Molecular Analysis of a Transposon-Induced Deletion of the *nivea* Locus in *Antirrhinum majus*

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ABSTRACT

The transposable element Tam*3* of *Antirrhinum majus* is capable of causing large-scale chromosomal restructuring. It induced a large deletion at the *nivea* locus, to produce the allele *niv⁻:529*. The deletion removed the entire *nivea* coding region while the element remains intact with the potential to induce further rearrangements. Genetic experiments showed that the endpoint of the deletion (called *x*) is closely linked to *nivea*. The DNA sequences of *niv⁻:529*, a genomic excision of Tam*3* from *niv⁻:529*, and the original genomic position of *x* have been determined. These data suggest that the deletion could have resulted from an abortive transposition or through breakage and religation.

TRANSPOSABLE elements, both prokaryotic and eukaryotic are well known for their ability to induce large and small-scale rearrangements as a result of transpositional or recombination events. Bacterial IS elements and transposons can transpose via a replicative mechanism. Association of an element with a new site of insertion is accompanied by replication of the element and integration. Intermolecular transposition results in cointegrate formation, while intramolecular transposition leads to either deletions or inversions. Inversions and deletions may also result from intramolecular recombination between elements, this process requiring host functions (reviewed by CALOS and MILLER 1980; IIDA, MEYER and ARBER 1983; HEFFRON 1983; KLECKNER 1983).

In yeast, large-scale rearrangements, induced by Ty elements and *delta* sequences, appear not to result from transposition events; instead deletions and inversions arise from intramolecular recombination, while intermolecular recombinations can also generate translocations and duplications (reviewed by ROEDER and FINK 1983).

In *Drosophila melanogaster P* elements are commonly found at the breakpoints of large-scale deletions and inversions (BERG, ENGELS and KREBER 1980; ENGELS and PRESTON 1984), suggesting that breakage occurs at or near elements with subsequent rearrangement of the intervening sequences, although generation of these is not necessarily associated with transposition of the elements. Another group of *D. melanogaster* elements, called *FB*, often form composite elements (*TE*) which may induce very large, cytologically detectable deletions of flanking sequences on excision (ISING and BLOCK 1981). MCCLINTOCK (1953, 1954), by genetic means, detected large-scale deletions of chromosomal regions flanking *Ds* elements in maize. These deletions occurred only in the presence of an active *Ac* element. Smaller-scale deletions of flanking sequences induced by *Ac* have also been observed (DOONER, ENGLISH and RALSTON 1988), and are apparently a result of aberrant steps during transposition.

Tam*3*, a transposon that moves by excision and reintegration, has also induced a variety of large-scale rearrangements, which may modify or abolish the activity of an adjacent gene: deletions, dispersions and insertions have been described at *nivea* (MARTIN, MACKAY and CARPENTER 1988) and an inversion at *pallida* (ROBBINS, CARPENTER and COEN 1989). Analysis of these rearrangements and the small sequence rearrangements resulting from excisions of Tam*3* (from the *nivea* and *pallida* loci) has allowed a model for Tam*3* excision and transposition to be formulated (COEN, CARPENTER and MARTIN 1986; COEN and CARPENTER 1988; ROBBINS, CARPENTER and COEN 1989). It is proposed that staggered nicks are made in the sequences immediately flanking the element which, on ligation, form hairpins. Nicking of these hairpins and the subsequent religation and DNA repair would be responsible for the small sequence alterations at Tam*3* excision sites. A further aspect of this model demands a physical association between the donor and recipient sites before transposition occurs, making transposition to linked sites most likely. It is suggested that a break occurs in the flanking sequences at one end of the element, the element then invades at a new position along the chromosome, this is followed by breakage at the other end of the element and its transfer to the new integration site. An

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alternative model for plant transposable element excision has been proposed by SAEDLER and NEVERS (1985) which involves formation of a DNA-transposase complex where the inverted termini of the element are brought together. Staggered nicks occur in the sequences flanking the element which are then susceptible to the action of DNA repair enzymes, causing the small rearrangements at transposable element excision sites.

In the phenotypically unstable line J.I. 98, Tam*3* has inserted in the promoter region of the *nivea* gene that encodes the enzyme chalcone synthase. The transposon is inserted 29 bp upstream from the TATA box and creates a 8-bp duplication of the target sequence. The phenotype of J.I. 98 is pale red because of the reduction in chalcone synthase gene expression caused by the insertion, with full red flakes or spots caused by somatic excision of the element from the gene, allowing restoration of full gene expression (SOMMER *et al.* 1985). Germinal excision of the element can result in full red revertant flowers. Other alleles of the *nivea* locus may be generated through imprecise excisions of the element (CARPENTER, MARTIN and COEN 1987). A similar allelic series has been observed at *pallida* where Tam*3* is also inserted in the promoter region (COEN, CARPENTER and MARTIN 1986). Indeed imprecise excisions are a common feature of plant transposable elements (SCHWARZ-SOMMER *et al.* 1985; SAEDLER and NEVERS 1985; SAEDLER, SCHWARZ-SOMMER and GIERL 1985).

In this paper one rearrangement, a deletion of the *nivea* coding region (allele *niv⁻:529*), was analysed in detail by cloning and DNA sequencing and the exact breakpoints of the deletion were defined. Some of the genetic properties of the deleted sequences were characterized. Subsequent activity of Tam*3* in this deletion-derivative was analyzed, including precise and imprecise excisions and a secondary large-scale rearrangement of DNA. The analysis supports the previously proposed model of COEN, CARPENTER and MARTIN (1986); COEN and CARPENTER (1988) and ROBBINS, CARPENTER and COEN (1989) for Tam*3* transposition, suggesting that the deletion arose as a result of an abortive transposition, however an alternative mechanism involving breakage at a specific sequence is also conceivable.

MATERIALS AND METHODS

Antirrhinum majus stocks: Most of the alleles described are derived from the *niv^rec:98* allele of J.I. stock 98 (HARRISON and CARPENTER 1979, SOMMER *et al.* 1985 and MARTIN, MACKAY and CARPENTER 1988). *niv⁻:529* and *niv⁻:570* were maintained as heterozygotes, due to homozygous lethality, with a pigmented allele *niv:532* (MARTIN, MACKAY and CARPENTER 1988). Stock J.I. 45 carries the white *niv⁻:45* allele, the mutation is due to a stable insertion into the *nivea* coding region (CARPENTER, MARTIN and COEN 1987). *niv⁻:560* is a stable white allele, caused by an imprecise excision of Tam*3* from the *nivea* gene (MARTIN, MACKAY and CARPENTER 1988). J.I. stock 5A (*pal^rec:5A*) has been described by HARRISON and FINCHAM (1964).

Isolation of *Antirrhinum majus* genomic DNA: Genomic DNA was prepared from frozen leaves as described by MARTIN *et al.* (1985).

Southern hybridization of plant genomic DNA: Southern hybridizations were carried out according to SOUTHERN (1975) and WAHL, STERN and STARK (1979), as modified by SOMMER *et al.* (1985). Filters were washed twice in 0.1 × SSC (0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0), 0.5% SDS at 65° for 30 min.

Cloning of plant genomic DNA into λEMBL4: An aliquot of 100–200 μg of genomic DNA was partially digested with *Eco*RI and size fractionated on a 0.6% agarose gel. Fragments between approximately 15 and 20 kb were isolated by electroelution and purified over a "NACS" column (MANIATIS, FRITSCH and SAMBROOK 1981). λEMBL4 *Eco*RI arms were prepared by digestion with *Eco*RI and *Bam*HI, followed by isopropanol precipitation. Ligations between 500-ng vector arms and genomic fragments were optimized. The ligations were packaged *in vitro* and plated on *Escherichia coli* strain LE 392. Libraries, blotted onto nitrocellulose filters, were screened with the appropriate fragments. For more detailed analysis fragments were subcloned into pUC18 and M13 vectors for DNA sequence determination.

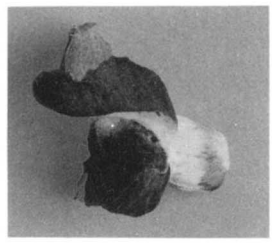
Phage DNA preparation: Phage DNA was prepared from liquid lysates by PEG 6000 precipitation and purification on CsCl gradients, followed by phenol:chloroform (1:1) extraction and ethanol precipitation, modified from the method of WILL, BAYEV and FINNEGAN (1981).

Plasmid DNA preparation: Plasmid was prepared as described by MANIATIS, FRITSCH and SAMBROOK (1982). Restriction fragments were isolated from agarose gels by a "freeze-squeeze" method.

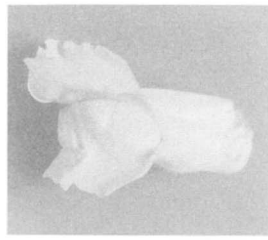
DNA sequence determination: Fragments to be sequenced were subcloned into the appropriate M13 vectors. Template preparation, sequencing reactions (using [^{35}S]-dATP) and buffer gradient electrophoresis were modified from the methods of SANGER, NICKLEN and COULSEN (1977) and BIGGIN, GIBSON and HONG (1983).

RESULTS

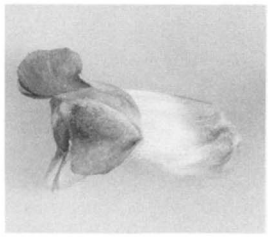
Genetical analysis of the *niv⁻:529* allele: The allele *niv⁻:529* arose from a cross between J.I. 98 and J.I. 45. It has been shown to be a deletion of sequence downstream from the insertion site of Tam*3* at *nivea*, removing the coding region of the gene, while the element remained apparently intact and active (MARTIN, MACKAY and CARPENTER 1988). Attempts to establish this allele in a homozygous form were completely unsuccessful, indicating that the allele was lethal when homozygous. This suggested that the deletion was large, involving loss of essential genes adjacent to *nivea*. The *niv⁻:529* allele was subsequently maintained as a heterozygote with another *niv* allele (*niv:532*). *niv:532* was also derived from J.I. 98 and is an imprecise excision of Tam*3* from *nivea*, removing 56 bases in total from the sequences flanking the insertion site of the element. Homozygous *niv:532* flowers are a uniform red, considerably paler than wild-type dark red flowers. In a heterozygote



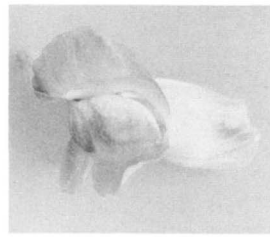
J.I. 98



niv⁻:45 / *niv*⁻:529



niv:532 / *niv*:532



niv:532 / *niv*⁻:529

FIGURE 1.—The mutant allele *niv⁻:529* arose from the unstable line J.I. 98. This allele is homozygous lethal and was maintained as a heterozygote with a pigmented allele *niv:532*.

with *niv⁻:529* the flowers are paler still, allowing phenotypic identification of those plants carrying the *niv⁻:529* (Figure 1).

Experiments were carried out to determine whether the lethality was due to loss of viability in the pollen or female gametophyte. Reciprocal crosses were carried out between the *niv⁻:529/niv:532* heterozygote and a white homozygote (*niv⁻:560*) and the number of white and pale red progeny scored. White progeny would arise as the result of inheritance of the *niv⁻:529* allele as the *nivea* gene is deleted in this allele. A deficiency in the pollen would be indicated by a reduction in the number of white progeny when the *niv⁻:529/niv:532* was the male parent, while a reduction in the number of white progeny with the heterozygote as the female parent would indicate loss of viability in the female gametophyte. The results of these crosses are presented in Table 1 and suggest that there is no loss of viability in either the pollen or the female gametophyte. This might suggest that the lack of viability of the homozygote, due to this deletion, may involve loss of a factor(s) involved in fertilization, seed development or germination.

Restriction mapping of *niv⁻:529*: In the *niv^{rec}:98* allele Tam*3* is inserted into the *nivea* locus between

TABLE 1

Results of reciprocal crosses between *niv⁻:529/niv:532* and *niv⁻:560/niv⁻:560*

	Cross A		Cross B
	$\frac{niv^-:560}{niv^-:560}$ ♀ × $\frac{niv^-:529}{niv:532}$ ♂		$\frac{niv^-:529}{niv:532}$ ♀ × $\frac{niv^-:560}{niv^-:560}$ ♂
F_1 Progeny			
Genotype	$\frac{niv^-:560}{niv^-:529}$		$\frac{niv^-:560}{niv:532}$
Phenotype	White		Pale red
Expected	1		1
Observed	Cross A	27	15
	Cross B	19	8

The data indicate that the deletion in *niv⁻:529* does not affect either the pollen or female gametophyte viability.

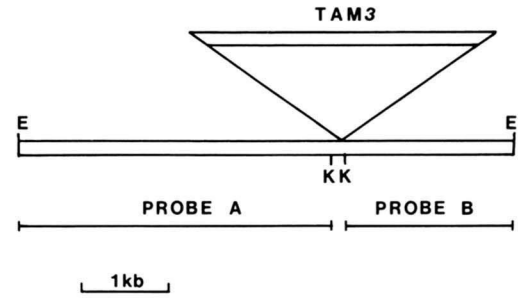


FIGURE 2.—Partial restriction map of JAM1, indicating the *nivea* promoter (A) and coding region (B) probes. E = *Eco*RI, K = *Kpn*I.

two *Eco*RI sites, giving rise to a 9.2-kb restriction fragment consisting of 5.7 kb of *nivea* sequence and 3.5 kb of Tam*3*. This has been cloned (SOMMER *et al.* 1985) into the plasmid pJAM1. Tam*3* is flanked by two *Kpn*I sites so digestion of pJAM1 with *Eco*RI plus *Kpn*I yields suitable probes for the *nivea* sequences upstream (probe A) and downstream (probe B) of Tam*3* (Figure 2). Genomic DNA from the heterozygote (*niv⁻:529/niv:532*) was digested with *Eco*RI and hybridized to probe A. A new *Eco*RI fragment of 13.5 kb was detected. No hybridization was observed with Probe B to allele *niv⁻:529*, suggesting that all sequences downstream from the insertion site of the element had been lost (MARTIN, MACKAY and CARPENTER 1988). Both probes A and B hybridized to *niv:532*, as only 56 bases have been deleted from this allele.

To determine whether Tam*3* was intact in *niv⁻:529*, Southern blots of double digestions of *niv⁻:529/niv:532* genomic DNA (*Eco*RI plus restriction enzymes cutting within the element) were hybridized to probe A. The fragments from the *niv⁻:529* allele that hybridized to the probe were identical to those from *niv^{rec}:98*, indicating that Tam*3* was intact.

To map restriction sites in the new sequence flanking Tam*3* in *niv⁻:529*, Probe A was used to hybridize to Southern blots of *niv⁻:529/niv:532* genomic DNA

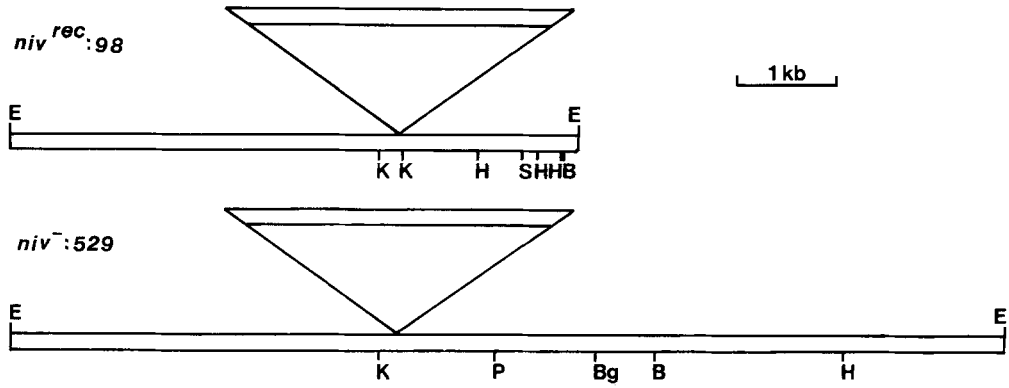


FIGURE 3.—Restriction maps of *niv*^{rec}*:98* and *niv*$^{-}$*:529*. Sites in Tam*3* are not shown. B = *Bam*HI, Bg = *Bgl*I, E = *Eco*RI, H = *Hin*dIII, K = *Kpn*I, P = *Pvu*II, S = *Sma*I.

digested with *Eco*RI plus restriction enzymes that do not cut within the transposon. In this way a restriction map of the flanking sequence was established (Figure 3).

Cloning and sequence analysis of *niv*$^{-}$*:529*: The 13.5-kb *Eco*RI fragment of *niv*$^{-}$*:529* was isolated from a genomic library of *Eco*RI partially digested *niv*$^{-}$*: 529/niv:532* DNA in λEMBL4 (see MATERIALS AND METHODS). Approximately 120,000 plaques were screened with probe A and two hybridizing clones were isolated. Hybridization of these two clones indicated that one was *niv*$^{-}$*:529* (known as JAM529-31: Figure 4) and the other was *niv:532*. The 13.5-kb *Eco*RI insert in JAM529-31 was subcloned into pUC18 as two *Eco*RI-*Bam*HI fragments, yielding pJAM529-4 and pJAM529-1. The two plasmids were digested with several restriction enzymes and probed with radioactively labeled DNA from the *niv*$^{-}$*:529/niv:532* heterozygote to determine whether the new sequences flanking Tam*3* were repeated elsewhere in the Antirrhinum genome. The Southern blots indicated that this sequence is not highly repetitive: the only strong hybridization was to Tam*3*-containing fragments.

To determine the new sequence flanking Tam*3* and the ends of the element, small restriction fragments from pJAM529-4, containing each end of Tam*3* and adjacent sequences, were subcloned into M13mp19 and sequenced (see MATERIALS AND METHODS). The sequence data confirmed that both ends of the element were intact and that the deletion had completely removed the 8 bases duplicated at the site of the Tam*3* insertion at *nivea* (Figure 5). The *nivea* sequence 5′ to the insertion site of Tam*3* was unaltered indicating that breakage and religation had not occurred at this end of the element.

Linkage analysis of *nivea* and the new flanking sequence: Linkage analysis was carried out to determine whether *nivea* and the new flanking sequence (called *x*) were genetically linked in the progenitor line, J.I. 98. A *Bam*HI restriction fragment length polymorphism (RFLP) at *x* was identified between J.I. 98 and another, unrelated, line J.I. 5A (*pal*^{rec}*:5A*) when the 3.5-kb *Bam*HI-*Eco*RI fragment from JAM529-31 (probe B-E) was used as a probe. J.I. 98 and J.I. 5A were crossed (R. CARPENTER, personal communication) and 35 homozygous *niv*^{rec} F_2 progeny were examined. If *nivea* and *x* were closely linked then there would be very little or no recombination between the two loci. No recombinants between *nivea* and *x* were found, while the RFLP was shown to segregate as predicted in homozygous *pal*^{rec} and heterozygous *niv*^{rec}*/pal*^{rec} plants (Figure 6). These results suggest that *nivea* and *x* are very closely linked. Although an RFLP between *niv*^{rec}*:98* and *pal*^{rec}*:5A* was identified, no RFLP was observed between *niv*^{rec}*:98* and four lines of a related species *A. glutinosum*. For all restriction enzymes tested the sequences were conserved between J.I. 98 and *A. glutinosum* and were also present in single copy. Our experience with Antirrhinum genes suggests that most are closely flanked by repeated sequence DNA (within 2–5 kb). The conservation of restriction sites and the occurrence of this sequence as single copy in two species might suggest that this region encodes a gene product.

Cloning and sequence analysis of *x* from the progenitor line J.I. 98: Using the *Bgl*I-*Bgl*I fragment from JAM529-31 (probe B-B) to hybridize to single restriction enzyme digests of genomic DNA from the progenitor line, J.I. 98, the original genomic position of *x* was identified on an 8.2-kb *Eco*RI fragment and on a 5.4-kb *Hin*dIII fragment.

To determine whether a copy of Tam*3* was present in the progenitor sequence probe B-B was used to probe double digestions of J.I. 98 genomic DNA (*Eco*RI plus restriction enzymes cutting within the element). This experiment suggested that no copy of Tam*3* was present.

A genomic library of *Eco*RI partially digested J.I. 98 DNA was prepared in λEMBL4 (see MATERIALS AND METHODS). Approximately 70,000 plaques were screened with probe B-B. Three hybridizing clones were isolated and the 5.4-kb *Hin*dIII fragment from

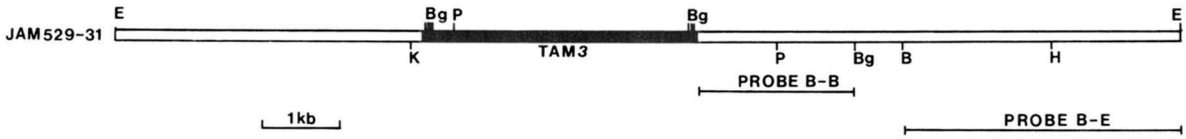


FIGURE 4.—Restriction map of JAM529-31. B = *Bam*HI, Bg = *Bgl*I, E = *Eco*RI, H = *Hin*dIII, K = *Kpn*I, P = *Pvu*II, S = *Sma*I.

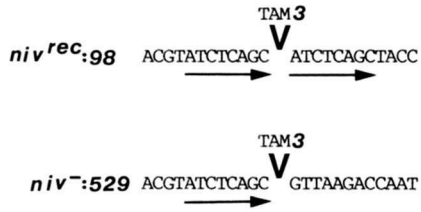


FIGURE 5.—DNA sequence data of the sequences flanking Tam*3* in *niv*^{rec}*:98* and *niv*$^{-}$*:529*. Arrows indicate the bases duplicated on insertion of the element.

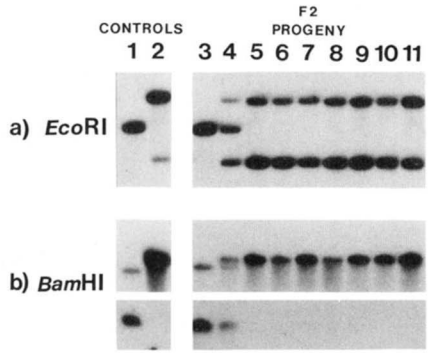


FIGURE 6.—Restriction enzyme digests of genomic DNAs from single plants, probed with fragments which indicate the RFLPs identified between lines J.I. 98 and J.I. 5A, a) *Eco*RI digests, hybridized to probe A; b) *Bam*HI digests, hybridized to probe B-E. 1, J.I. 5A; 2, J.I. 98; 3, homozygous *pal*^{rec} plant; 4, heterozygous *niv*^{rec}*/pal*^{rec} plant; 5–11, homozygous *niv*^{rec} plants.

FIGURE 7.—a, DNA sequence data of the breakpoint of the deletion in *niv*$^{-}$*:529*, compared with the progenitor sequence *x*. Arrow indicates inverted termini of Tam*3*. b, Comparison of the inverted termini of Tam*3* and *Ac* with the sequence of the breakpoint of the deletion. Underlined bases indicate homology.

one of these was subcloned into pUC18 to give pJAM98-529p.

To sequence across the junction between the new DNA adjacent to Tam*3* in *niv*$^{-}$*:529* and the sequence adjacent to it in the progenitor line, a *Hin*dII fragment from pJAM98-529p was subcloned into the *Sma*I site of M13mp19 and sequenced. The sequence data (Figure 7a) confirmed that no copy of Tam*3* was present in the progenitor sequence. However, the breakpoint of the rearrangement has strong homology to the terminal inverted duplications of Tam*3* and also to those of the maize element *Ac* (POHLMAN, FEDOROFF and MESSING 1984; MÜLLER-NEUMANN, YODER and STARLINGER 1984) suggesting that transposase may recognize this sequence as the terminus of an element and is cutting at this site in error (Figure 7b). Alternatively the deletion may have resulted from an abortive transposition by Tam*3* in which one end of the element caused breakage and invaded the chromosome at this position. In either instance the deletion would then be generated by ligation between a hairpin loop at *x* and one end of Tam*3*. The intervening sequence would be lost because of the lack of a centromere.

Isolation, cloning and sequence analysis of an excision of Tam*3* from *niv*$^{-}$*:529*: The activity of Tam*3* in *niv*$^{-}$*:529* can no longer be detected phenotypically because of the deletion of *nivea* coding sequence. However, hybridization of probe A to a faint band 3.5 kb smaller than the *niv*$^{-}$*:529* fragment suggested that somatic excision could still occur at high frequency (Figure 8). The result of a germinal excision event of Tam*3* from *niv*$^{-}$*:529* was isolated by random screening of 15 *niv*$^{-}$*:529/niv:532* heterozygotes. One plant appeared to carry an excision of Tam*3*. This allele (*niv*$^{-}$*-570*) was cloned into λEMBL4 (see MATERIALS AND METHODS). A *Kpn*I-*Pvu*II restriction fragment spanning the region from which Tam*3* had excised was subcloned directly into M13mp19 digested with *Kpn*I and *Hin*dII and sequenced. The sequence data (Figure 9a) showed that excision of the element had produced a small rearrangement at its former insertion site. There was no net loss or addition of bases but an inversion of two bases had occurred. This structure can be accounted for by the model of COEN, CARPENTER and MARTIN (1986), COEN and CARPENTER (1988) and ROBBINS, CARPENTER and COEN (1989), as shown in Figure 9b.

The excision of Tam*3* from *niv*$^{-}$*:529* demonstrates that the 8 bp duplication of target sequence (in *niv*^{rec}*:98*) is not exsential for excision of the element, this has also been shown by ROBBINS, CARPENTER and COEN (1989).

Mapping of secondary rearrangements of *niv*$^{-}$*:529*: As described above, Tam*3* is able to excise from *niv*$^{-}$*:529* but it may also induce further re-

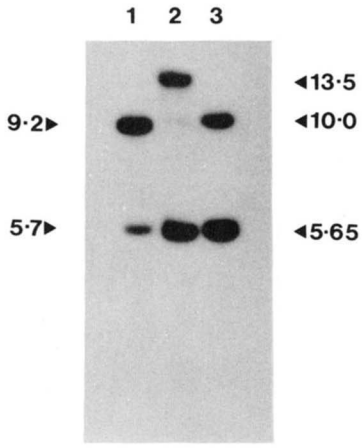


FIGURE 8.—*Eco*RI digests of genomics DNAs hybridized to probe A showing somatic and germinal excision of Tam*3* from *niv^{rec}:98* and *niv^{-}:529*. 1, *niv^{rec}:98*; 2, *niv:532/niv^{-}:529*; 3, *niv:532/niv^{-}:570*. The 5.65-kb band in 2 and 3 comes from *niv:532* which is an imprecise excision of Tam*3* from *niv^{rec}:98* removing 56 bases of flanking sequence.

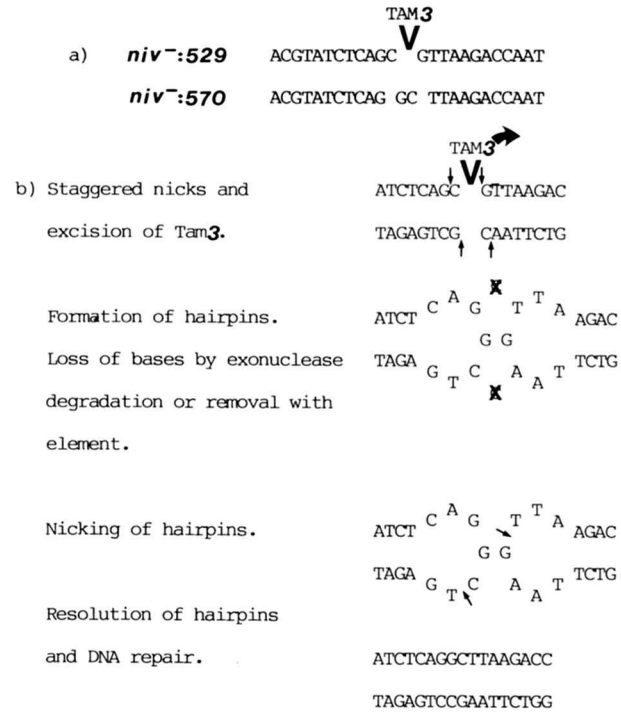


FIGURE 9.—a, DNA sequence data of the sequences flanking Tam*3* at *niv^{-}:529* and the rearrangement of sequence resulting from excision of the element in *niv^{-}:570*. b, Model for Tam*3* excision from *niv^{-}:529*.

arrangements of the surrounding sequences. Two such rearrangements have been analyzed: an excision of Tam*3* which deletes sequences adjacent to the element and a duplication of part of the remaining *nivea* promoter region. These rearrangements were identified by random screening of plants carrying the *niv^{-}:529* allele, because phenotypic selection is not possible in this line.

In the line containing the excision, Tam*3* removed approximately 2.0–2.5 kb of the sequences flanking its former insertion site. The extent of the deletion on the 3′ side of the former insertion site of the element was mapped to between the *Pvu*II and *Bgl*I restriction sites. On the 5′ side the insertion was mapped to between the *Ava*II and *Dde*I restriction sites (Figure 10a).

A second rearrangement involves the duplication of probe A-hybridizing sequences (*i.e.*, the promoter region of *nivea*) and is seen as an additional 4.8-kb *Eco*RI fragment. The duplicated DNA is linked to *niv^{-}:529* locus. The duplication was mapped from the 5′ *Eco*RI site and extends downstream to at least the *Kpn*I site 167 bp upstream from the insertion site of Tam*3* (Figure 10b). The duplicated region does not hybridize to probe B. Digestion of genomic DNA from this line with *Dde*I and probing with a *Dde*I fragment from the *nivea* promoter indicated that the *Dde*I site immediately adjacent to Tam*3* (actually part of the 8-bp duplication) was lost or rearranged. This suggests that the duplication resulted from Tam*3* activity because the sequences flanking Tam*3* were disturbed, although transposition of the element has not occurred. No somatic excision of Tam*3* from the *niv^{-}:529* locus in the line carrying the duplication was observed (Figure 10b, track B) compared with *niv^{-}:529* (track A). This suggests that Tam*3* was also disturbed by the duplication and this has affected its ability to excise. No data are available on how far the duplication extends beyond the *Eco*RI site.

DISCUSSION

Plant transposable elements are well known for their ability to cause small-scale rearrangements of a gene on excision. These rearrangements are the result of imprecise excision of the element and are thought to have a role in gene evolution (SCHWARZ-SOMMER *et al.* 1985; SAEDLER and NEVERS 1985; SAEDLER, SCHWARZ-SOMMER and GIERL 1985). Imprecise excisions usually leave some of the bases that were duplicated on insertion of the element, although more complex rearrangements can also occur. These additional bases may affect the gene product by adding amino acids or by altering the gene product if inserted into an intron; or they may alter the expression of a gene if the element has inserted into the regulatory region of the gene. A recent example of this in maize shows how *Ds*-induced mutations of the *bronze* locus can affect both the quantity and quality of the gene product (SCHIEFELBEIN *et al.* 1988). Large-scale rearrangements may also have a profound effect, even affecting the viability of plants, as in the case of the deletion described here. The cause of the lethality of *niv^{-}:529* has not been fully determined but may involve failure at fertilization, seed development or germination.

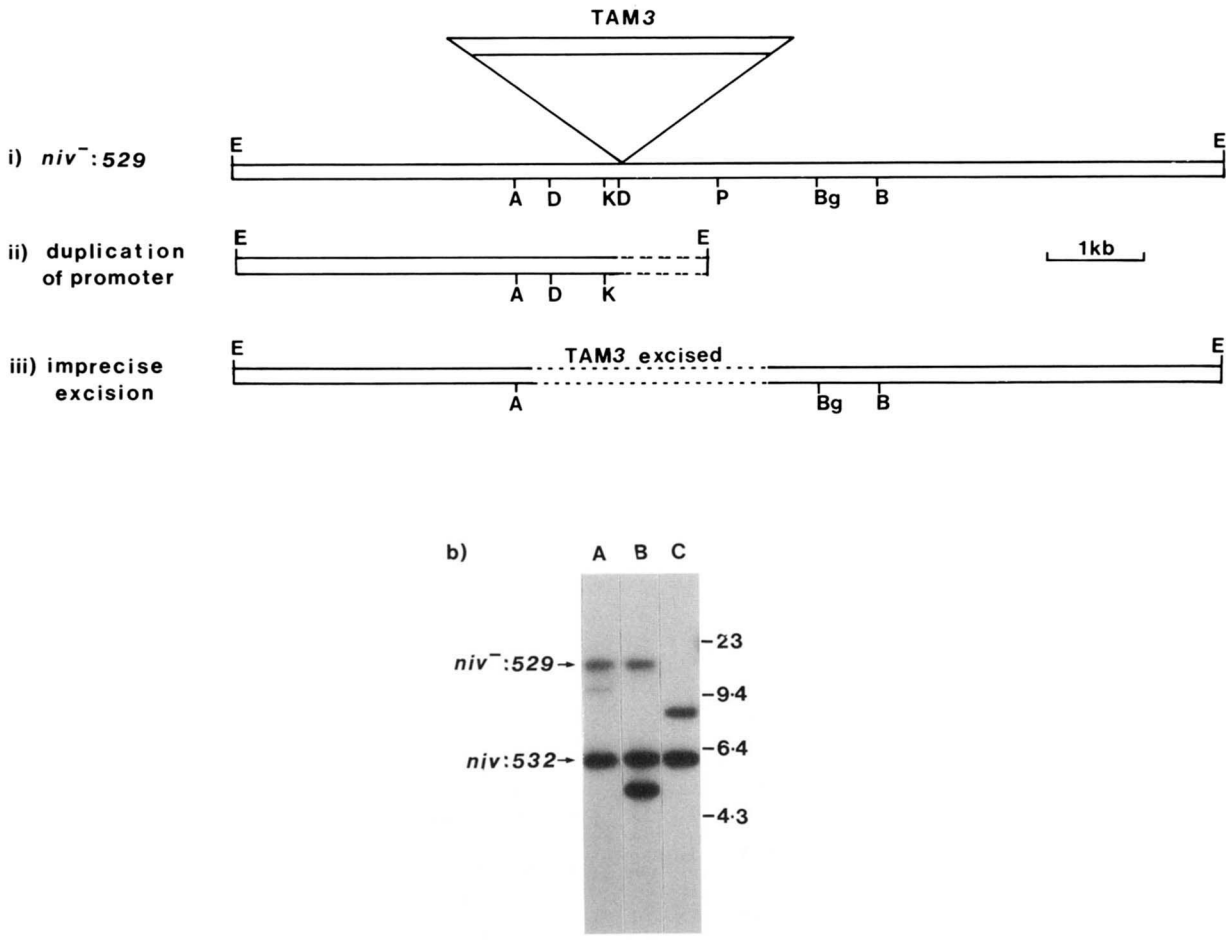


FIGURE 10.—a, Restriction maps of i) *niv⁻:529*; ii) sequences remaining after the imprecise excision of Tam*3* from *niv⁻:529*; and iii) the 4.8-kb *Eco*RI fragment carrying the duplicated *nivea* promoter. Sites in the element are not shown, likewise all other *Dde*I sites. A = *Ava*II, B = *Bam*HI, Bg = *Bgl*I, D = *Dde*I, E = *Eco*RI, K = *Kpn*I. b, Southern blot of *Eco*RI digested genomic DNAs, hybridized to probe A. A) *niv⁻:529*; B) duplication of *nivea* promoter; and C) imprecise excision of Tam*3* from *niv⁻:529*.

Tam*3* induces a variety of rearrangements at a high frequency (MARTIN, MACKAY and CARPENTER 1988) and, provided that the element is unaffected by a rearrangement, it may excise or induce further rearrangements at a relatively high frequency, suggesting an inherent instability in lines where a rearrangement has already occurred. In the *niv⁻:529* deletion Tam*3* remains intact and active, while the 8-bp target sequence that was duplicated on insertion of the element was removed. Our analyses of germinal and somatic excision indicates that the duplicated bases are not required for excision of the element. This has also been observed in other Tam*3*-induced rearrangements (ROBBINS, CARPENTER and COEN 1989). A very similar situation was reported in maize where a deletion adjacent to *Ac* removed the eight duplicated bases but left the element intact and able to transpose (DOONER, ENGLISH and RALSTON 1988).

Tam*3* commonly inserts into active genes, as at *pallida* (MARTIN *et al.* 1985) and *nivea* (SOMMER *et al.* 1985). It is, however, impossible to determine genetically whether Tam*3* has a strong preference for such insertions, although phenotypically identifiable transposon-induced mutations are observed at a high frequency (HARRISON and CARPENTER 1979). There is also evidence that Tam*3* may have a preference for insertion into single copy DNA from the rearrangement described here, that of ROBBINS, CARPENTER and COEN (1989) and another rearrangement under investigation (C. LISTER, unpublished results) where the new pieces of DNA flanking Tam*3* at the rearrangement are present in single copy. A possible explanation is that Tam*3* requires the chromatin to be of a certain "open" structure to allow invasion of the element. Insertion of the element into an active gene may prevent suppression of its activity by methylation; it has been shown that copies of Tam*3* which have inserted in repetitive DNA are unable to transpose and this inability is correlated with heavy methylation of the element (MARTIN *et al.* 1989). Sequence *x* appears to be hypomethylated compared to repetitive DNA.

There are several mechanisms by which this deletion may have formed, the simplest being recombi-

nation. Intramolecular recombination between two elements, in the same orientation, would result in a deletion of the intervening sequence; however, although the endpoints of the deletion are linked, there is no evidence of a second copy of Tam*3* being involved, unless one proposes a brief encounter with a second element. However, because the *niv*$^{-}$*:529* allele was derived from *niv*^{rec}*:98* in a single generation, any transient association of Tam*3* with *x* would probably have been observed. An alternative mechanism would involve breakage at the ends of two elements and the subsequent loss of the intervening sequence. Such a mechanism was proposed for large-scale rearrangements induced by the *P* element of *D. melanogaster* by ENGELS and PRESTON (1984) who observed that *P* element-induced inversions usually arise by a single multibreak event with subsequent random rearrangement of the chromosome segments; this mechanism could also explain the formation of large deletions. These rearrangements may result from transposition of the element but, because *P* elements often remain at the site of the rearrangements, this might suggest aberrant transposition events. We have shown that there is no second copy of Tam*3*. However, because the sequence beyond the breakpoint of the rearrangement strongly resembles the inverted termini of Tam*3* the transposase may be recognizing this sequence as the end of an element and causing breakage, with breakage also occurring at the 3′ end of the element at *nivea*. This might suggest that the Tam*3* transposase could recognize a sequence that is not completely homologous to its termini and cause breakage; indeed a copy of Tam*3*, known as Tam*3:105*, has a 2-bp mismatch in its inverted termini yet it is still able to transpose (MARTIN *et al.* 1989).

The model for Tam*3* transposition proposed by ROBBINS, CARPENTER and COEN (1989) was deduced from the analysis of a large Tam*3*-induced inversion at the *pallida* locus. They suggest that, in a normal transposition, the donor and recipient sites are brought into close association. Sequential breakage and transfer of the ends of the element to the recipient site occurs with the formation of hairpin loops at the broken ends. This is followed by nicking of the hairpins with ligation and DNA repair of the free ends. Inversions and deletions may be formed by this mechanism if transfer and ligation of only one end of the element to the recipient site occurs, with the intervening sequence being lost or inverted.

From the data obtained from the analysis of *niv*$^{-}$*:529* it is difficult to differentiate between a single multibreak event and the model proposed by ROBBINS, CARPENTER and COEN (1989) as to the mechanism of formation of this deletion. In both models it would be expected that the transposase or the element would generate a duplication of sequence at *x*; however, because the intervening sequence between *nivea* and *x* has been lost, this duplication would not be seen.

It has been suggested by ROBBINS, CARPENTER and COEN (1989) that Tam*3* has a preference for transposition to closely-linked sites, and the very close linkage of *nivea* and *x* could support this model. This would not however rule out a mechanism of breakage and religation; the breakpoint of which could be more distantly linked or unlinked as in a translocation.

There is no evidence that Tam*3* has a preference for insertion sites that are homologous to its termini. This would therefore suggest that the strong homology (9/12 bases) of *x* to the Tam*3* inverted termini was significant and the transposase recognized the sequence at *x* and caused breakage there. The homology of *x* to Tam*3* does not extend beyond the termini and it would therefore be necessary to separate the "breakage activity" of the transposase from the "transposition activity." It has been shown that sequences inside the inverted termini of the maize element *Ac* are also required for transposition (COUPLAND *et al.* 1988). This is relevant as Tam*3* and *Ac* are likely to be related transposable elements and have homology at the amino acid level in the transposase (SOMMER *et al.* 1988).

We are therefore able to present two possible models for the formation of the deletion in *niv*$^{-}$*:529*. These are not mutually exclusive when considering the formation of deletions by transposons in general. The simplest involves the recognition by the Tam*3* transposase of the sequence at *x* as the inverted termini of a copy of Tam*3* and causing breakage. Breakage also occurs at the 3′ end of Tam*3* of *nivea*. This could be followed by ligation between Tam*3* and *x* with the intervening fragment being lost. Alternatively the deletion could have been formed by an aberrant transposition of Tam*3* from *nivea*. Breakage could occur at the 3′ end of the element and the free end could invade at *x*. If there was no breakage and transfer from the 5′ end of the element ligation could occur between Tam*3* and *x*, with loss of the intervening fragment.

Tam*3* is able to generate new mutant phenotypes at a high frequency. That is, by actual insertion into a gene, resulting from a successful transposition but also through aberrant transpositions where large regions of the chromosome may be rearranged or lost. Transposable elements are being developed as genetic tools. Transposon tagging is now well established as a mechanism for cloning genes, but transposable elements may also be used to generate an ordered sequence of relatively large deletions, *in vivo*, which can be used for chromosome mapping and the identification of genes flanking a known gene. Tam*3* has induced a number of large deletions at the *nivea* locus,

including *niv⁻:529*, which is known to delete an essential gene. Analysis and cloning of the sequences delimiting these deletions and examination of phenotypic effect of such deletions should provide considerable information about the chromosomal region around the *nivea* locus.

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