ATG 1) of the ORFa protein is located in an unusual sequence context for eukaryotic translation initiation (6–8). It has no guanosine in position +4 nor a purine in position −3. The same is true for the second ATG (ATG 2) at codon 21. The next four ATG codons open small ORFs in different reading frames, while the seventh through ninth ATG codons (ATG 7–9) are in ORFb. Only the tenth ATG (ATG 10) is again in frame with ORFa and located here at codon 102. It also is in a good sequence context for a start ATG.

A peculiarity of the amino acid sequence of the putative Ac protein is a 10-fold repeat of a Pro-Gln or Pro-Glu dipeptide. ATG 10 is located five amino acids in front of this potentially unstructured sequence that may separate two protein domains.

We report here studies on mutations in the N-terminal region of the putative Ac protein. We wanted to answer the following questions. (*i*) Does an improvement of the sequence context of ATG 1 significantly raise the excision frequency of *Ac*? (*ii*) Is translation of ORFb necessary for excision of *Ac*? (*iii*) Is a truncated ORFa protein initiated at ATG 10 capable of excision of the transposable element?

MATERIALS AND METHODS

Plant Material. *Nicotiana tabacum* cv. Petit Havana SR1 and its transformants SR1/pKU4 or SR1/30-1 were used for the excision assay of *Ac* mutants. Plant SR1/pKU4 was obtained by stably transforming SR1 with pKU4, carrying a 1.6-kb internal *Hin*dIII deletion of *Ac* (designated AcΔ) inserted in the untranslated leader of the neomycin phosphotransferase gene NPT II, and was used to test for transposase activity of *Ac* and its mutants (9). Plant SR1/30-1 was a SR1 stably transformed with an *Ac* element and was used to test for cis-acting sequences necessary for excision of *Ac* mutants by wild-type Ac protein (9, 10).

Tobacco plantlets for protoplast preparation were grown on 0.8% agar containing 50% MS medium (11) and 3% saccharose in glass pots in a 26°C light room.

Site-Directed Mutagenesis and Plasmid Construction. The point mutations were made by a procedure described by Kramer and Fritz (12). The following three oligonucleotides were used to create mutations (bases deviating from the wild-type sequence are italicized): (*i*) the 28-mer GACTCCATTCCT*AA*GATG*G*CGCCTCCGG introduces mutations at positions 985 and 991 in *Ac*; (*ii*) the 52-mer GCAGTAGTAA*A*GCAAA*A*GGTACAGCTACAGATCCGAGTCAAGAAGATATGGC mutates *Ac* positions 1364, 1370, and 1397; and (*iii*) the 24-mer GGAAATTGAAGTAGAGGTCGATGG causes a base change at position 1556. To alter the flanking sequences of ATG 1, the *Bam*HI–*Hin*dIII fragment from the Ac cDNA in pcAcP (4) was inserted into the phage M13mp9 (13), and oligonucleotide 1 was used as the mutagenic primer. In the cases of mutations in ORFb, a shorter *Acc* I–*Hin*dIII fragment of the Ac cDNA in pcAcP was cloned between the *Sma* I and *Hin*dIII sites of M13mp9. Primers 2 and 3 were utilized to mutate ATG codons 7–9 and to introduce an amber codon at codon 65 in ORFb, respectively. Mutant phages were identified by dideoxy sequencing (14). Appropriate DNA fragments in pKU3 (15) were exchanged with the equivalent but mutated fragments from M13 phage either directly or, where necessary, in two steps with the help of an intermediate plasmid. Details can be obtained from the authors upon request. The cauliflower mosaic virus (CaMV) 35S promoter was used as a 1.6-kb *Hin*dIII–*Bam*HI fragment of plasmid pDO432 (16). Soybean upstream heat-shock elements were used on a 189-base-pair (bp) *Hae* III–*Mnl* I fragment of gene *hs6871* (17) in plasmid pKU109A and as a

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Abbreviations: ORF, open reading frame; URF, upstream reading frame; CaMV, cauliflower mosaic virus.

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278-bp *Hae* III–*Mnl* I partial digestion product carrying all of the heat-shock elements in plasmids pKU111A and pKU115A. The latter fragment also carries the TATA box of the heat-shock gene. The deletions between *Bam*HI or *Acc* I and *Pvu* II were created by a fill-in reaction of the former site, followed by ligation. The deletion in pKU111A is created by insertion of the heat-shock promoter fragment into pKU33 (9).

Excision Assay. Transfer of the resulting cointegrate plasmids into *Agrobacterium*, transformation of tobacco protoplasts, and selection on kanamycin-containing medium were performed as described by Baker *et al.* (15). The number of calli obtained is a measure of excision of *Ac* or its derivatives and should be compared with the number obtained with wild-type *Ac* in pKU3 (Tables 1 and 2).

Regeneration of Plants. Plants were regenerated from some transformed calli. The presence of the *Ac* derivatives used for transformation was confirmed by Southern analysis or PCR sequencing from genomic DNA (data not shown), or both.

DNA Amplification and Polymerase Chain Reaction (PCR) Sequencing. The PCR was performed in a 1.5-ml Eppendorf tube with *Thermophilus aquaticus* DNA polymerase under the conditions recommended by the manufacturer (Perkin–Elmer/Cetus) with minor modifications. The purified double-stranded DNA was directly sequenced by using Sequenase (United States Biochemical).

RESULTS

Altered Sequence of ATG 1 Does Not Increase Transposition Significantly. The sequence surrounding ATG 1 reads as follows: 5′-TCAGATGACG-3′. Replacement of the cytidine at position −3 with an adenosine and of the adenosine at position +4 with a guanosine in plasmid pKU125 did not increase the number of kanamycin-resistant calli significantly. The average transposition increase factor for six experiments was 1.4 (Fig. 1, lines 2 and 4). The problems involved in reliable measurements of increases of excision frequency will be discussed below.

Mutations Affecting ORFb Show No Effect on Transposition. ORFb is completely enclosed in ORFa, which encodes the 807-amino acid protein. The 102-amino acid peptide encoded by ORFb is opened by ATG 7, with ATG 8 and ATG 9 in frame. The next ATG is located at codon 18 of this reading frame.

Table 2. Ability of mutated *Ac* to excise itself and an AcΔ in trans

Plasmid name	Kanamycin-resistant calli, no.						
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Average
pKU108B	5	3	2	6	—	—	4
pKU108A	109;157	100	—	229	288	280	194
pKU2	84	114	87	210	400	300	199
pKU3	73	86	51	92	194	145	106
pKU4	5	0	—	—	—	1	3

These experiments were done in protoplasts from plants containing pKU4. Data are presented as described in Table 1.

We have mutated ATG codons 7, 8, and 9 each time by replacing the thymidine residue with an adenosine. A translation start at ATG 11 encoding 18 amino acids of ORFb would abolish 17 amino acids at the N terminus (pKU101). We have also replaced Guo-1556 with an adenosine. This introduces an amber codon instead of codon 65, thus removing the last 38 amino acids from the protein (pKU102).

No significant alteration in excision frequency was observed with either mutant (Fig. 1, lines 2, 5, and 6). Incidentally, this showed that the replacement of two asparagine residues with lysines and one aspartic acid residue with glutamic acid in the N terminus of the ORFa protein encoded by the large ORF did not have a negative influence on excision either.

To confirm the presence of the mutations in the transposed *Ac* element still carried by the kanamycin-resistant calli obtained in these experiments, a few of the calli were grown into plants and analyzed by Southern blotting and DNA sequencing of the relevant sites in *Ac* by the PCR. In all cases, Southern blotting showed the presence of the expected excision bands, and PCR sequencing showed that the mutations were present in the *Ac* elements (data not shown).

N Terminus of The 807-Amino Acid Protein Is Dispensable for Transposition. The lack of effects on transposition of three point mutations in the N-terminal part of the 807-amino acid protein prompted us to test whether the N terminus of the protein is dispensable for transposition. To allow unhindered translation start at ATG 10, two constructs were used. In pKU107, a deletion extending from the Acc I site at position 1051 to the *Pvu* II site at 1320 altered the reading frame of ATG 1 and ATG 2 and removed ATG codons 3–6. ATG codons 7–9 were mutated as described above (Fig. 1, line 7).

Table 1. Ability of mutated *Ac* to excise itself

Plasmid name	Kanamycin-resistant calli, no.										
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8	Exp. 9	Exp. 10	Average
pKU125	50	52	90	88 126	—	—	—	159	—	57;10	79
pKU101	26	72	24	111	—	25	—	132	—	25;66	60
pKU102	60	43	120	83 74	—	25	—	94	—	50;57	67
pKU107	—	—	—	—	40	—	38	151	—	37;48	63
pKU114	—	—	—	—	—	—	66	243	80	76	116
pKU103	2	9	2	0	—	—	—	0	—	1	2
pKU104	2	5	0	0	—	—	—	0	—	0	1
pKU106	2	6	10	4	—	—	—	0	0;6	0	3
pKU109A	0;1	—	—	—	—	—	—	—	—	—	0.5
pKU111A	4	—	—	—	—	—	—	—	—	—	4
pKU2	—	—	26	122	—	—	—	244	107	178	162
pKU3	—	—	26	76	25	32	31	121	30	50;50	51
pKU4	—	—	0	0	0	0	—	0	0	0	0

Raw data from all experiments show the degree of correspondence between controls and experiments, while absolute values vary from experiment to experiment (e.g., experiments 5 and 8). The values were averaged, and excision frequencies were calculated as a percentage of the pKU2 value. Two values in a single experiment are from two plates obtained from the same batch of infected protoplasts and are separated by a semicolon.

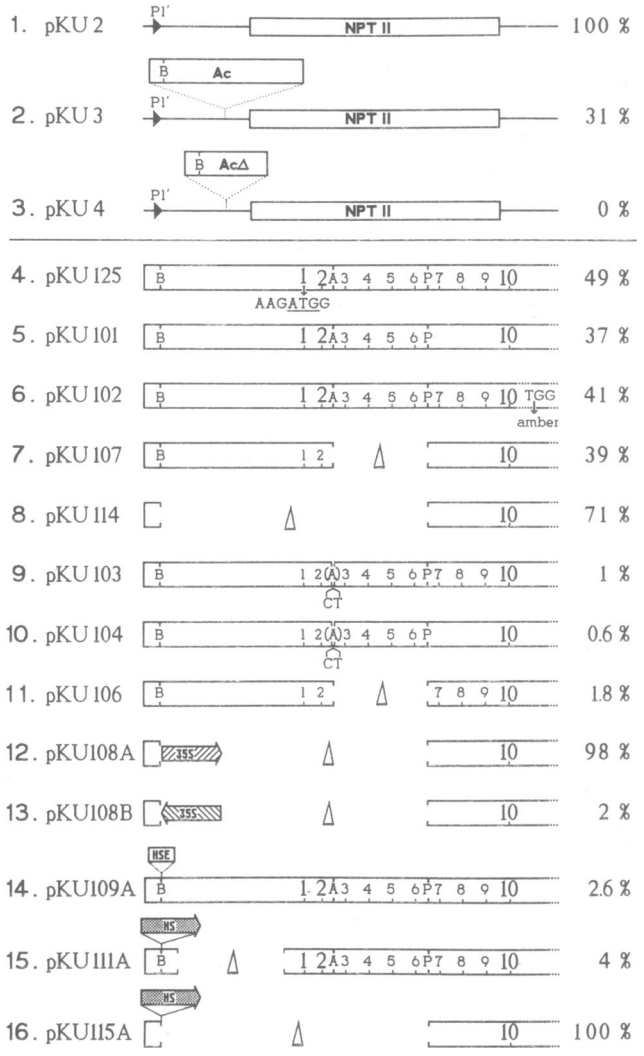

FIG. 1. Mutants in *Ac* and their effect on excision. The sketches are intended to show the relevant features of the constructions and are not drawn to scale. The complete *Ac* element inserted in the leader of the *NPT* II gene is 4.56 kb; AcΔ is created by the removal of a central 1.6-kb *Hin*dIII fragment. The sketches from pKU125 to pKU115A show only the left end of *Ac* from position 1 to 1785. ORFa starts at ATG 1 at position 988, and the following ATGs are indicated by numbers; large numbers indicate ATG codons in frame with ORFa, whereas missing numbers indicate point mutations. For exact locations, see figure 6 in ref. 4. Restriction sites in the sketches are: B, *Bam*HI at 181; A, *Acc* I at 1051; P, *Pvu* II at 1320. The deletion (Δ) in pKU115 starts at position 246 and ends at position 736. 35S is the CaMV 355 promoter; HSE is the heat-shock element, and HSP is the heat-shock promoter. The arrows of the promoter fragments indicate the direction of transcription. CT below lines 9 and 10 indicates the insertion of two bases by a fill-in reaction at the *Acc* I site. The excision frequencies at the end of each line are compiled from the Tables and show the average numbers of kanamycin-resistant transformants as a percentage of the average number obtained for pKU2 that needs no excision for expression of kanamycin resistance.

In a second construct, pKU114, a deletion extending from the *Bam*HI site at position 181 to *Pvu* II site at 1320 was followed by point mutations in ATG codons 7–9 (Fig. 1, line 8).

Both constructs gave excision rates as high (pKU107) or even higher than (pKU114) those obtained with an intact *Ac* element. Southern blot analysis showed that *Ac* in pKU107 is still able to transpose to a new position (data not shown). While this experiment shows that 101 amino acids at the N terminus of the 807-amino acid protein are dispensable for the excision reaction, both constructs pose problems with regard to the expression of this gene. pKU107 has two out-of-frame ATG codons created by the *Acc* I–*Pvu* II deletion in front of ATG 10. pKU114 carries a deletion extending more than 100 bp upstream of the ordinary transcription start of the protein (4) and, thus, should be lacking the wild-type promoter of the Ac transcript. To investigate these problems, additional mutations were constructed.

Out-of-Frame ATG Codons Can Influence Excision Negatively. Coupland *et al.* (9) have shown that a deletion removing ATG codons 1 and 2 but leaving ATG codons 3–9 in position abolishes excision. As the previous experiments seem to indicate that the N terminus of the 807-amino acid protein is dispensable for excision, we considered the possibility that the negative effect of the above-mentioned deletion was exerted not at the level of protein structure but at the level of translation of the mRNA. We tested this by constructing a number of mutants, leaving various combinations of out-of-frame ATG codons in front of ATG 10. pKU103 puts ATG codons 1 and 2 out of frame by a fill-in reaction of the *Acc* I site at position 1051. pKU104 carries the same mutation in this *Acc* I site in addition to point mutations of ATG codons 7–9. pKU106 carries a deletion from the *Acc* I site at 1051 to the *Pvu* II site at 1320, thus altering the reading frame of ATG codons 1 and 2 and removing ATG codons 3–6.

All three mutations show a low excision frequency. In several experiments no calli were visible (Fig. 1, lines 2, 9, 10, and 11).

Influence of Promoter Strength on Excision Is Not Yet Clear. Experiments with pKU114 gave an excision rate higher than that obtained with the wild-type *Ac* in spite of the fact that this construct is devoid of the putative *Ac* promoter. The activity of this *Ac* derivative may be due to read-through transcription from the adjacent 1′ promoter. We have not yet carried out transcription studies, which would have to await the generation of sufficient plant material. However, we have added a fragment carrying the CaMV 35S promoter in the correct or in the inverse orientation in the *Bam*HI site. As the *Bam*HI site at position 181 is in a region necessary in cis for transposition (ref. 18; M.-L. and P.S., unpublished data), any effect of the insertion on transposition could be due to either a cis effect or an effect of insertion of the transcription of the truncated ORFa. To separate these effects, we used protoplasts derived from tobacco plants pretransformed with pKU4. Low numbers of kanamycin-resistant calli would then indicate failure to excise a cis-competent AcΔ from pKU4.

The insertion in the correct orientation (pKU108A) gave an excision frequency not significantly different from that obtained with pKU2, amounting to excision in 100% of the transformants. Low excision levels were observed in the presence of pKU108B (inverse orientation of the 35S promoter) (Fig. 1, lines 12 and 13). While this experiment is not sufficient to prove that read-through transcription from the outside of *Ac* is sufficient to generate the high excision rate observed with pKU114, the negative effect of the CaMV 35S promoter in the reverse orientation does not contradict such an assumption.

Truncated Ac Protein Has an Altered Target Site Requirement. The high excision rate observed with pKU114 is interesting from a second point of view. Coupland *et al.* (18) and Chatterjee (19) have reported that a deletion in *Ac* extending from position 186 to position 807 (pCP40), which removes a large part of the leader sequence and 150 bp upstream of the transcription start but leaves the large ORF intact, severely depresses the excision rate. No such decrease was observed with an *Ac* element carrying a deletion between positions 207 and 796 (pCP30). In both cases, the deletion-carrying element was tested for passive transposability in an *Ac*-carrying SR1/30-1 plant. This was interpreted

to mean that sequences between positions 186 and 207 are important in cis for a normal transposition frequency. In view of the results obtained with pKU114, we have to consider the possibility that this cis-acting site is dispensable for excision if the ORFa protein is truncated at the N terminus. In support of this hypothesis, we have carried out experiments in which a fragment carrying the heat-shock element was inserted in the *Bam*HI site (pKU109A; Fig. 1, line 14) or the whole heat-shock promoter was inserted at the same site (pKU111A; Fig. 1, line 15). Neither construct was able to excise itself at normal frequency autonomously or to be excised by an *Ac* that had been introduced previously into the tobacco plant, giving rise to the protoplasts used in this experiment (see Table 3). However, the plasmid pKU111A was able to promote the excision of an AcΔ element from pKU4 (data not shown). A similar experiment as with pKU111A was performed with plasmid pKU115A (Fig. 1, line 16). Here, however, the protein had to start at ATG 10 because the sequence between the *Bam*HI site at 181 and the *Pvu* II site at 1320 is deleted and ATG codons 7–9 are mutated. In this case, an excision frequency not significantly different from that of *Ac* was observed in the presence of an active *Ac* (no experiment was done in the absence of the active *Ac*). This experiment supports the hypothesis that a transposable element lacking cis-acting sequences 3′ to the *Bam*HI site at position 181 or having the sequence around the *Bam*HI site interrupted by the insertion of a DNA fragment can be excised by the truncated protein but not by the protein initiated properly at ATG 1.

DISCUSSION

All experiments were done with the phenotypic assay described by Baker *et al.* (15). This assay is suitable for detecting the loss of excision or the decrease of excision frequency by factors of 10–30. Smaller decreases are harder to detect because of the variability of individual experiments. Also, it is not clear whether increases in excision frequency can be detected. The average excision frequency of wild-type *Ac* is 30% of the transformants and can be higher in individual experiments. Thus, the excision frequency can increase at most by a factor of 3, which is already difficult to measure in a statistically significant way. In addition, we do not know whether 100% excision is attainable at all or whether there is a lower saturation level. For these reasons, we only consider strong decreases of excision frequency as certain and take small changes or increases as indications at best.

The alteration of ATG 1 of the *Ac* transcript into a sequence resembling the translation initiation consensus sequence (6, 20, 21) does not have a visible effect on excision frequency, but for the reasons mentioned above we do not conclude from this experiment that the amount of Ac protein produced by wild type is saturating. Also, Lehto and Dawson (22) have not found an increased expression of the 30-kDa protein-encoding gene of tobacco mosaic virus when they improved the start ATG context. We have not yet compared the amount of Ac protein produced by wild-type *Ac* and pKU125.

Two sets of mutants introduced into ORFb do not detectably decrease *Ac* excision. One of these mutations introduces a stop codon into the central region of the ORF, and the other removes the first three ATG codons. Biological activity of the ORFb translation product must thus be confined to its central region or else its role must be a subtle one.

Firmer conclusions can be drawn from the experiments leading to the truncation of ORFa. These experiments can be summarized as follows (Fig. 2): The N terminus of 100 amino acids is dispensible for the excision of Ac or AcΔ as long as all of the sequences necessary in cis for excision are present. A difference between the full-length ORFa product and its truncated version is apparent when a cis-acting region located 3′ to the *Bam*HI site at position 181 is removed from the

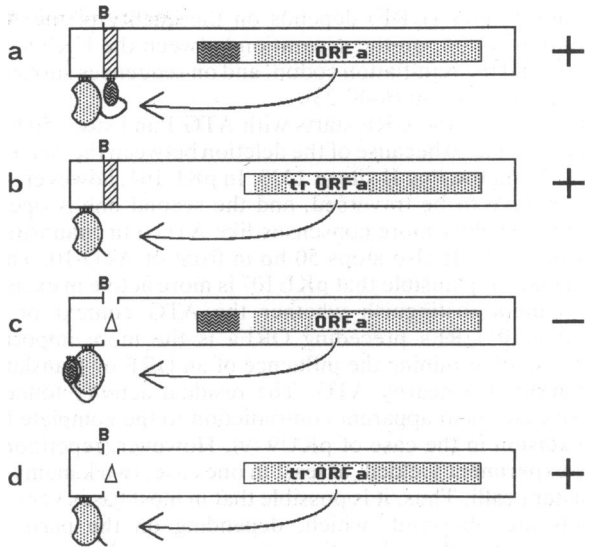

FIG. 2. Hypothetic model for Ac protein action. The C terminus (stippled) is in the active conformation when the N terminus (wavy) is held to the hatched site (B) at left (*a*) or when no N terminus is present because of truncation (*b* and *d*). In the absence (Δ) of the hatched site (*c* and *d*), the N terminus blocks the active conformation (*c*).

sequences located 5′ to the *Bam*HI site (Table 3). Although the product of the complete ORFa cannot excise such an element, the truncated ORFa product is capable of this excision. It is conceivable that binding of the N terminus to sequences located 3′ to the *Bam*HI site alters the conformation of the ORFa protein such as to allow excision. Removal of the N terminus might then have the same conformational effect and might thus make the presence of the cis-acting site unnecessary.

These conclusions are based on the interpretation of the effects of a number of different mutations. We will now discuss the validity of these conclusions in detail.

Deletion of ATG codons 1–6 and point mutation of ATG codons 7–9 in pKU114 allows excision at wild-type levels or even higher, as also has been seen with pKU33 (9). Thus, the N terminus does not seem to be needed for this reaction. Experiments with constructs removing ATG codons 1 and 2 or altering their reading frame but leaving combinations of ATG codons 3–9 in front of ATG 10 lead to a strongly reduced excision frequency. As these ATG codons are out of frame with ORFa, a plausible explanation would be that initiation at these start codons inhibits or precludes initiation at ATG 10. In the case of ATG codons 7–9, this is easily conceivable because the frame opened by these codons extends into the region covered by ORFa. In the case of ATG codons 3–6, this is less obvious because these codons open short reading frames terminating in front of ATG 10. The effect of upstream

Table 3. Function of a sequence around base pair 181 in cis position

Plasmid name	Kanamycin-resistant calli, no.		
	Exp. 1	Exp. 2	Average
pKU114	66	138	102
pKU109A	3;2	4	3
pKU111A	6;3	6	5
pKU115A	—	123	123
pKU4	69;37	135	80
pKU2	94;52	220	122

These experiments were done with protoplasts from plants containing *Ac*. Data are presented as described in Table 1.

reading frames (URF) depends on the quality of the ATG initiation codon, on the separation between the URF's stop codon and the reinitiation codon, and on sequences surrounding the stop codon (6–8, 23).

In pKU107, the URF starts with ATG 1 and stops 50 bp in front of ATG 10 because of the deletion between the *Acc* I site at 1051 and the *Pvu* II site at 1320. In pKU104, however, two URFs have to be traversed, and the second one is opened with the slightly more consensus-like ATG 4 (a guanosine in position −4). It also stops 50 bp in front of ATG 10. These data make it plausible that pKU107 is more active in excision but cannot distinguish whether the ATG context or the number of URFs preceding ORFa is the more important factor in determining the influence of an URF on translation initiation at a nearby ATG. The residual activity found in these cases is in apparent contradiction to the complete loss of excision in the case of pKU9 (9). However, repetition of this experiment yielded, at least in one case, two kanamycin-resistant calli. Thus, it is possible that in most cases very low levels are observed, which, depending on the particular experiment, allow detection of only a small number of transformants or none of them.

Self-excision frequency of *Ac* is similar, whether pKU114 is used to transform *Ac*-free SR1 protoplasts or *Ac*-containing SR1/30-1 protoplasts. This similarity indicates that the presence of wild-type Ac protein does not inhibit the specific action of the truncated protein. This may indicate either that the protein is not oligomeric (and for this reason, negative complementation does not occur) or that the amount of truncated protein is so large that it is not negatively complemented by the Ac protein provided by the wild-type *Ac* present in SR1 30-1 protoplasts.

An interesting difference between the putative full-length Ac protein and its truncated version is seen by the action of pKU114. The deletion extending from the inside to position 181 does not decrease the ability of the truncated protein to excise *Ac*. Thus, sequences located 3′ to the *Bam*HI site at position 181 do not seem to be important when in the cis position. This is in contrast to the pronounced decrease in transposability of plasmid pCP40 (18), in which sequences 3′ to position 186 are deleted. In this plasmid, the full-length Ac protein should be made according to the presence of its undisturbed coding sequence. Comparison of plasmids pCP30 and pCP40 (18) narrows the sequence necessary in cis to positions 186–207.

It might be argued that the difference between pKU114 and pCP40 lies in the base pairs 3′ to the *Bam*HI site at position 181, which start at position 807 in pCP40 and at the *Pvu* II site at 1320 in pKU114. This is made less likely, however, by the experiments with pKU109A, pKU111A, and pKU115A. Here, the 189 bp located 3′ to the *Bam*HI site at 181 are identical, and thus the difference between pKU109A and pKU111A on the one hand and pKU115A on the other hand is more likely to be caused by the presence or absence of the truncated ORFa. Eventually, the effect of an *Ac* element encoding a truncated ORFa and incapable of self-excision will have to be tested in trans on pCP40.

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1. McClintock, B. (1951) *Cold Spring Harbor Symp. Quant. Biol.* **16,** 13–47.
2. McClintock, B. (1984) *Science* **226,** 792–801.
3. Jones, J. D. G., Carland, F. M., Maliga, P. & Dooner, H. K. (1989) *Science* **244,** 204–207.
4. Kunze, R., Stochaj, U., Laufs, J. & Starlinger, P. (1987) *EMBO J.* **6,** 1555–1563.
5. Fusswinkel, H., Müller-Neumann, M., Both, C., Doerfler, W. & Starlinger, P. (1988) *Maize Gen. Coop. Newslett.* **62,** 47.
6. Kozak, M. (1986) *Cell* **44,** 283–292.
7. Kozak, M. (1987) *Nucleic Acids Res.* **15,** 8125–8133.
8. Kozak, M. (1987) *Mol. Cell. Biol.* **7,** 3438–3445.
9. Coupland, G., Baker, B., Schell, J. & Starlinger, P. (1988) *EMBO J.* **7,** 3653–3659.
10. Baker, B., Schell, J., Lörz, H. & Fedoroff, N. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4844–4848.
11. Murashige, T. & Skoog, F. (1962) *Physiol. Plant* **15,** 473–497.
12. Kramer, W. & Fritz, H.-J. (1987) *Methods Enzymol.* **154,** 350–367.
13. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
14. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
15. Baker, B., Coupland, G., Fedoroff, N., Starlinger, P. & Schell, J. (1987) *EMBO J.* **6,** 1547–1554.
16. Ow, D. W., Wood, K. V., DeLua, M., de Wet, J. R., Helinsk, D. R. & Howell, S. H. (1986) *Science* **234,** 856–859.
17. Baumann, G., Raschke, E., Bevan, M. & Schöffl, F. (1987) *EMBO J.* **6,** 1161–1166.
18. Coupland, G., Plum, C., Chatterjee, H., Post, A. & Starlinger, P. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9385–9388.
19. Chatterjee, S. (1989) Thesis (Univ. of Cologne, Cologne, F.R.G.).
20. Heidecker, G. & Messing, J. (1986) *Annu. Rev. Plant Physiol.* **37,** 439–507.
21. Lütcke, H. A., Chow, K. C., Mickels, F. S., Moss, K., Kern, H. F. & Scheele, G. A. (1987) *EMBO J.* **6,** 43–48.
22. Lehto, K. & Dawson, W. (1990) *Virology* **174,** 169–176.
23. Müller, P. & Hinnebusch, A. G. (1986) *Cell* **45,** 201–207.