Efficient Mobilization of *mariner in Vivo* Requires Multiple Internal Sequences

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ABSTRACT

Aberrant products of *mariner* excision that have an impaired ability to be mobilized often include internal deletions that do not encroach on either of the inverted repeats. Analysis of 13 such deletions, as well as 7 additional internal deletions obtained by various methods, has revealed at least three internal regions whose integrity is necessary for efficient *mariner* mobilization. Within the 1286-bp element, the essential regions are contained in the intervals bounded by coordinates 229–586, 735–765, and 939–1066, numbering in base pairs from the extreme 5′ end of the element. These regions may contain sequences that are necessary for transposase binding or that are needed to maintain proper spacing between binding sites. The isolation of excision-defective elements with point mutations at nucleotide positions 993 and 161/179 supports the hypothesis of sequence requirements, but the reduced mobility of transformation vectors with insertions into the *Sac*I site at position 790 supports the hypothesis of spacing requirements. The finding of multiple internal regions that are essential for efficient *mariner* mobilization *in vivo* contrasts with reports that mini-elements with as little as 43 bp of DNA between the inverted repeats can transpose efficiently *in vitro*.

MANY natural populations contain internally deleted copies of type II transposons (DNA transposons with inverted repeats). In Drosophila, the best known examples are the *P* element (Bingham *et al.* 1982; Black *et al.* 1987), the *hobo* element (Periquet *et al.* 1989; Pascual and Periquet 1991; Kim and Kim 1999), and *mariner* (Maruyama and Hartl 1991; Brunet *et al.* 2001). In the case of the *P* element, the internal deletions can arise as a product of abortive transposition. Transposition of the *P* element is mediated by the *P* transposase encoded within the element that functions in DNA cleavage and strand transfer. Transposition proceeds by a "cut and paste" mechanism (Kaufman and Rio 1992), and the resulting gap in the donor molecule is repaired by template-directed gap repair (TDGR), using as template the sister chromatid, the homologous chromosome, or an ectopic site (Engels *et al.* 1990; Gloor *et al.* 1991; Nassif *et al.* 1994). Incomplete repair by TDGR results in a class of *P*-element mutations that are internally deleted, but the terminal 16 ± 1 bp of the inverted repeats are rarely included in the deletions (Staveley *et al.* 1995). *In vitro* studies have shown that *P* transposase makes a staggered cut within the 31-bp inverted repeats, leaving the extreme 17 nucleotides of each *P*-element end single stranded (Beall and Rio 1997). The single-stranded DNA is bound by an inverted-repeat binding protein (IRBP; Rio and Rubin 1988). It has been proposed that IRBP binding protects the *P*-element termini from exonuclease degradation (Staveley *et al.* 1995), thereby explaining the retention of the terminal inverted repeats in the deletion derivatives.

Most of the internal deletions of the *P* element are missing all or part of the transposase-coding region and hence are unable to produce functional transposase. They are, however, able to be mobilized *in trans* by a *P* element that does encode a functional transposase. It is the mobilization of such defective *P* elements that results in efficient *P*-element-mediated insertional mutagenesis (Cooley *et al.* 1988a,b).

The generality of TDGR in transposition of inverted-repeat DNA transposons is supported by evidence from excision of the *mariner* element, a member of the *mariner/Tc*1 superfamily (Hartl *et al.* 1997; Hartl 2001). Homolog-dependent gap repair frequently accompanies *mariner* excision (Lohe *et al.* 2000). The ratio of excision events that leave a characteristic 5-bp footprint, compared to gap repair using a copy on the homologous chromosome, was estimated at 2.4:1 (Lohe *et al.* 2000). Therefore, at least 30% of all *mariner* excision events undergo TDGR.

On the other hand, recent experiments suggest that the details of the process of transposition leading to TDGR are quite different in the *P*-element and *mariner* systems. In a screen for aberrant products of excision of the *mariner* element denoted *peach*, we uncovered a high frequency of deletions near the termini of the inverted repeats (Lohe *et al.* 2000). The *peach* element is a *mariner* element that is inserted into the 5′ untrans-

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lated region of the *white* gene in the *wpch* allele. The insertion changes the phenotype from bright red to peach (Jacobson *et al.* 1986). The *peach* element does not produce a functional transposase, but it can be excised efficiently in response to active transposase supplied *in trans*. Somatic activity of the transposase in *wpch* flies results in a mosaic eye color consisting of red sectors on a peach background. Germline transposase activity results in excision of *peach* and restoration of the wild-type *white* gene function in the progeny (Bryan *et al.* 1987). When under control of the dual *hsp70::Mos1* promoter, the *Mos1 mariner* transposase is strongly expressed in the germline and soma even in the absence of heat shock. In flies carrying the *wpch* allele, *hsp70::Mos1* induces high levels of germline reversion of *peach* and extreme mosaicism in the eyes (Garza *et al.* 1991).

In the presence of *hsp70::Mos1* transposase, the frequency of excision-defective mutations in peach is ~0.2% per exposed *wpch* allele per generation. The most frequent aberrations (51/68 events) could be explained by exonuclease degradation of the inverted repeats during host-mediated repair of a double-stranded gap made by transposase at either the 5′ junction or the 3′ junction of the *peach* element and genomic DNA. An analogous class of mutations has not been reported to arise from *P*-element transposition (Staveley *et al.* 1995).

In this article we report on 20 internal deletions in *peach* obtained by a variety of methods, ranging in size from 31 to 899 bp. The deletions overlap in such a way as to include all sequences internal to the inverted repeats. The fact that *peach* elements with a variety of internal deletions have an impaired ability to excise in the presence of active transposase seems to imply that, *in vivo*, the *mariner* transposase, or some complex between the *mariner* transposase and one or more host factors, must interact with internal sequences to catalyze efficient excision. Furthermore, from the overlaps of the deletions we can identify three distinct internal regions that are required for efficient excision, either because they contain essential binding sites or because their presence is needed to maintain the correct spacing between binding sites. A model requiring correct spacing is supported by evidence in a companion article (Lozovsky *et al.* 2001), which shows that insertions of exogenous DNA at any of a number of internal sites also impair *mariner* mobility.

MATERIALS AND METHODS

The *peach*/*Mos1* two-element system: Although the *peach* and *Mos1* elements both have an uninterrupted open reading frame, they differ at 11 nucleotide positions out of 1286 bp, and only *Mos1* encodes a functional transposase. Both elements can excise and transpose in response to *Mos1* transposase, which is active in the soma as well as the germline. The insertion of *peach* into the 5′ untranslated region of the *white* gene alters the eye color from red to peach (Jacobson *et al.* 1986). With *Mos1* in the genetic background, the *peach* element excises and white gene function is restored. Excision of *peach* in ommatidia results in red spots or sectors on a peach background in the eye, and *peach* excision in germline cells results in full restoration of the wild-type red eye color in the progeny (Bryan *et al.* 1987). The degree of somatic mosaicism is a semiquantitative assay for the rate of *peach* excision and correlates well with the rate of germline excision.

The *wpch* allele was originally discovered in *Drosophila mauritiana* (Jacobson *et al.* 1986) but was transferred onto the X chromosome of *D. melanogaster* by cloning and *P*-element transformation (Garza *et al.* 1991). Thus, in *D. melanogaster*, the *peach* element is part of a *P*-element construct that is a composite of sequences from the *D. melanogaster white* gene and the *D. mauritiana wpch* allele (Garza *et al.* 1991).

The *wpch* eye-color mosaicism screen: A mutagenesis screen for *peach* mutations utilized the two-element *peach*/*Mos1* system with the *wpch* allele as a scorable marker for *Mos1*-induced *peach* excision leading to eye-color mosaicism (Lohe *et al.* 2000). The source of transposase was not *Mos1* itself but an *hsp70::Mos1* construct that is unable to be mobilized because it lacks the 5′ inverted repeat (Lohe *et al.* 1995). In the absence of functional transposase, the *wpch* allele yields a uniform peach-colored phenotype and there is no somatic mosaicism. In the presence of *hsp70::Mos1*, even without heat shock, somatic excision of *peach* restores wild-type white gene function and the eye phenotype is mosaic with hundreds of red ommatidia per eye on a peach background (Lohe *et al.* 1995). The particular *hsp70::Mos1* construct used is designated *Mr182*, which consists of a *P*-element transformation vector containing *hsp70::Mos1* inserted in chromosome 2 (Lohe *et al.* 1995). New mutations in the *wpch* allele affecting the ability of the *peach* element to excise were identified by a decrease in the level of eye-color mosaicism. Germline reversion rates of *wpch* were measured to verify that *peach* excision was also reduced in the germline and that the eye color of the revertants was wild type. Mutagenized *wpch* males were crossed to 10–15 *y* w^-; *Mr182*/*Mr182* females in bottles, and female progeny were scored for somatic mosaicism. Stocks of *wpch* mutants were each made from a single male using the *FM7a* balancer chromosome, and *Mr182* was immediately removed from the genetic background.

Methods of mutagenesis: Three mutagenesis schemes were employed.

1. *Mos1* mutagenesis was carried using the *Mr182 hsp70::Mos1* construct as described above, selecting for mutants of *wpch* that showed markedly reduced levels of *peach* excision.
2. *P*-element mutagenesis made use of the fact that the *wpch* allele is present in a *P*-element vector and that excision of a *P* transposon and aberrant template-directed gap repair can result in internal deletions in the newly synthesized copy (Gloor *et al.* 1991). With the *wpch* allele, internal deletions or other mutations in the *peach* element can be recovered. In the present experiments, the stable source of *P* transposase was the *PΔ2–3(99B)* element (Robertson *et al.* 1988). Before commencing the experiments, we verified that the *P* transposase does not mobilize the *peach* element even at low levels (data not shown).
3. Chemical and radiation mutagenesis made use of EMS (ethyl methanesulfonate), DEB (diethylbutane), or X rays. EMS mutagenesis was carried out as described (Lohe *et al.* 1996). About 100 crosses were set up with 4–5 mutagenized *wpch* males and 10–15 *y* w^-; *Mr182*/*Mr182* females per bottle, and ~20,000 female progeny were scored for somatic mosaicism. From this screen, 13 *wpch* mutants were recovered with reduced somatic mosaicism and low germline reversion rates. DEB mutagenesis was carried out using a protocol similar to the EMS mutagenesis, except that the concentration of DEB was 10 mm (Crosby and Meyero-

witz 1986). This resulted in a high loss of males and only ~4000 female progeny were screened, which yielded 4 *wpch* mutants. For X-ray mutagenesis, 300 males (4–5 days old) were exposed to 4000 R from a cesium-137 source kindly provided by the CSIRO Division of Entomology. Groups of 4–5 mutagenized *wpch* males were crossed with 10–15 *y w*$^{-}$; *Mr182/Mr182* females per bottle. Approximately 6000 female progeny were screened for somatic mosaicism and 4 *wpch* mutants were recovered.

Molecular analysis: Analysis of *wpch* mutants and DNA sequencing were carried out using primers and PCR amplification of genomic DNA from single flies, as described (Lohe *et al.* 2000).

RESULTS

Internally deleted *peach* elements produced by the *mariner Mos1* transposase: In the *peach/Mos1* two-element system, the *peach* element is tested for its ability to excise by means of a phenotypic screen for the observed level of eye-color mosaicism in *wpch* flies. This screen appears to be relatively free from biases in phenotypic selection, because the selection assays the ability of the target element to excise, not the phenotypic effect of the excision on a nearby gene. In the analysis of mutant *peach* elements arising from aberrant effects of the *Mos1* transposase, two major classes of mutants were recovered. One class consisted of relatively small deletions at the extreme termini of either the 5′ or the 3′ inverted repeat, though primarily affecting the 5′ inverted repeat (Lohe *et al.* 2000), and the other class consisted of the internal deletions in the element reported here. Among 68 mutant *peach* elements recovered in the *peach/Mos1* screen, 13 proved to have internal deletions.

The internal deletions resulting from aberrant effects of *Mos1* transposase are listed in Table 1, along with internal deletions from other sources described below. Although most of the *Mos1*-associated aberrations are simple deletions, a few also had a few nucleotides of "filler" DNA sequence inserted between the breakpoints, similar to some of the terminal deletions reported earlier (Lohe *et al.* 2000). Among the internal deletions the length of the filler sequence was 1 bp (Δ*16R* and Δ*57R*), 2 bp (Δ*49R* and Δ*J2*), 8 bp (Δ*72R*), or 16 bp (Δ*35R*); all are much shorter than the length of the deletion.

Among the 13 *Mos1*-associated deletions, only Δ*99R* contained a deletion endpoint that removed part of one of the inverted repeats. This deletion is 705 bp in length and extends from nucleotides 577–1281, leaving only the terminal 6 bp of the 3′ inverted repeat intact. Not surprisingly, Δ*99R* shows no somatic mosaicism.

Unexpectedly, while none of the remaining 12 internal deletions encroaches on either of the terminal inverted repeats, their effects on *peach* excision are profound. The level of somatic mosaicism is reduced by at least a factor of 10, and in many cases somatic mosaicism is no longer observed.

Internally deleted *peach* elements produced by *P*-element transposase: It is possible that the internal deletions produced by the *mariner* transposase may be nonrandom in such a way as to yield a high frequency of *peach* derivatives with a drastically impaired ability to excise. For example, the breakpoints may be in regions in which there are essential contacts between the target element and the transposase. To evaluate this possibility we also examined mutant *peach* elements generated by mechanisms unrelated to the *mariner* transposase, and once again we found internally deleted elements.

Table 1 lists five mutant *peach* elements generated from aberrant effects of the *P*-element transposase. The *wpch* allele is present in a *P*-element construct that contains the *peach* element inserted into the 5′ untranslated region of the *white* gene in a *P*-element vector (Garza *et al.* 1991). The action of *P* transposase on the *wpch* allele should induce template-directed gap repair and result, on rare occasions, in internal deletions within the transposon including all or part of the *peach* element. Accordingly, crosses were carried out with *wpch* in the same genome as a stable source of *P*-element transposase (*PΔ2–3*), and the *wpch* progeny were tested for mosaicism by crossing to a strain carrying *hsp70::Mos1*. In this test, defective *peach* elements induced by the *P* transposase could be distinguished from the rare defective *peach* element produced by the *Mos1* transposase, because in the former case (*P* element) all of the progeny show reduced mosaicism, whereas in the latter case (*Mos1*) only ~1/500 progeny shows a mutant phenotype.

This experiment yielded five *wpch* mutants with reduced or absent somatic mosaicism, in which the *wpch* allele remained at its original chromosomal location. All five mutants had an internal deletion within *peach* (Table 1). The deletions cause a reduction in level of somatic mosaicism of *wpch* of at least a factor of 10, although two of the four deletions that have the most drastic effects on somatic mosaicism have one breakpoint near the 5′ inverted repeat, namely Δ*C1* and Δ*D1*. (In fact, the deletion in Δ*C1* includes the innermost nucleotide of the 5′ inverted repeat.)

Defective *peach* elements produced by chemical mutagens and radiation: To generate still more *peach* mutations with impairments in somatic excision, the *wpch* allele was mutagenized with three different mutagens: EMS, DEB, or X rays. These mutagens are expected to induce a different spectrum of mutations, ranging from point mutations (EMS) through small deletions (DEB) to large and small deletions (X rays).

Five additional *peach* deletions were recovered from these screens, three of which affected the inverted repeats. Two small deletions were recovered from EMS treatment: One was of 5 bp at the extreme 5′ end of the element (including the TA duplication and the first three nucleotides of the 5′ inverted repeat), and the other was of 1 bp near the extreme 3′ end of the element (deleting nucleotide 1284). In both of these mutants

TABLE 1

Internal deletions of *peach*

Mutant	Source	Size (bp)	Location (bp)	Phenotype[a]
peach	None	NA	NA	100 (>200)
Δ*4R*	*Mos1*	128	939–1066	0.2 (0–1)
Δ*9R*	*Mos1*	666	495–1160	0.2 (0–1)
Δ*16R*[b]	*Mos1*	900	207–1106	0 (0)
Δ*31R*	*Mos1*	617	532–1148	0.2 (0–1)
Δ*35R*[b]	*Mos1*	411	586–994	0.2 (0–1)
Δ*37R*	*Mos1*	473	574–1046	0 (0)
Δ*49R*[b]	*Mos1*	141	725–865	10 (10–30)
Δ*57R*[b]	*Mos1*	341	638–978	2 (0–10)
Δ*63R*	*Mos1*	402	856–1257	0 (0)
Δ*72R*[b]	*Mos1*	530	149–678	0 (0)
Δ*80R*	*Mos1*	394	721–1114	0 (0)
Δ*97R*	*Mos1*	425	160–584	0 (0)
Δ*99R*	*Mos1*	705	577–1281	0 (0)
Δ*B1*	*PΔ2–3*	357	229–586	10 (~20)
Δ*C1*	*PΔ2–3*	378	28–405	0 (0)
Δ*D1*	*PΔ2–3*	828	31–858	0 (0)
Δ*F2*	*PΔ2–3*	648	134–781	0 (0)
Δ*J2*[b]	*PΔ2–3*	600	274–873	1 (0–5)
Δ*DEB-342*	DEB	31	735–765	10 (10–30)
Δ*XRAY-234*	X rays	461	318–778	10 (10–30)

NA, not applicable.
[a] Percentage (and approximate number of mosaic spots per eye) relative to nonmutant *peach*.
[b] Also has small insertion at site of deletion.

the level of somatic mosaicism was reduced by a factor of at least 10. The third small deletion was induced by DEB and deleted nucleotides 5–6 (or perhaps 7–8) from the 5′ inverted repeat; this deletion reduced somatic mosaicism to ~0.2% of the control level.

The other two deletions recovered from the mutagen screens are shown in Table 1. One small one (31 bp) was induced by DEB and one relatively large one (461 bp) by X rays. Both of these reduce somatic mosaicism of *wpch* by a factor of ~10.

EMS-induced internal point mutations affecting *peach* excision: EMS mutagenesis of *wpch* also produced four mutations with one or two nucleotide changes in which the *peach* element had reduced somatic excision. Three of these (*EMS-15*, *EMS-36*, and *EMS-118*) had identical G-to-A transitions at nucleotide position 993. The fourth (*EMS-9*) was a double mutant with C-to-A transversions at both nucleotide positions 161 and 179. All four of these mutant *peach* elements showed a level of *wpch* excision that was reduced by more than an order of magnitude, suggesting that nucleotides 161 and/or 179, and nucleotide 993, are key nucleotides required in the excision reaction of *peach*. None of these sites is close to either of the inverted repeats.

Transposase-induced recombination between mutant *peach* alleles: We previously reported that, in females, the rate of recombination at the site of a homozygous peach element is increased in the presence of active transposase by a factor of ~200 (Lohe *et al.* 2000). This estimate assumes that the transposase-induced increase in recombination occurs across the region of the 1.3-kb peach element only, but not more uniformly across adjacent regions. To test this assumption, we examined rates of recombination in females carrying two different mutant peach elements, in the presence or absence of an hsp70::Mos1 source of transposase. Progeny were scored for high levels of somatic mosaicism. Because both mutant peach elements in the parental female have low levels of somatic mosaicism, restoration of a normal level requires recombination in the interval between the mutations. (We use the term recombination for any mechanisms of information exchange between homologs, including gene conversion associated with template-directed gap repair.)

Four mutant *peach* elements were tested individually in heteroallelic combinations with *EMS-118*, which has a single nucleotide substitution (C-to-A) at position 993 of *peach*. The phenotype of *EMS-118* is a weak mosaic, showing 10–20 mosaic spots per eye in the presence of *Mos1* transposase. The results are shown in Table 2. The *peach* mutations tested in combination with *EMS-118* were Δ*B1*, Δ*65R*, Δ*66R*, and Δ*68R*. Δ*B1* is a deletion of *peach* that includes nucleotides 229–586 (Table 1), Δ*65R* is a deletion/insertion in which the first 18 bp of the 5′ inverted repeat has been deleted and replaced with 25 bp of unrelated sequence, *D66R* is another deletion/insertion in which 123 bp at the 5′ end of *peach* has been deleted and replaced with 9 bp of unrelated se-

TABLE 2

Recombination between mutant *peach* alleles

Series	Genotype	No. of recombinants	Total progeny	% recombination
Experimental	Δ*B1/EMS-118*; *Mr182/+*	30	17,320	0.17
Control	Δ*B1/EMS-118*; *+/+*	0	10,527	0
Experimental	Δ*65R/EMS-118*; *Mr182/+*	10	16,204	0.06
Control	Δ*65R/EMS-118*; *+/+*	1	1,360	0.07
Experimental	Δ*66R/EMS-118*; *Mr182/+*	2	2,436	0.08
Control	Δ*66R/EMS-118*; *+/+*	0	2,149	0
Experimental	Δ*68R/EMS-118*; *Mr182/+*	3	6,577	0.04
Control	Δ*68R/EMS-118*; *+/+*	ND	ND	ND

ND, not determined.

quence, and Δ*68R* is a 9-bp deletion that includes 6 bp of flanking sequence and the first 3 bp of the 5′ inverted repeat (Lohe *et al.* 2000).

The highest level of recombination was obtained with Δ*B1/EMS-118* (0.17% in Table 2) and this recombination was dependent on the presence of *Mos1* transposase. Two recombinant elements from this genotype were verified as authentic *peach* by DNA sequencing. Since the value 0.17% includes only one of the two recombinant classes, the recombination rate between the mutant alleles is actually 0.34%. Because Δ*B1* has a 357-bp deletion between nucleotides 229 and 586 inclusive, any recombination that restores somatic mosaicism recombination must have occurred in the 406-bp region between the 3′ breakpoint of Δ*B1* and position 993.

Previous results have also suggested that the *Mos1* transposase preferentially attacks the 5′ inverted repeat of *peach*. If this is the case, then we would also expect disruption of the 5′ end to reduce the rate of transposase-induced recombination in heteroallelic mutant *peach* combinations. This prediction is borne out by the results with Δ*65R*, Δ*66R*, and Δ*68R*, all three of which have a mutant 5′ inverted repeat. The total distance between the lesion in Δ*68R* and that in *EMS-993* is 989 nucleotides, approximately threefold greater than that between the lesions in Δ*B1* and *EMS-993*, yet the rate of transposase-induced recombination is reduced by a factor of more than four. (The unexpected recombinant obtained in the control cross between Δ*65R* and *EMS-118* is most easily explained by a maternal effect of *Mos1* transposase present in the tested females, since their mothers were heterozygous for *Mr182*.)

DISCUSSION

Hotspots of recombination: The results in Table 2 confirm that, in the presence of transposase, *mariner* elements are hotspots of recombination in the Drosophila genome, as they also are, at least at some sites, in the human genome (Kiyosawa and Chance 1996; Reiter *et al.* 1996). It should be emphasized, however, that there is as yet no direct evidence for an active *mariner* transposase produced in human cells. The Drosophila results also demonstrate that, in the presence of transposase, high rates of recombination between allelic, defective elements can restore excision competency to mutant elements. In the genotype Δ*B1/EMS-118*, the rate of recombination is 0.34 map units across the 406 bp between the mutational lesions, which implies an overall rate of recombination of 1 map unit per 1.2 kb. The *wpch* transgene is located in the region of the X chromosome between *singed* and *lozenge*, in which the normal rate of recombination is 1 map unit per 250 kb (Heino *et al.* 1994). Hence the rate of recombination within *mariner* elements is increased by a factor of ~200.

Most of the recombinant *mariner* elements probably result from template-directed gap repair rather than from the usual homologous recombination pathway. This inference is supported by the observation in Table 2 that mutant *mariner* elements missing all or part of the 5′ inverted repeat show less recombination than mutant elements, like Δ*B1*, with internal deletions. The difference is statistically significant ($\chi^2 = 12.5$, $P < 0.01$). This finding also supports a previous inference, based on other evidence, that the *mariner* transposase makes its initial attack on the 5′ inverted repeat (Lohe *et al.* 2000).

Differences between *mariner* transposition and *P*-element transposition: Our results indicate many points of difference between *mariner* transposition and *P*-element transposition. For example, the major class of defective elements recovered as aberrant products of *mariner* transposition have alterations in the 5′ inverted repeat or, to a lesser extent, in the 3′ inverted repeat. These alterations are most easily interpreted as arising from exonuclease degradation initiated at the site of a double-stranded cleavage made by transposase near the junction of the element and genomic DNA, usually between nucleotides 2 and 3 in the 5′ inverted repeat, which is repaired by host enzymes with or without insertion of filler sequences (Lohe *et al.* 2000). An analogous class of mutations affecting the extreme ends of the *P*-element inverted repeats has not been reported (Staveley *et al.*

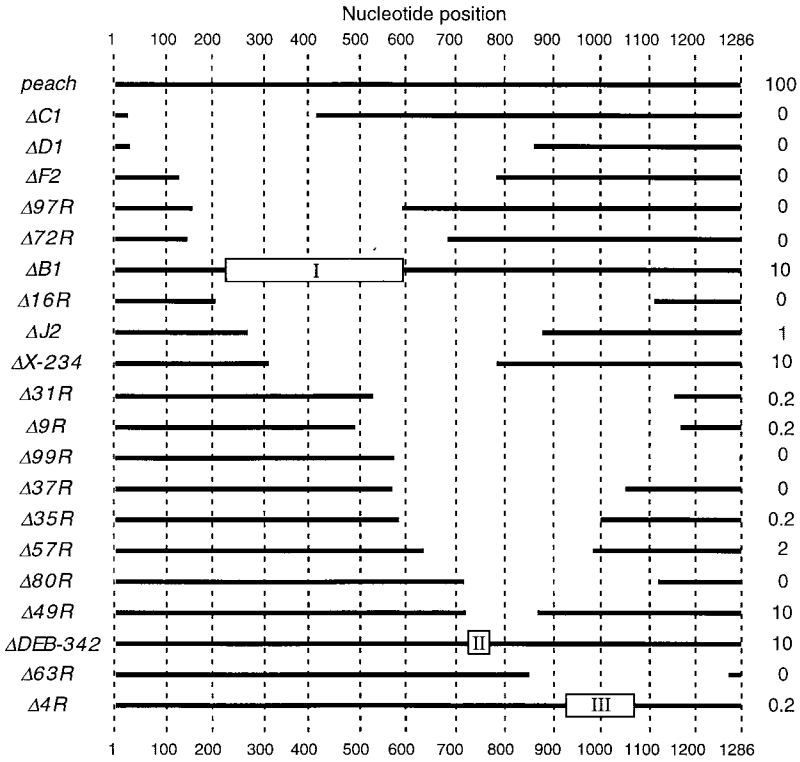

FIGURE 1.—Location of internal deletions (gaps) within the *peach* element, with the nondeleted element across the top for comparison. The numbers at the right are the rates of excision of each deleted element (in percentages), relative to the nondeleted element. The rectangles indicate the critical regions I, II, and III defined by the deletions Δ*B1*, Δ*DEB-342*, and Δ*4R*, respectively.

1995). The *P*-element transposase generates asymmetrical double-stranded cleavage with 3′ single-stranded overhangs of the terminal 17 bp of each of the inverted repeats. Aberrant deletion products almost always retain the terminal 15–17 bp of the inverted repeats, which are apparently protected following cleavage by an IRBP (RIO and RUBIN 1988; STAVELEY *et al.* 1995; BEALL and RIO 1997). Our results with *mariner* are most easily interpreted by supposing that the IRBP does not bind to the *mariner* inverted repeats. Alternatively, if IRBP does bind to the *mariner* inverted repeats, then there is a different interaction with the transposase such that the bound ends are not protected from exonuclease attack.

The characteristics of internal deletions also indicate a major difference between the mechanisms of *mariner* and *P*-element transposition. Internal deletions of *mariner* resulting from aberrant excision appear to have endpoints scattered at random throughout the element (Table 1). Among the 13 *Mos1*-associated internal deletions in Table 1, only one (Δ*99R*) extends into one of the inverted repeats. In contrast, internal *P*-element deletions usually terminate within one of the inverted repeats, and sometimes both endpoints lie within the inverted repeats (STAVELEY *et al.* 1995).

Internal sequences and/or spacing of sequences required for efficient *mariner* excision: The most unexpected result of our study is the apparent presence of internal sites, far removed from the inverted repeats, that appear to be necessary for efficient *mariner* excision. The locations of the deletions in Table 1 are represented graphically in Figure 1. Because each of these deletions reduces the level of excision by at least a factor of 10, either each deletion must be missing sites that are essential for efficient transposition or, alternatively, each deletion alters an essential spacing between binding sites that flank the deletion. The remarkable finding is that the deletions are scattered throughout the element. We emphasize that these deletions were selected on the basis of their reduced levels of transposition; hence there may be other deletions that do not markedly affect the rate of excision. Nevertheless, if internal deletions that affected excision were rare or were restricted to a particular region, we would neither have expected to recover so many of them (13/68 mutant *peach* elements recovered in the *peach/Mos1* screen had internal deletions) nor have expected them to be scattered throughout the entire element (Figure 1).

Inspection of Figure 1 indicates that, generally speaking, the larger the deletion the smaller the rate of element excision. (The numbers on the right are the rates of excision, in percentages, relative to excision of the *peach* element.) On the assumption that there might be discrete internal regions that are required for efficient element excision, three regions can be identified on the basis of the three smallest deletions: Δ*B1*, Δ*DEB-342*, and Δ*4R*. The Δ*B1* deletion implicates the region 229–586 bp (which we denote as region I), Δ*DEB-342* implicates the region 735–765 bp (region II), and Δ*4R* implicates the region 939–1066 bp (region III). Deletion of any of these regions reduces the efficiency of element excision by more than an order of magnitude. Sequences flanking these regions may also be important. For exam-

ple, the deletion Δ*72R* completely abolishes element excision, yet it is only slightly larger than the deletion Δ*B1*. We emphasize that internal regions other than I, II, and III may also be essential for efficient excision but have escaped identification and also that the sequences required for recognition may be large enough to overlap regions I and II or regions II and III. A more extensive mutational analysis will be required to define the minimal regions required for efficient excision.

It is possible that regions I, II, and III are necessary for efficient excision not because they contain essential binding sites but because they maintain an essential spacing between binding sites. Evidence for length dependence in the case of the *Escherichia coli* insertion sequence IS*50* has been reported by Goryshin *et al.* (1994), who show that elements <64 bp are severely impaired in transposition, whereas those >200 bp exhibit efficient transposition with essentially no length dependence. In our experiments, the lengths of the deleted *peach* elements are ⩾200 bp, ranging from 386 to 1255 bp. The hypothesis of critical internal binding sites is also supported by the point mutations recovered in the EMS screen. *EMS-118* is a single-nucleotide substitution at position 993, which is very near the middle of region III. Clearly a sequence in this region is necessary for efficient excision, but we cannot exclude the possibility that the region is also necessary for spacing. *EMS-9* is a double mutant with single-nucleotide substitutions at positions 161 and 179. None of these lie in regions I, II, or III, but it may be of some interest that both of them lie in the region immediately to the left of region I in the interval defined by the left-hand breakpoints of Δ*72R* and Δ*B1*. This result again suggests that Δ*72R* contains essential sequences not present in region I, and it is sequence, not spacing, that is important.

On the other hand, correct spacing between essential binding sites also appears to be important for efficient *mariner* mobilization. Evidence is presented in an accompanying article (Lozovsky *et al.* 2001), in which we report that *mariner* vectors with exogenous DNA fragments of 1–5 kb inserted into any of the unique internal restriction sites *Sal*I, *Sph*I, or *Cla*I are severely compromised in their ability to excise and transpose.

Comparison with *mariner* deletions found in natural populations: Brunet *et al.* (2001) recently studied deletions of *Mos1*-related *mariner* elements in natural populations of multiple species of *Drosophilidae*, which were detected by means of PCR amplification using the *Mos1* inverted repeats as primers. They found 27 distinct deletions, 21 of which were >50 bp. Among these, 10 overlap our region I; 5 overlap regions I and II; 2 overlap regions II and III; and 4 overlap regions I, II, and III. Furthermore, among the 6 deletions <50 bp, 2 (of lengths 27 and 45 bp) are in the critical region identified between the left-hand breakpoints of deletions Δ*72R* and Δ*B1*, and one (of length 17 bp) overlaps region II. Judging from the locations of these deletions and the impaired mobility of the deletions that overlap regions I, II, and III (Figure 1), it seems very likely that most of the deletions found in natural populations are unable to undergo excision or transposition at anywhere near normal levels. This finding supports the conclusion of Brunet *et al.* (2001) that many of the deletions found in nature are created spontaneously by mechanisms unrelated to transposase activity. For example, one-third of the naturally occurring deletions occur between short direct repeats of five to eight nucleotides (Brunet *et al.* 2001).

Comparison with *mariner* transposition *in vitro*: Our results appear to conflict with those of Tosi and Beverley (2000), who reported that a *Mos1* element with as little as 43 bp of DNA between the inverted repeats could transpose *in vitro* as efficiently as the complete element. However, the mini-element carried a kanamycin-resistance gene inserted between the inverted repeats, and the complete element carried a kanamycin-resistance gene inserted into the *Sac*I site (Tosi and Beverley 2000). Because the complete element forms the baseline for the comparison, the result implies that only the mini-element transposes *in vitro* as efficiently as an element with an insertion into the *Sac*I site.

It may be that vectors with insertions into the *Sac*I site undergo relatively efficient transposition *in vitro*. However, we have shown that, *in vivo*, *Mos1* constructs with insertions into the *Sac*I site are highly refractory to either excision or transposition by *Mos1* transposase (Lohe and Hartl 1996). These results caution against extrapolation from data obtained *in vitro* to estimate the rate of transposition *in vivo*. We have shown in this article that the continuity of several internal regions of *mariner* is essential for efficient transposition *in vivo* and that excision of *peach* is abolished by many internal deletions > ~400 bp (Figure 1). Furthermore, *in vitro* reactions are carried out with purified DNA, whereas *in vivo* the transposase protein must interact with chromatin.

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