Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 7094–7098, August 1993
Genetics

Dominant transposition-deficient mutants of maize *Activator* (*Ac*) transposase

(*Zea mays*/transient expression/petunia/negative complementation/transposable element)

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Communicated by Peter Starlinger, May 3, 1993 (received for review November 3, 1992)

ABSTRACT **The maize transposable element *Activator* (*Ac*) encodes a transposase (TPase) protein, whose DNA-binding domain is located in a basic region around aa 200. The N-terminal 102 aa of the TPase are not required for the transposition reaction. In transfected petunia protoplasts, we analyzed the protein levels of the N-terminally truncated TPase and mutants thereof and the corresponding transposition frequencies. The TPase protein forms large insoluble aggregates at high expression levels. There is no proportionality observed between TPase levels and transposition frequency. Twenty-one mutations (of 26), which are distributed over the whole length of the protein, inactivate the TPase completely. By coexpressing inactive mutant and active truncated TPase, it was found that several mutations have a trans-dominant inhibitory effect. Among those are two DNA-binding-deficient mutants, indicating that inhibition of the active TPase is not caused by competition for the binding sites on the transposon. Accordingly, *Ac* TPase acts as an oligo- or multimer formed by protein–protein interactions. Peculiarly, two mutants lacking 53 and 98 aa from the C terminus that are themselves transpositionally inactive lead to an increased excision frequency when they are coexpressed with the active truncated TPase.**

Forty-six years ago Barbara McClintock (1, 2) discovered the maize transposable element *Activator* (*Ac*). Since then, *Ac* has been studied genetically and molecularly in great detail (for review, see refs. 3 and 4). The *Ac* element is 4565 bp long, has 11-bp terminal inverted repeats, and creates 8-bp target site duplications upon insertion. The sequence of the inverted repeats of *Ac* is similar to sequences of the inverted repeats of transposable elements *Tam3* from *Antirrhinum majus* (5), *hobo* and *P* from *Drosophila* (6, 7), and several other eukaryotic transposons. All these elements supposedly transpose via a "cut-and-paste" mechanism (8). In addition, the *Ac* transposase (TPase) shows homologies to the putative TPases of *Tam3* and *hobo* (9–11), suggesting that these elements have similar transposition mechanisms.

A further step toward understanding the molecular mechanism of transposition is the analysis of the proteins mediating the reactions. The *Ac* TPase is encoded by the 807-aa open reading frame a (ORFa) from the only *Ac* mRNA (12). The ORFa protein was shown to be the *Ac* TPase, as it is necessary to mobilize a *Ds* element in heterologous plants (13) and a transient excision assay in petunia protoplasts (14). *Ac* TPase with an apparent molecular mass of 112 kDa was detected by Western blot analysis in nuclear extracts from maize. In addition to the full-length TPase, a smaller less-abundant ORFa-derived protein of ≈70 kDa was also identified. This polypeptide lacks an N-terminal segment of the TPase and appears to be processed from the full-length ORFa protein (15). It is not known whether this derivative is involved in or has an influence on the transposition reaction.

The TPase binds *in vitro* to short sequence motifs that are reiterated, but differently arranged, at both ends of *Ac*. The occupation of the binding motifs with TPase is concentration-dependent, resulting in complexes that are heterogeneous in size and composition (16). The TPase domain that recognizes the subterminal DNA motifs is high in positively charged amino acids, some of which are essential for DNA binding. However, it has no sequence homology to other DNA-binding proteins (11).

In this paper, we define functional polypeptide segments of the *Ac* TPase required, in addition to the DNA binding domain, to catalyze excision. In particular, we show that several mutant derivatives of the TPase protein can act as dominant negative repressors of transposition, which suggests that several TPase molecules are acting simultaneously during the transposition reaction.

MATERIALS AND METHODS

Construction of Plasmids. The plant reporter plasmid pNT150:Ds carries a *Ds* insertion between the 1′ promoter of the octopine TR-DNA and the β-glucuronidase (GUS) coding region as described (17).

All plant expression vectors are based on plasmid pNT600 (14). Plasmid pcATG1 contains the full-length TPase reading frame (*Ac* nt 967–4224 minus introns). Mutations were introduced into two expression vectors encoding an N-terminally truncated TPase starting with aa 103. In pcATG3-10 the *Ac* cDNA *Acc* I fragment (*Ac* nt 1052–4196 minus introns) was inserted into the *Bam*HI site of pNT600 (17). pcATG10 has *Ac* nt 1401–4224 minus introns inserted into the same site. For simplicity abbreviated names are used for mutant plasmids that indicate the alteration in truncated TPase protein (e.g., plasmid p297PR encodes mutant TPase 297PR; for the nomenclature of mutant proteins see Fig. 3). pcATG10-based mutant plasmids are pΔ(109–128), pΔ(103–135), pΔ(103–188), pΔ(228–237), pK174A/K176A, pH182A/H183A, pH191R/R193H, p249(S)AD, p297PR, p341RG, p388CR, p524PR, and p663DR. pcATG3-10-based mutants are p270TR, p369TR, p390RV, p445TR, p462TR, p577RV, p585TR, p623TR, p709RV, p754TR, p771RV, pΔ(710–807), and pΔ(755–807). All 2-aa insertion mutants were constructed by ligating 6-bp linkers into either pcATG3-10 linearized by partial digestion with *Mae* I or into pcATG10 linearized by (partial) digestion with *Rsa* I, *Bal* I, *Bsa*BI, or *Bcl* I. In plasmids pΔ(755–807) and pΔ(710–807), a stop codon linker was inserted behind *Ac* nt 3290 and 3426. Mutants pΔ(103–135), pΔ(109–128), pΔ(228–237), pK174A/K176A, pH182A/H183A, and pH191R/R193H were gener-

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Abbreviations: TPase, transposase; ORF, open reading frame; GUS, β-glucuronidase.

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ated by PCR-based site-specific mutagenesis as described (11).

All mutants were sequenced across all restriction sites and PCR-amplified segments that were used or generated during the subcloning steps. For most of the inactive 2-aa insertion mutants, two independent isolates from the linker-ligation step were tested. Details of the cloning procedures and DNA sequences can be obtained from the authors upon request.

Transient *Ds* Excision Assay. Seed material of *Petunia hybrida* [ssp. RL01 × ssp. Blue Titan] was kindly provided by Peter Meyer (Max-Delbrück Laboratory, Cologne-Vogelsang). Protoplast isolation and transfection were performed as described (14, 17). Due to the variability in absolute numbers of blue-stained GUS-positive protoplasts obtained from one batch of protoplasts to another (17), we repeated each cotransfection experiment three to six times and counted two platings per cotransfection. The activities shown in Fig. 3 are the averages of these values relative to the number of GUS-positive protoplasts obtained with 10 μg or 3 μg of pcATG10, which were taken as 100%. Standard deviations are usually between 15% and 50% of the values shown. Exceptionally high are the standard deviations for p663DR (77%) alone and for p297PR (64%), pΔ(710–807) (63%), and pΔ(755–807) (76%) when cotransfected with pcATG10.

Protein Extraction from Protoplasts and Western Blot Analysis. About 8 × 10^5 transfected protoplasts were diluted in 0.24 M $CaCl_2$ and sedimented by centrifugation. After resuspending in 100 μl of 20 mM Tris·HCl, pH 8.3/600 mM NaCl/20 mM $MgCl_2$/0.6 mM phenylmethylsulfonyl fluoride/aprotinin (2 μg/ml)/leupeptin (2 μg/ml)/pepstatin A (2 μg/ml)/antipain (2 μg/ml), protoplasts were homogenized for 10 sec with a Polytron PT1200C. The suspension was centrifuged for 10 min at 1000 × *g*, and the resulting pellet was kept on ice. Protein from the supernatant was acetone-precipitated. Proteins were size-fractionated by SDS/PAGE and subsequently electrotransferred to positively charged nylon membranes (Hybond-N^+; Amersham). Membranes were blocked overnight at 4°C in blocking buffer [1% blocking reagent (Boehringer Mannheim) in 0.1 M maleic acid/0.15 M NaCl, pH 7.5]. Incubation with primary antiserum (diluted 1:1000 in blocking buffer) was for 1 h at room temperature. After washing for four 10-min periods in phosphate-buffered saline/0.1% Tween 20, membranes were incubated for 1 h at room temperature with secondary antibodies [alkaline phosphatase-coupled goat anti-rabbit immunoglobulin (Boehringer Mannheim) diluted 1:5000 in blocking buffer]. Membranes were washed as before, followed by four 5-min washes in 0.1 M diethanolamine/1 mM $MgCl_2$, pH 10. Subsequently, membranes were incubated 5 min at 37°C with 0.24 mM disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2′-(5′-chloro)tricyclo[3.3.1.$1^{3,7}$]decan}-4-yl)phenyl phosphate (CSPD) (Tropix, Bedford, MA) and exposed to x-ray film (0.5–5 min).

RESULTS

Recently, Feldmar and Kunze (11) have described the *in vitro* DNA binding properties of a number of artificial *Ac* TPase derivatives. To investigate the functional consequences of these and other mutations *in vivo*, we tested them in a transient *Ds* excision assay developed by Houba-Hérin *et al.* (14). Three questions were addressed: (*i*) Are the mutated TPase proteins correctly expressed in transfected protoplasts? (*ii*) Can mutated TPase molecules directly catalyze *Ds* excision? (*iii*) What is the effect of coexpression of mutated and wild-type TPase molecules on excision?

Expression Levels of (Mutant) TPase in Transfected Protoplasts. We based our TPase mutants on a derivative lacking 102 aa from its N terminus (18). This derivative is fully *Ds*-excision-competent in the tobacco stable transformation assay (18, 19). In the petunia transient *Ds* excision assay, it induces even higher excision rates than the full-length TPase (14, 17). For simplicity, we will refer to this N-terminally truncated TPase, the ORFa-(103–807) protein, as "wild-type". The nomenclature for the mutants is explained in Fig. 3.

The mutants that were constructed for this study fall into two groups. The first group is derived from the "wild-type" TPase plant expression plasmid pcATG10 (see Fig. 3) whose first methionine codon behind the T-DNA 2′-promoter opens the TPase reading frame ORFa-(103–807). The second group of constructs is based on a different "wild-type" TPase plasmid, pcATG3-10, that carries seven out-of-frame ATG codons upstream of the ORFa-(103–807) methionine codon. These two versions of "wild-type" plasmids mobilize *Ds* with the same frequency in the excision assay.

To assess whether relative (mutant) TPase activities and protein expression levels are correlated, we analyzed the two "wild-type" plasmids and each mutant in the transfected protoplasts on a Western blot. For this purpose crude protein extracts were prepared from 8 × 10^5 transfected protoplasts. By centrifuging 10 min at 1000 × *g*, the extracts were separated into a pellet and supernatant and then analyzed on a Western blot. Rather strong protein bands of the expected sizes were detected with pcATG10 and all 13 mutants derived thereof (examples are shown in Fig. 1). In all cases, (mutant) TPase protein was detected predominantly or even exclusively in the pellet fraction, indicating that the TPase either strongly aggregates or associates with large insoluble cellular components. Only two of these mutants are transpositionally active (p388CR and p663DR; see Fig. 3).

In contrast, with pcATG3-10 and its derivatives, only weak [pcATG3-10, p270TR, p445TR, p623TR, p709RV, p754TR, and pΔ(755–807)] or even no [p369TR, p390RV, p462TR, p577RV, p585TR, p771RV, and pΔ(710–807)] (mutant) TPase bands were detected, although pcATG3-10 itself and three mutants [p623TR, p754TR, and p771RV] mobilize *Ds* as efficiently as the active constructs from the highly expressed group (see Fig. 3). Accordingly, there is no correlation between transpositional activity and a signal on the Western blots. We conclude from these experiments that TPase amounts below our detection limit are sufficient for *Ds* excisions and that much higher amounts neither enhance nor diminish the excision frequency.

The latter finding was corroborated by transfecting protoplasts with increasing amounts of "wild-type" plasmid pcATG10. In spite of the fact that the protein level continually increased with the amount of plasmid DNA (Fig. 2*A*), the *Ds* excision frequency reached a maximum with 3 μg of DNA and remained about constant when 10 μg or 18 μg of DNA was used (Fig. 2*b*).

Transposition Activity of TPase Mutants. The excision frequencies obtained with the mutants are schematically summarized in Fig. 3. The approximate locations of the mutations relative to structural and functional features of

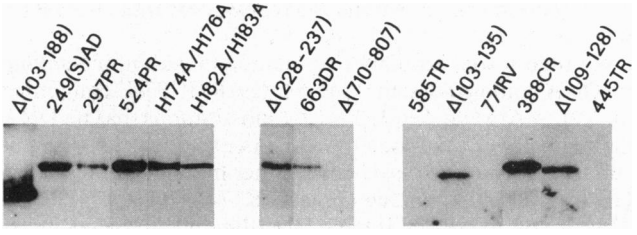

FIG. 1. Expression of mutant derivatives of *Ac* TPase in transfected petunia protoplasts. The pellet fraction of total protein extracts from 8 × 10^5 petunia protoplasts transfected with 10 μg of DNA from the indicated plasmids was analyzed on Western blots.

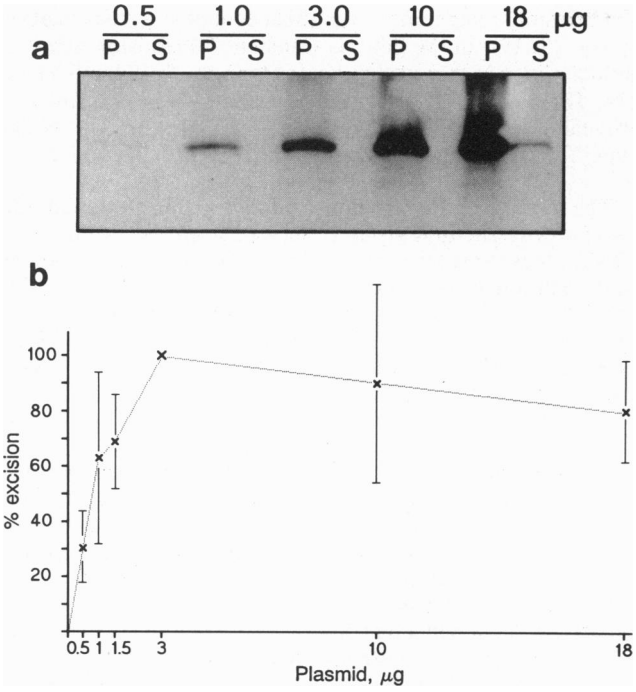

FIG. 2. (*a*) "Wild-type" TPase levels in transfected protoplasts. Total protein extracts from 8×10^5 petunia protoplasts transfected with the indicated amounts of "wild-type" TPase plasmid pcATG10 were analyzed on a Western blot. Lanes: P, pellet fraction; S, supernatant fraction. (*b*) Relative frequency of *Ds* excisions depending on the amount of cotransfected TPase plasmid. The indicated amounts of "wild-type" TPase expression plasmid were cotransfected with 10 μg of the reporter plasmid. The numbers of blue-stained protoplasts were determined in four to six experiments. In each experiment the number obtained with 3 μg of TPase plasmid was taken as 100%. The relative excision frequencies (mean ± SD) are shown.

TPase are indicated. The average *Ds* excision frequencies relative to "wild-type" TPase are shown.

We first asked whether TPase activity tolerates N-terminal deletion beyond aa 103. Three N-terminal deletions were tested [Δ(103–135), Δ(109–128), and Δ(103–188)], and all had lost TPase activity. This result was expected for mutant Δ(103–188) as it is DNA-binding-deficient (11). Mutant Δ(103–135) starts behind the "PQ repeat"—a 10-fold repeat of the dipeptide Pro-Gln or Pro-Glu (12)—whereas in mutant Δ(109–128) the PQ repeat is precisely deleted. We infer that the PQ repeat is required for TPase activity, although it is not involved in binding of the TPase to its AAACGG target motifs (11).

C-terminal deletion mutants Δ(755–807) and Δ(710–807), removing 53 and 98 aa, also abrogated *Ds* excision. Since Coupland *et al.* (13) have shown that a serendipitous random exchange of 4 C-terminal amino acids does not abolish excision activity, we conclude that the C terminus of the ORFa protein from positions 755 to 803 is essential for TPase function.

We then tested a number of mutations within or flanking the DNA binding domain. Mutant H191R/R193H, containing His-191 → Arg and Arg-193 → His substitutions, has no DNA binding activity (data not shown). As expected, its transposition function is also lost. Three other mutants in sequences flanking the DNA binding domain [K174A/K176A, H182A/H183A, and Δ(228–237)] have DNA binding activities indistinguishable from that of the "wild-type" TPase (11). However, all three are transposition-defective.

C-terminal to the DNA binding domain, the *Ac* TPase has a high degree of homology with the putative TPase of *Tam3*

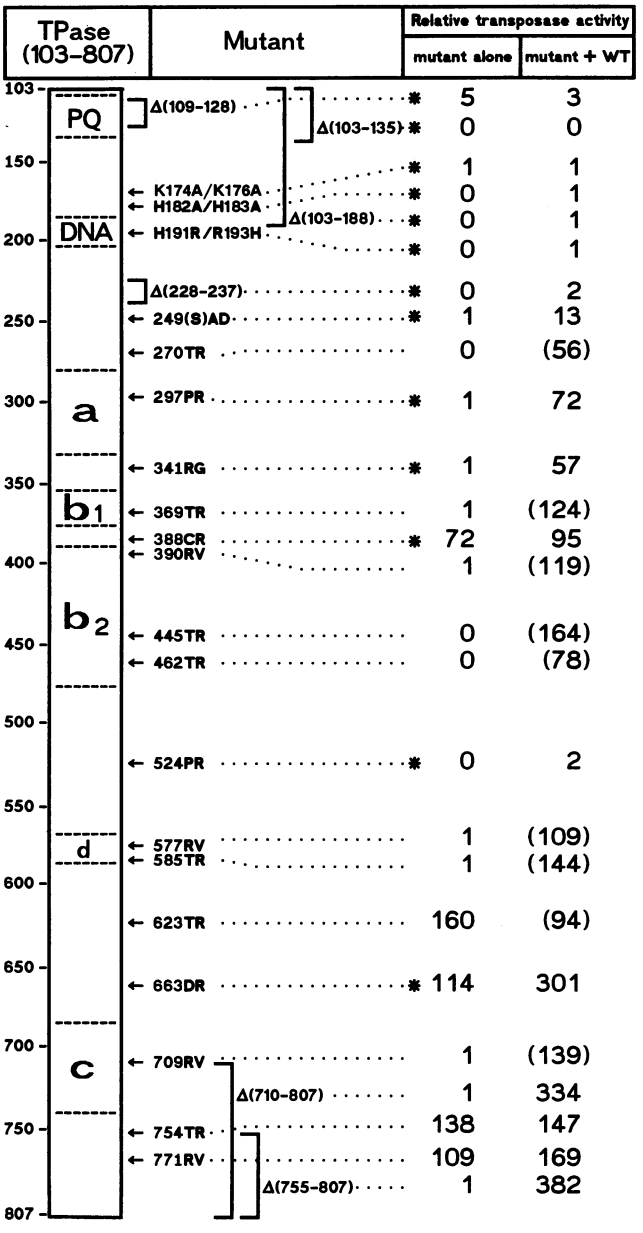

FIG. 3. Distribution and relative activities of mutations along the ORFa-(103–807) TPase. The column labeled TPase(103–807) schematically displays the "wild-type" *Ac* TPase with the following segments highlighted: PQ, $P^{109}QPQPQPQPEPQPQPQPEPE^{128}$; DNA, DNA binding domain; a–d, protein regions with >30% sequence identity between the *Ac* TPase and the putative *Tam3* TPase (a, residues 280–332; b_1, residues 355–375; b_2, residues 389–477; c, residues 680–741; d, residues 568–586). The column labeled Mutant shows the approximate locations of individual mutations. For mutations, the single-letter amino acid code is used as follows: Δ(*n*–*m*) = deletion of ORFa residues *n* to *m*. H*n*A = substitution of His^n with Ala. *n*PR = insertion of Pro and Arg behind ORFa residue *n*. 249(S)AD = replacement of Tyr^{250} with Ser and insertion of Ala and Asp. TPase mutants derived from the highly expressed plasmid pcATG10 are marked with asterisks. Protoplasts were cotransfected with 10 μg of reporter plasmid pNT150Ds and either 10 μg of (mutant) TPase plasmid alone (column labeled mutant alone) or 3 μg of "wild-type" TPase plasmid pcATG10 plus 15 μg of mutant TPase plasmid (column labeled mutant + WT). The number of blue-stained (i.e., GUS-positive) protoplasts obtained with "wild-type" pcATG10 plasmid alone was taken as 100%. The values shown in columns labeled mutant alone and mutant + WT are the averages of three to six cotransfections and two platings per transfection. Values in parentheses indicate that expression of mutants is possibly too weak to cause effects (see *Results*).

(9), suggesting that the C-terminal regions of ≈600 aa of the two proteins are structurally and, potentially, functionally similar. We constructed 17 mutants with insertions of 2 aa at different positions throughout this region [mutants 249(S)AD to 771RV]. Twelve of these mutants are transpositionally inactive, whereas 5 entirely retain *Ds* excision capability. These 5 all carry insertions in regions less well conserved between *Ac* and *Tam3*, and 4 of them are clustered at the C terminus. Although ≈50 aa from the C terminus are required for excisions (see above), it appears that the C-terminal 200 residues of the TPase protein, which include this segment, are insensitive to disturbances of local structure.

Trans-Dominant Inactive TPase Mutants. It is unknown whether the *Ac* TPase is active as a monomeric protein or as an oligo- or multimer. To test whether some of our transpositionally inactive TPase mutants functionally interact with "wild-type" TPase, we triple-transfected petunia protoplasts with the *Ds* reporter plasmid, the "wild-type" TPase expression plasmid, and an expression vector encoding one of the TPase mutants. Since the variance in the number of blue-stained protoplasts between individual transfection experiments does not allow the detection of subtle changes in *Ds* excision frequency (17), we used a 5-fold excess of mutant DNA (15 μg) over "wild-type" TPase plasmid (3 μg). The results are schematically summarized in Fig. 3.

The transposition-positive mutants 388CR, 623TR, 663DR, 754TR, and 771RV were cotransfected as positive controls. Except with mutant 663DR, excision frequencies upon cotransfection are similar to the frequency obtained with the "wild-type" TPase plasmid alone. Mutant 663DR, however, causes a pronounced increase in the number of blue-stained protoplasts.

Interestingly, all mutations within the N-terminal ≈130 aa of the truncated *Ac* TPase and mutant 524PR almost completely inhibit TPase function (Fig. 3). To test the extent to which the degree of inhibition is dependent on the ratio between mutant and "wild-type" plasmid, we also cotransfected equimolar mixtures (3 μg plus 3 μg) of each of these dominant mutants (except p524PR) and the "wild-type" pcATG10 plasmid. In all cases, the *Ds* excision frequencies were reduced to similarly low levels (1–9% of "wild-type" TPase activity) as with a 5-fold excess of mutant DNA.

These results can be explained in one of two ways. The mutant TPase derivative could compete with "wild-type" TPase for the TPase binding sites on the transposon. Alternatively, the mutant protein interacts with the "wild-type" TPase and forms nonfunctional heterooligomers. Since among the dominant inactive mutants are the two DNA-binding-deficient mutants H191R/R193H and Δ(103–188), the first mechanism of inhibition of transposition can be excluded at least in these two cases.

These conclusions are only relevant if the additional N-terminal 102 aa of the full-length TPase are not crucially involved in the oligomerization process. To test whether the full-length TPase activity is affected like that of the truncated "wild-type" TPase, we cotransfected the full-length TPase plasmid pcATG1 with the inactive dominant mutant pH191R/R193H. Indeed, in a 5-fold excess, this DNA-binding-deficient protein inhibited the activities of the full-length TPase and the truncated "wild-type" TPase to similar extents (≈7%), and equimolar amounts of the mutant caused a reduction to 13%. Hence, the full-length TPase is also active as an oligomer.

Coexpression of mutant 249(S)AD reduces "wild-type" TPase function to an intermediate level, indicating that it also is capable of interacting with the "wild-type" TPase. The reductions in excision frequencies observed with mutants 270TR, 297PR, 341RG, and 462TR are not pronounced enough to allow an unambiguous distinction from "wild-type" activity.

None of the other inactive 2-aa insertion mutants negatively affects the functionality of the "wild-type" TPase. Since they all are pcATG3-10 derivatives, it seems possible that their expression level is too low to cause a recognizable alteration in *Ds* excision frequency. Therefore, the respective values in Fig. 3 are shown in parentheses.

Surprisingly, Δ(710–807) and Δ(755–807), the two mutants that are also weakly expressed pcATG3-10-derivatives and are completely inactive in the absence of "wild-type" TPase, cause an increase of excision events in the presence of "wild-type" TPase. We conclude that, rather paradoxically, the C-terminal ≈50 aa from the TPase are absolutely required for its function, yet their absence in some TPase monomers of the transposition complex enhances excision.

DISCUSSION

High Amounts of TPase Do Not Lead to a Further Increase in Transposition. We observed that the *Ac* TPase in the cellular extracts forms large insoluble aggregates that sediment during low-speed centrifugation. Most likely, these aggregates are not preparation artefacts, because TPase aggregates were microscopically detected *in situ* (Manfred Heinlein, personal communication).

The TPase level in petunia protoplasts transfected with pcATG3-10 or its derivatives is significantly lower than in protoplasts transfected with pcATG10 (or its derivatives). This is expected, because the seven upstream out-of-frame ATG codons in pcATG3-10 should hinder correct initiation of translation (17). Interestingly, however, the low protein levels expressed by pcATG3-10 and its derivatives (p623TR, p754TR, and p771RV) are sufficient to mobilize *Ds* as efficiently as the high protein levels expressed by the "wild-type" plasmid pcATG10 and its derivatives (p388CR and p663DR). In accordance with this result, "wild-type" TPase expression in the cells above a certain concentration threshold does not lead to a further increase of transposition frequency (Fig. 2). This phenomenon can only be explained by a posttranslational mechanism that limits the level of TPase activity. Such a mechanism was discussed by Scofield *et al.* (20), who proposed that *Ac* TPase can catalyze transposition reactions only up to a certain concentration threshold.

***Ac* TPase Acts as an Oligo- or Multimer in the Transposition Complex.** When cotransfected with "wild-type" TPase, a number of TPase mutants entirely inhibit transposition reactions. This dominance of transpositionally inactive TPase derivatives over "wild-type" TPase activity indicates that more than one TPase molecule is acting during transposition. With two exceptions, the DNA binding domains of the inactive dominant mutants are not affected. Thus, it is conceivable that they repress formation of an active transposition complex by blocking the DNA binding sites of *Ac* or *Ds*. However, as mutant 297PR has no inhibitory effect, our results suggest that the intact DNA binding domain is not sufficient to inhibit transposition. The two mutants H191R/R193H and Δ(103–188) lack active DNA-binding domains (data not shown; ref. 11). Consequently, their capability to dominantly inhibit "wild-type" TPase activity cannot be explained by competition for the DNA binding sites. Rather it has to be caused by formation of nonfunctional heterooligomers between defective and intact protein. As mutant Δ(103–188) is able to interact with "wild-type" TPase in this way, the responsible protein interface(s) has (have) to be located on the C-terminal side of TPase residue 189.

A second aspect of the Δ(103–188) mutant is that it could be structurally similar to the 70-kDa *Ac* protein in maize, which has been found beside the full-length TPase (15). Both proteins have about the same electrophoretic mobility in SDS-containing gels, and they are recognized by five poly-

clonal antisera directed against different TPase segments including the C terminus. In contrast, neither one is recognized by an antiserum directed against the 97 N-terminal TPase residues. These similarities are consistent with the assumption that the 70-kDa maize protein is lacking ≈200 aa from the TPase N terminus, including the DNA binding domain. As the Δ(103–188) protein is an inhibitor of transposition in the *in vivo* excision assay, it is conceivable that the 70-kDa protein in maize acts as a negative regulator of transposition and that it might be involved in the *Ac* dosage effect (2, 21). The 70-kDa protein is clearly less abundant than the full-length TPase in maize endosperm (15), and we do not know whether this low concentration would suffice to notably alter the variegation pattern on the kernels. We observed that an equimolar amount of pΔ(103–188) plasmid causes a reduction of N-terminally truncated "wild-type"—as well as full-length—TPase activity to 13% or less.

The principle of negative regulation of transposition via a transposon-encoded protein product is used by several transposons. For the *Drosophila P* element, an inhibitor protein is either translated from an alternatively spliced mRNA (22) or encoded by a mutant *P* element (23–26). In fact, the alternative models explaining dominant inhibitory transposase mutations discussed by Rio (27) for the *Drosophila P* element are also fully applicable to *Ac*. Transposon-encoded inhibitor proteins are also involved in the transposition regulation of *Escherichia coli* elements IS*1* (28) and Tn*5* (29, 30).

Inactive TPase Mutants Can Enhance *Ds* Excision in Trans. Surprisingly, three mutants [663DR, Δ(710–807), and Δ(755–807)] considerably enhance the excision frequency when cotransfected with the "wild-type" TPase (Fig. 3). Mutant 663DR is a functional TPase itself. Even more astonishing is the finding that the two completely inactive C-terminal TPase deletion mutants, Δ(755–807) and Δ(710–807), apparently enhance the excision frequency also. This means that on the one hand at least 50 aa from the *Ac* TPases C terminus are absolutely required for functionality, but on the other hand the C terminus has a negative effect if present in every molecule of the transposition complex.

We thank Peter Meyer (Max-Delbrück Laboratory, Cologne-Vogelsang) for the generous supply of *Petunia hybrida* seed, Shivani Chatterjee for help with the transfections, and Peter Starlinger, Jeff Dangl, and Sarah Grant for critically reading the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft through SFB 274.

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