

## P element excision in *Drosophila melanogaster* and related drosophilids

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**Summary.** The frequency of P element excision and the structure of the resulting excision products were determined in three drosophilid species, *Drosophila melanogaster*, *D. virilis*, and *Chymomyza procnemis*. A transient P element mobility assay was conducted in the cells of developing insect embryos, but unlike previous assays, this mobility assay permitted the recovery of excision products from plasmids regardless of whether the excision event was precise or imprecise. Both quantitative and qualitative differences between the products of excision in the various species studied were observed. The frequency with which P element excision products were recovered from *D. melanogaster* was 10-fold greater than from *D. virilis* and *C. procnemis*; however, the proportion of all excision events resulting in the reversion of a P-induced mutant phenotype was the same. Virtually all excision products recovered, including those resulting in a reversion of the mutant phenotype, did not result in the exact restoration of the original target sequence. Sequence analysis suggested that duplex cleavage at the 3' and 5' termini of the P element, or their subsequent modification, occurred asymmetrically and interdependently. P element-encoded transposase was not absolutely required for P element excision.

**Key words:** Transposable elements – P elements – Excision – *Drosophila melanogaster* – drosophilids

### Introduction

P elements from *Drosophila melanogaster* have been well characterized both genetically and structurally, yet little is known about the mechanism(s) by which these transposable elements move (for a review see Engels 1989). For example, P elements are capable of excision and transposition yet the relationship between these pro-

cesses remains unclear. Two general mechanisms for P element transposition have been considered. First, P elements may transpose via a conservative mechanism whereby the element is cut from its resident site and inserted into a second site. Alternatively, the element may undergo replication with subsequent transposition of the copy. It has been argued that conservative transposition would require the precise excision of P elements and that the frequency of new P element insertions should be less than or equal to the frequency of precise excision. Estimates of the frequency of precise excision have been less than the transposition frequency and therefore the two processes were not considered to be mechanistically related (Engels 1989). Difficulties with this interpretation arise when one considers the definition of a precise excision and the way in which they are detected. Precise excisions have often been defined as events that result in the phenotypic restoration of a P-induced mutation. In many cases such a reversion results from not just the removal of the P element but also the 8 bases that were duplicated during the initial integration. Therefore, based on structural criteria this is not a precise excision of a P element but a precise restoration of the target sequence. A precise excision of a P element, based on structural criteria, is expected to leave both copies of the 8 bp duplicated target sequence behind. If the original insertion occurred in an open reading frame of a gene the additional 8 bases would result in a frame shift and the excision product would not be recovered as a phenotype reversion. Furthermore, without recovering and sequencing excised P elements it is not possible to deduce their structure from the sequence of the "empty" target site. An imprecise excision resulting in P element sequences remaining at the target site may have arisen as a result of staggered double-stranded cleavages at the P/target junction resulting in terminal single-stranded overhangs. Subsequent repair of the terminal unpaired nucleotides could result in the restoration of a complete excised P element and the retention of P sequences in the insertion site. Genetic evidence reported by Steller and Pirrotta (1986)

and Hawley et al. (1988) have suggested that conservative transposition of P elements (requiring excision) can occur, although other explanations could not be ruled out. Engels et al. (1990) have recently proposed a model for the conservative transposition of P elements which accounts for the disparity between the observed precise excision and transposition rates and for a number of other features of P element-mediated hybrid dysgenesis.

In addition to our incomplete understanding of the relationship between P element excision and transposition we do not know the precise role of the P element-encoded "transposase", or the role, if any, of host-encoded factors in the excision/transposition process. An important step in elucidating the mechanism(s) by which P elements excise and transpose is the analysis of the products of these reactions. Here we report the results of a sequence analysis of P excision products collected from three drosophilid species, *D. melanogaster*, *D. virilis*, and *Chymomyza procnemis*. The major excision products recovered in the species studied were similar but the frequencies with which they were observed were different. In addition, evidence is presented which indicates that P element transposase is not absolutely required for P element excision, although its presence greatly facilitates this process.

## Materials and methods

### Insects

*Drosophila melanogaster* *Adh*<sup>fn23</sup> *cn*; *ry*<sup>506</sup>. This is an M strain devoid of endogenous P element sequences and capable of supporting P element movement. This stock has been used previously in the P element excision assay described below (O'Brochta and Handler 1988).

*Drosophila virilis*. The flies used in this study originated from a wild-type strain originally obtained from the Bowling Green *Drosophila* Species Stock Center (Bowling Green, Ohio, USA; stock number 150101051). P element sequences have not been detected in *D. virilis* (Stacey et al. 1986) and Southern analysis of genomic DNA from the insects used in this study, using the complete P element as a probe under high stringency wash conditions, confirmed these observations (D.A. O'Brochta, unpublished results).

*Chymomyza procnemis*. These flies were originally obtained from the Bowling Green *Drosophila* Species Stock Center (stock number 200002631). As with *D. virilis*, Southern analysis of genomic DNA from these insects also failed to detect endogenous P-homologous sequences (D.A. O'Brochta unpublished results).

### Excision assay

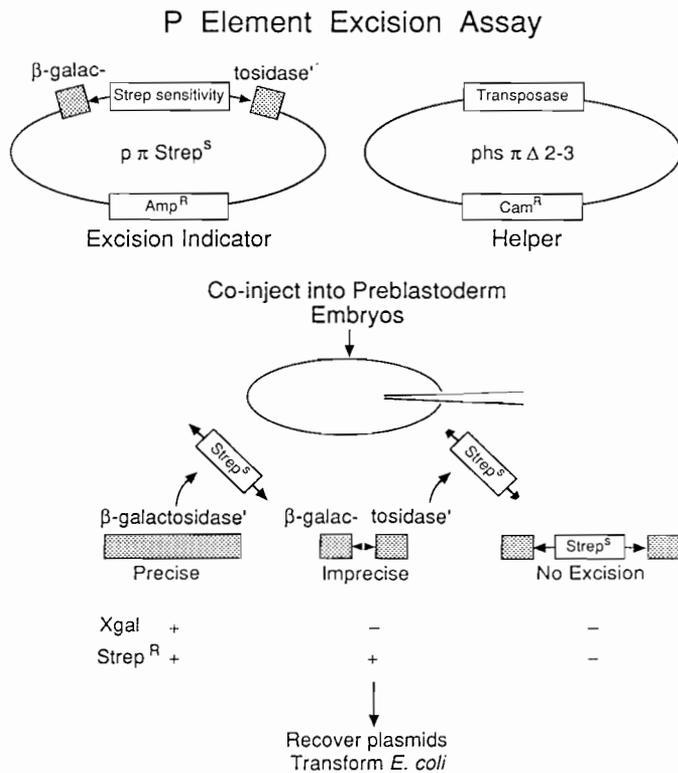
*Plasmids*. The P element mobility assay used in these experiments was essentially as described (Rio et al. 1986; O'Brochta and Handler 1988; Fig. 1); however, the original excision indicator plasmid, pISP-2, which permitted detection of excisions only after the restoration of the LacZ  $\alpha$ -complementing ability of the vector (Rio et al.

1986), was modified to allow virtually all excision events to be detected and recovered. The modification involved the insertion of the *Escherichia coli* S12 ribosomal protein gene, which confers sensitivity to streptomycin, into the *XmnI* site of the non-autonomous P element in pISP-2 (Rio et al. 1986), to create a new indicator plasmid, p $\pi$ strep<sup>s</sup>. The insertion site is 450 bp from the 3' terminus of the P element and is known not to be essential for P element mobility (O'Hare and Rubin 1983; Mullins et al. 1989). The S12 gene was removed from the plasmid pNO1523 (Dean 1981) as a *Bam*HI-*Nru*I fragment and inserted into pISP-2 after the ends had been filled using the Klenow fragment of DNA polymerase I. Certain mutations in the S12 gene result in streptomycin resistance; however, expression of a non-mutated gene *in trans* in a resistant host can restore the original streptomycin-sensitive phenotype (Dean 1981). Therefore, in the appropriate host grown in the presence of streptomycin, the S12 gene acts as a dominant lethal locus. Excision indicator plasmids which have lost the S12 gene due to precise or imprecise P element excision are recovered in streptomycin-resistant hosts when selected on streptomycin and ampicillin (Fig. 1). The plasmid pACYChs $\pi$  $\Delta$ 2-3 was used as a helper plasmid to provide P element transposase (Laski et al. 1986; O'Brochta and Handler 1988). This plasmid contains a modified transposase gene which, when expressed, results in functional P element transposase in somatic as well as germ tissue.

*Assay*. The plasmids p $\pi$ strep<sup>s</sup> (0.5 mg/ml) and the P element transposase-encoding helper plasmid pACYChs $\pi$  $\Delta$ 2-3 (0.5 mg/ml) were coinjected into preblastoderm embryos. The injected embryos were incubated at 23° C in an oxygen saturated atmosphere for approximately 18 h and heat-shocked for 1 h at 37° C, followed by a 1 h recovery at 23° C in the presence of oxygen. Injected plasmids were recovered as described by O'Brochta and Handler (1988) and introduced into the *E. coli* strains MC1009 [(*lacI*POZYa)X74, *galU*, *galK*, *strA*, (*ara*, *leu*)7697, *RecA*] (cells obtained from Pharmacia, Piscataway, New Jersey) or JM83 [*ara*, $\Delta$ (*lac-proAB*), *rspL*,  $\Phi$ 80, *lacZ* $\Delta$ M15, (*r*<sup>+</sup>, *m*<sup>+</sup>)] (Yanisch-Perron et al. 1985), both of which are streptomycin-resistant as a result of a mutation in the S12 locus (denoted as *strA* or *rspL*, respectively). Transformants were selected on LB containing ampicillin (75  $\mu$ g/ml) and streptomycin (100  $\mu$ g/ml). Transformants only containing pACYChs $\pi$  $\Delta$ 2-3 are ampicillin-sensitive and were not recovered under these selection conditions. Amp<sup>r</sup> Strep<sup>r</sup> transformants containing p $\pi$ strep<sup>s</sup> plasmids with detectable deletions, as determined by supercoiled plasmid mobility in agarose gels, were retained and their plasmids sequenced. Double-stranded DNA sequencing was done using Sequenase<sup>TM</sup> according to the manufacturer's specifications (US Biochemicals, Cleveland, Ohio).

## Results

Using a total excision assay (Fig. 1), which permits the recovery of a wide range of P element excision products,

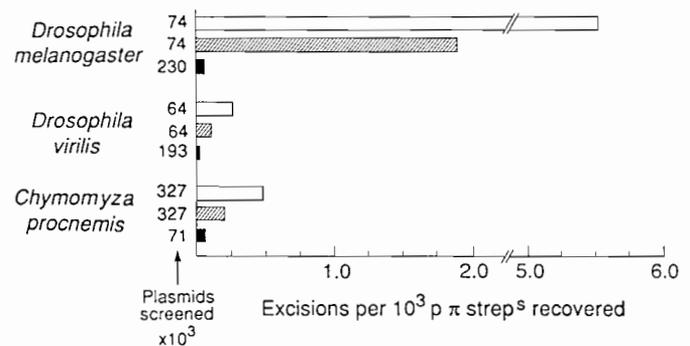


**Fig. 1.** P element mobility assay permitting the detection and recovery of P element excision products resulting from precise or imprecise excisions. The assay involves the coinjection of the excision indicator plasmid  $p\pi\text{strep}^s$  and P element transposase-encoding  $p\text{ACYChs}\pi\Delta 2-3$ . Plasmids are subsequently recovered from late stage embryos and excision products recovered as streptomycin- and ampicillin-resistant *Escherichia coli* transformants

we determined the frequency of P element excision from plasmids and the sequence of the resulting products recovered from three drosophilid species, *D. melanogaster*, *D. virilis*, and *Chymomyza procnemis*. None of the strains used contain endogenous P element sequences based on Southern analysis of genomic DNA (Stacey et al. 1986; D.A. O'Brochta unpublished results).

### Frequency of P excision

P elements were found to excise from plasmids transiently maintained in the embryonic soma of *D. melanogaster* at a frequency of  $5.5 \times 10^{-3}$  (Fig. 2). Approximately 35% of these resulted in the phenotypic reversion of the mutated lacZ  $\alpha$ -peptide present on the excision indicator plasmid. In drosophilids distantly related to *D. melanogaster* the frequency of P element excision was much lower,  $0.26 \times 10^{-3}$  in *D. virilis* and  $0.47 \times 10^{-3}$  in *C. procnemis*. As in *D. melanogaster*, excisions resulting in the reversion of the lacZ  $\alpha$ -peptide mutation composed a minority of the products, 39% in *D. virilis* and 42% in *C. procnemis*. In the three species tested, P element excision products were recovered from embryos in which the P element transposase-encoding helper plasmid had not been injected. In *D. melanogaster* and *C.*



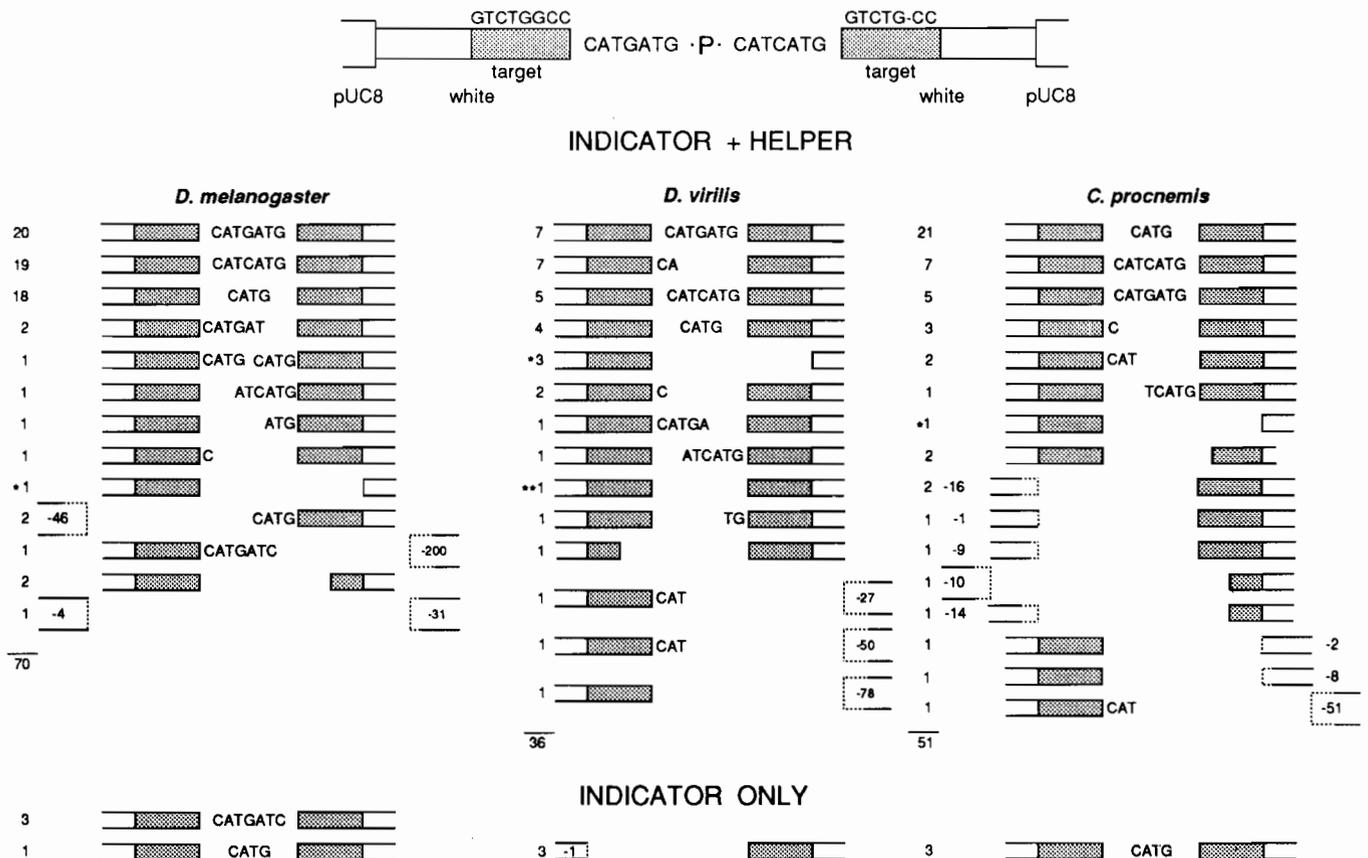
**Fig. 2.** Frequency with which excision products were recovered from *Drosophila melanogaster*, *D. virilis* and *Chymomyza procnemis*. Open bars indicate the total frequency of excisions observed when the transposase-encoding plasmid  $p\text{ACYChs}\pi\Delta 2-3$  was coinjected with  $p\pi\text{strep}^s$ . Shaded bars indicate the frequency of excisions which resulted in a restoration of lacZ  $\alpha$ -complementation to  $p\pi\text{strep}^s$ . Solid bars indicate the frequency of excisions which occurred in the absence of  $p\text{ACYChs}\pi\Delta 2-3$ . The numbers adjacent to each bar indicates the number of thousands of  $p\pi\text{strep}^s$  plasmids screened and represents the pooled results of at least three replicates of each experiment

*procnemis* transposase-independent excisions were recovered at a frequency of  $0.06 \times 10^{-3}$  while in *D. virilis* the frequency was  $0.02 \times 10^{-3}$  (Fig. 2). Control experiments where the excision indicator plasmid was directly introduced into *E. coli*, in both the presence and absence of  $p\text{ACYChs}\pi\Delta 2-3$ , did not yield any P element excision events after screening  $6.5 \times 10^5$  plasmids. This indicated that all excision products recovered in the experimental samples were generated in the injected embryos.

### Sequence analysis of excision products

Sequence analysis of P element excision products recovered from *D. melanogaster*, *D. virilis*, and *C. procnemis* revealed both similarities and differences in the structure of the excision products recovered from these species. In the species tested, the majority of the products appeared to result from internal deletions of the P element (90% in *D. melanogaster*, 78% in *D. virilis*, 76% in *C. procnemis*) whereby most of the P element excised leaving both copies of the duplicated 8 bp target site and up to 8 nucleotides from the termini (Fig. 3). While there was a large degree of similarity in the structure of excision products resulting from internal deletions of the P element among species, the relative frequency of specific products differed. Excision events which precisely restored the original target sequence as a result of excision of the P element and one copy of the 8 bp target site duplication were uncommon (1% in *D. melanogaster*, 8% in *D. virilis*, 2% in *C. procnemis*). Likewise, precise excision of the entire P element (leaving both copies of the 8 bp duplication) was observed only once after sequencing 157 excision products (Fig. 3).

Another class of excision products resulted from the complete or almost complete excision of the P element and sequences flanking the 8 bp target duplication (6%



**Fig. 3.** Summary of the excision products recovered in this study. Partial nucleotide sequence of  $\pi\text{strep}^{\text{R}}$  is shown and consists of four regions: the P element, the 8 bp duplicated target sites, the sequence surrounding the target sites and vector sequences. The 3' duplicated 8 bp target site has a single base deletion at the position indicated by the dash. This deletion was present in (unpublished observation), the parent plasmid of  $\pi\text{strep}^{\text{R}}$ ,  $\pi\text{ISP-2}$  (Rio et al. 1986), and was probably generated during construction of this plasmid. The P element is inserted in a short, approximately 50 bp, segment of the *D. melanogaster* white gene (Rio et al. 1986). Excision products containing sequences from the termini of the P element but the origin of which, i.e. 3' or 5', is unknown are shown with the remaining P element sequences equally spaced be-

tween the duplicated target sites. When the origin of the P element sequences left at the donor site could be confidently determined their position is indicated. Deletions of target sequences are indicated by a shaded bar of reduced length. Deletions extending into the white gene sequences flanking the 8 bp target site are indicated by broken lines and the number of bases deleted from this region is indicated. Similarly, deletions extending into the vector (pUC8) are indicated with broken lines and the size of the vector deletion is indicated. Numbers adjacent to each product indicate the number of products recovered with this sequence. A single asterisk denotes an excision product with an exact restoration of the original target sequence. A double asterisk denotes an excision product resulting from the precise removal of the entire P element

in *D. melanogaster*, 8% in *D. virilis*, 18% in *C. procnemis*). While this class constituted 10% of all excision products recovered from the three species, large deletions resulting in the removal of essential sequences required for plasmid replication or deletions removing both DNA sequencing primer sites were excluded. Therefore, these estimates should be considered minimum values. Some of the excision products had one of the deletion breakpoints in flanking DNA sequences located tens or hundreds of bases from the P element terminus. While these breakpoints occurred proximal to the 5' or 3' ends of the P element, the second deletion breakpoint was usually at or near the opposite P/target site junction. Only one excision product ( $n=157$ ) arose as a result of both deletion breakpoints occurring in sequences flanking the P element (Fig. 3). It should be realized that the excision products sequenced may have arisen simply from the removal of sequences followed

by immediate religation of the gapped target sequence, or ligation may have been preceded by end-processing steps which could either add or remove nucleotides.

Because the termini of the P element are perfect 31 bp inverted repeats, the precise location of the excision breakpoints for most (106/157) of the recovered products remained uncertain. For example, the most common excision product recovered from *D. melanogaster* was an internal deletion of the P element leaving the sequence CATGATG. While it is clear that these seven nucleotides were from the termini of the P element the precise location of the original deletion breakpoint is ambiguous. Any of the following excision breakpoints is consistent with the structure of the observed product: CATG/ATG; CATGA/TG; CATGAT/G; CATGATG/.

For approximately 30% (51/157) of the excision products, however, the breakpoints can be confidently located. In most of these products (50/51) the deletion

breakpoints were asymmetrically located with respect to the P element termini. An analysis of these excision products revealed some aspects of P element excision which may provide insight into the mechanism by which this process takes place. One common characteristic of the excision breakpoints was that the preferred breakpoint was at the junction of the terminus of the P element and the flanking target sequence. Eighty-four percent ( $n=51$ ) of the excision products within this class had a breakpoint at either the 5' or 3' P/target junction, occurring with almost equal frequency at the 5' or 3' termini (35% and 47%, respectively). Rarely were excision products recovered which had a breakpoint at both the 5' and 3' P/target junctions (1/157).

Transposase-independent excision of the P element resulted in excision products which were indistinguishable from those recovered in the presence of P element transposase (Fig. 3). In the case of *D. melanogaster* and *C. procnemis* the products recovered were identical to the most common class of products recovered in the presence of P element transposase. While these events were rare they suggest that P element transposase was not absolutely essential for P element excision.

## Discussion

The P element from *D. melanogaster* is one of the best characterized eukaryotic transposable elements. Interest in the mechanisms responsible for P element movement and the regulation of that movement stems not only from a desire to understand the biology of these interesting genetic elements but also from the desire to employ these elements as gene vectors in insects other than *Drosophila* (Handler and O'Brochta 1991). Thus far there has been no reported evidence for P element mobility following their introduction into nondrosophilid species (O'Brochta and Handler 1988). Elucidating the mechanisms of P element movement and regulation should facilitate identification of the basis for P element dysfunction in nondrosophilids. The results reported here provide information relevant to understanding the mechanism of P element excision by determining the frequency of excision and the structure of the resulting products recovered from *D. melanogaster* and two distantly related species.

### Frequency of P element excision

The structure of the P element excision products and the frequency with which they were recovered from various drosophilid species indicated that the host's cellular environment influenced both the number and type of excision products recovered. In general, the processes associated with P element excision in the three species tested appeared to be similar based on the common classes of excision products observed. Notably, P element excision resulted almost exclusively in an imprecise restoration of the original target sequence. While imprecise excision occurred frequently, based on the structure

of the "empty" target site, 35%–40% of the excision products resulted in the phenotypic reversion of the P element-induced mutant phenotype. These results are in general agreement with those of Daniels et al. (1985) who estimated, using a sensitive genetic test, that at least 75% of the P element excisions recovered were imprecise based on restriction map analysis. Because of the limitations of this method their estimates of imprecise excision rates were assumed to be minimum values. Using less sensitive genetic methods, Voelker et al. (1984) estimated a minimum value of imprecise P element excision to be 35%. The proportion of imprecise excision events which result in reversion of a mutant phenotype is expected to vary depending on the gene that contains the P element and the location of the element within the gene. In the experiments reported here approximately one-third of the excision products containing P element sequences after excision resulted in the reversion of the P-induced mutant phenotype.

Differences in the frequency with which revertants of the plasmid-borne, P-induced mutation were recovered from various drosophilid species have been reported (O'Brochta and Handler 1988). Because the assay originally used to monitor P element mobility only permitted excision products resulting in phenotype restoration, it was not known if the differences observed between species reflected differences in the total frequency of excision or differences in types of excision products (O'Brochta and Handler 1988). The total excision frequency in the species tested may have been equal; however, species distantly related to *D. melanogaster* may have supported a greater proportion of imprecise excisions. The results reported here indicate that this was not the case. Despite the phylogenetic distance between the species studied, imprecise excision products were no more common, relative to the total number of excisions, in *D. virilis* and *C. procnemis* than in *D. melanogaster* based upon either genetic or structural criteria. The observed differences in the frequency and type of the excision products recovered may reflect differences in the action of transposase and/or differences in subsequent steps which might include processing of the ends of the cleaved duplex (perhaps by exonucleases or repair enzymes) and religation of the ends. We reason that because there is no evidence for post-cleavage processing of the ends of the excision products, and no reason *a priori* to assume there is a difference in the ability of these drosophilids to repair gapped plasmid molecules, that the observed differences in the type and number of excision products reflect differences in transposase activity.

### Excision product structure

Internal deletions of the P element comprised the majority of excision products in all species studied. This resulted in a small number of nucleotides from the termini of the P element (1–8 bp) being left at the insertion site, as well as both copies of the 8 bp of duplicated sequence created upon initial insertion of the P element. Similar

excision products were reported by Rio et al. (1986) using a related plasmid excision assay; however, few comparisons with the data reported here can be made since their excision products were recovered from insect cell lines, their selection system permitted only the recovery of products resulting in the restoration of an open reading frame, and their sample size was small. Tsubota and Schedl (1986) and Daniels et al. (1985), after recovering chromosomes from which a P element excised also found frequent internal deletions of P. These resulted in as few as 8 or as many as 40 bp of the P termini being left in the insertion site.

The terminal 31 bp of both the 5' and 3' ends of the P element are identical and as a result the precise location of the excision breakpoints could be identified in only 30% of the recovered products. It should be noted that the observed breakpoints may have arisen not only from cleavage of the duplex followed by religation but potentially from modification of the ends prior to ligation. Within this class of product a high degree of breakpoint specificity was observed, with 84% of the products having at least one breakpoint precisely at the P/target junction. Rarely (1/157) did both the 3' and 5' breakpoints occur at the P/target junction suggesting that there are two interdependent steps in excision breakpoint determination, one highly specific and the other less so. To account for the apparent lack of independence in 3' and 5' breakpoints we propose that the termini of the P element are interacting during the cleavage/processing steps. One way the ends could interact is by synapsis, since the sequence identity of the termini of the P element, in addition to the repeated 8 bp target sequence flanking the termini, could facilitate such interaction.

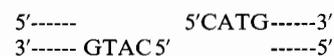
Ten to twenty percent of the excision breakpoints that could be confidently identified extended beyond the 8 bp target duplication and in some cases as much as 200 bp beyond the target site. Previous reports of deletions associated with P element excision have revealed sequence losses ranging from 1 to 6 kb and larger based on structural analysis (Tsubota and Schedl 1986; Salz et al. 1987). Deletions of this magnitude were excluded from this study because they would have either removed both sequencing primer sites or sequences essential for plasmid replication, thereby preventing the recovery of the excision products.

#### *Mechanisms for P mobility*

Engels et al. (1990) have proposed a model of P element transposition whereby a P element is excised from a site and reintegrated elsewhere. Following excision the resulting double-stranded gap is repaired and, depending on whether or not a homolog is present and its structure, double-stranded gap repair could result in the restoration of the original nonmutated target sequence, a complete restoration of the P insertion sequence, or an imperfect restoration of the original target sequence, usually containing a portion of the P termini. The P element excision assay described in this report most closely re-

sembles the situation described by Engels et al. (1990) in which the P element insertion mutation is not associated with a homolog. In this case, gap repair involving the use of a homolog as a template, as proposed by Engels et al. (1990), cannot occur and the gapped duplex resulting from P element excision is simply religated. Religation may be preceded by modification of the ends of the duplex; however there is no evidence for the occurrence of such modification during excision in *Drosophila*. The rare occurrence of excision products with precisely restored target sequences under these conditions is consistent with the predictions of the 'gap-repair' model. Furthermore, the frequency of excision observed using the plasmid excision assay is in general agreement with that observed by others in the absence of a homolog (Rubin and Spradling 1982; Woodruff et al. 1987; Engels et al. 1990). The plasmid excision assay, therefore, permits the initial steps in the excision reaction, such as duplex cleavage, to be observed in isolation of template-mediated gap repair and appears to reflect the behavior of P elements residing in chromosomes.

The data presented in this report is largely consistent with two general modes of duplex cleavage. The initial cleavage of the duplex may occur symmetrically at each terminus resulting in a 4 bp staggered cut with either a 3' or 5' overhang:



Religation of the ends would lead to the product ----CATG----, which comprises the most frequent class of product amongst the pooled data. Minor modifications of the 4 bp overhang followed by religation could result in a number of the other observed products. For example, filling the overhangs followed by the terminal deletion of a single base pair would result in the products ----CATGATG---- and ----CATCATG----. Note however that because of the symmetry of the P element termini the precise location of the duplex cleavage sites cannot be determined for many of the products recovered. In addition the mode of cleavage just proposed does not adequately explain the asymmetry of the duplex cleavage sites discussed below.

In those cases where the excision breakpoints can be confidently located there is a distinct asymmetry in their location. Again, this may reflect asymmetry in the choice of initial cleavage sites or asymmetry in any subsequent end-processing. Models involving asymmetrical cleavage sites and blunt-end cleavages could also account for the observed products. For example, one duplex cleavage event may occur precisely at the P/target junction while the second cleavage occurs with less precision, and rarely at the other P/target junction. As already discussed, synapsis of the P element termini during duplex cleavage could account for the observed interdependence of 3' and 5' cleavage events. The observation by Mullins et al. (1989) that the 3' and 5' termini of the P element are not interchangeable also supports the idea that the termini are not acted upon in an identical

manner during excision/transposition. Recovery and sequence analysis of the excised P element will be crucial in distinguishing modes of duplex cleavage.

Although the precise steps in the excision/transposition process remain unclear it has been thought that P element-encoded transposase was absolutely required. The data presented here demonstrate that P element excision can occur in the absence of transposase since excision products were recovered from embryos injected only with the excision indicator plasmid. Hence, transposase may not be directly involved in the initial duplex cleavages, although its presence facilitates the process. For example, the role of transposase may be one of configuring P element sequences to promote synapsis of the termini allowing endogenous cellular recombination machinery to function. This proposed role of transposase is consistent with the observed sequence-specific binding of transposase to P element DNA *in vitro*. Transposase does not bind to the termini of P elements, where duplex cleavage appears to occur, but to internal P element sequences (Kaufman et al. 1989). Furthermore, a non-P element-encoded protein has been found to bind specifically to the termini of P elements (Rio and Rubin 1988) and is more likely to be involved in duplex cleavage.

Transposase-independent excision has been reported for other transposable elements. For example, Tn10 from *E. coli* can excise in the absence of transposase. However, the mechanism used is believed to be distinct from transposase-mediated excision possibly occurring via template switching during DNA replication (Kleckner 1989). Unlike the situation with Tn10 the mechanism responsible for transposase-independent P element excision appears similar to transposase-mediated excision, based on the sequence of the products recovered in the absence of P element transposase.

An alternative explanation for the recovery of transposase-independent P element excision products is that there is a transposase-related protein in the species tested, perhaps encoded by another transposable element, which is capable of cross-mobilizing P elements at a low frequency. Thus far there is no evidence for cross-mobilization of transposable elements in *D. melanogaster* (Woodruff et al. 1987; Eggleston et al. 1988) although spontaneous (transposase-independent) excisions of P elements have been reported in this species (Osgood and Seward 1989).

P elements are structurally similar to the plant transposable elements Ac/Ds and Tam (Federoff 1989; Coen et al. 1989). That is, they are relatively small elements, unrelated to retroviruses, containing short terminal inverted repeats and cause small target-site duplications upon integration. There is evidence that both Ac and Tam transpose via a conservative mechanism with element excision being an intermediate step (Federoff 1989; Coen et al. 1989). Structurally, the excision products generated by Tam and Ac excision are distinct from those observed following P element excision. Excisions of Ac/Ds and Tam, unlike P elements, result in the complete removal of the elements, and occasionally include flanking DNA but usually leaving both copies of the duplicated target. Internal deletions of the elements are

uncommon. Excision of Ac/Ds and Tam elements also results in small inverted duplications of the target sequences. Similar excision products were never observed following P element excision. These results suggest that the mechanism by which these elements excise may be different.

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