

Self-Inflicted Wounds, Template-Directed Gap Repair and a Recombination Hotspot: Effects of the *mariner* Transposase

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ABSTRACT

Aberrant repair products of *mariner* transposition occur at a frequency of $\sim 1/500$ per target element per generation. Among 100 such mutations in the nonautonomous element *peach*, most had aberrations in the 5' end of *peach* (40 alleles), in the 3' end of *peach* (11 alleles), or a deletion of *peach* with or without deletion of flanking genomic DNA (29 alleles). Most *mariner* mutations can be explained by exonuclease "nibble" and host-mediated repair of the double-stranded gap created by the transposase, in contrast to analogous mutations in the *P* element. In *mariner*, mutations in the 5' inverted repeat are smaller and more frequent than those in the 3' inverted repeat, but secondary mutations in target elements with a 5' lesion usually had 3' lesions resembling those normally found at the 5' end. We suggest that the *mariner* transposase distinguishes between the 5' and 3' ends of the element, and that the 5' end is relatively more protected after strand scission. We also find: (1) that homolog-dependent gap repair is a frequent accompaniment to *mariner* excision, estimated as 30% of all excision events; and (2) that *mariner* is a hotspot of recombination in *Drosophila* females, but only in the presence of functional transposase.

ALMOST all *mariner* transposable elements isolated from natural populations are nonautonomous because of insertions, deletions, or missense mutations. The only naturally occurring *mariner* elements known to produce active transposase are closely related to the *Mos1* element, which was originally identified in *Drosophila mauritiana* using a genetic screen (Bryan *et al.* 1987). The inability of most *mariner* elements to encode active transposase is evident even in genomes that have a large number of copies (Robertson and MacLeod 1993; Robertson and Lampe 1995). Despite the identification of hundreds of copies of *mariner* elements from a wide variety of invertebrates (Robertson 1993; Garcia-Fernández *et al.* 1995), plants (Jarvik and Lark 1998), and the human genome (Augegouillou *et al.* 1995; Morgan 1995; Oosumi *et al.* 1995; Hartl 1996), few if any copies contain an open reading frame (Hartl *et al.* 1997a). The existing literature does not address the issue of what proportion of naturally occurring *mariner* elements is still capable of being mobilized *in trans*, because the use of PCR approaches has not allowed the inverted terminal repeats (ITRs) of most naturally occurring elements to be examined.

The evolutionary "natural history" of *mariner* elements is proposed to begin with horizontal transmission of an active element that invades the germline of a susceptible host species (Hartl *et al.* 1997b). Invasion is followed

by a period of increasing copy number that ensures the dispersal of active elements throughout the genome and, through sexual reproduction, eventually the entire population. Attainment of a high copy number of *mariner* elements is proposed to increase the chance of horizontal transmission to yet other hosts, so that an increase in copy number is an essential part of the long-term survival strategy of *mariner*. Subsequent inactivation of a high proportion of autonomous *mariner* elements by mutation may be essential to minimize the detrimental effects of transposon mutagenesis in the host genome that result from continuing mobilization. *Mariner* elements are active in the soma as well as in the germline, and a decrease in fitness of organisms containing autonomous elements has been detected under experimental conditions (Nikitin and Woodruff 1995).

Some transposable elements, in *Drosophila* most notably *P* (Engels 1989) and *hobo* (Blackman and Gelbart 1989), produce deletion derivatives at appreciable frequencies owing to abortive excision reactions. We undertook the present experiments to assess the extent to which *mariner* produces such aberrations and to compare the molecular nature of the aberrations with those produced particularly by the *P* element. The *peach* element used in the present experiments is a *mariner* element that is inserted into the 5' untranslated region of the *white* gene in the *w^{peh}* allele. The insertion changes the phenotype from bright red to peach (Jacobson *et al.* 1986). Although the *peach* element has an open reading frame, the element is nonautonomous: it produces either no transposase or a transposase incapable of excising *peach*. Nevertheless, *peach* can still be excised effi-

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ciently in response to active transposase supplied in *trans*. Somatic activity of *Mos1* in flies carrying the w^{pch} allele results in a mosaic eye color 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, *Mos1* transposase is strongly expressed in the germline and soma even in the absence of heat shock. In flies carrying the w^{pch} allele, *hsp70::Mos1* induces high levels of germline reversion of *peach* and extreme mosaicism in the eyes (Garza *et al.* 1991; Lohe *et al.* 1995).

A mutation screen for reduced levels of somatic mosaicism in w^{pch} ; *Mos1* flies has been used to isolate mutations in the *Mos1* transposase gene (Lohe and Hartl 1996; Lohe *et al.* 1997). Here we use a similar screen to identify mutations in w^{pch} with reduced somatic mosaicism due to lesions in the *peach* element that impair its ability either to be recognized by transposase or to be excised. In the absence of active transposase, such excision-defective mutations arise at a frequency no greater than the spontaneous mutation rate (Lohe *et al.* 1997). In the presence of active transposase, the frequency of excision-defective mutations in *peach* is $\sim 0.2\%$ per exposed w^{pch} allele per generation. Most of the molecular defects can be interpreted in terms of either aberrant mismatch repair or aberrant template-directed gap repair (TDGR). We call these lesions "self-inflicted wounds" because they are initiated by transposase-directed strand scission at one or both ends of *peach* that are aberrantly repaired using host functions. The major types of *peach* aberrations are completely different from those isolated in comparable experiments with the *P* element. In the case of *P* element, most lesions are internal deletions, and defects overlapping the last 15–17 bp of the ITRs are rare (Staveley *et al.* 1995). In the case of *peach*, the majority of mutations with defective excision have damage to either the 5' or 3' ITR. Only a few of the *peach* mutations have internal deletions. Moreover, these were large internal deletions, quite unlike the small deletions that characterize nonautonomous *mariner* elements in natural populations.

One excision-defective *peach* element afforded an opportunity to estimate the frequency of TDGR in the *mariner* system. We found that the ratio of homolog-directed gap repair to excision is $\sim 1:2$. We also demonstrate here that *peach* is a hotspot of recombination in *Drosophila* females, but only in the presence of functional transposase. This finding is of some interest in view of data suggesting that a human *mariner*-like element in chromosome *17p11.2-p12* is a hotspot of recombination (Kiyosawa and Chance 1996; Reiter *et al.* 1996).

MATERIALS AND METHODS

Transgenic stocks and genetic markers: The *wpch* transgene $P[w^{pch}, ry^+]$, described below, contains a nonautonomous *mar-*

ner element designated *peach* (Jacobson *et al.* 1986). The *peach* element was identified originally as an insertion into the 5' region of the *white* gene of *D. mauritiana* (Jacobson *et al.* 1986), yielding the w^{pch} allele; the *peach* insertion is at a position corresponding to coordinate 3643 in the *D. melanogaster* physical map of Levis *et al.* (1982; see Bryan *et al.* 1990). A *peach*-containing *Bam*HI restriction fragment was isolated from the w^{pch} allele and was used to replace the corresponding restriction fragment of the *white* gene of *D. melanogaster*. The resulting chimeric gene was inserted into a *P*-element germline transformation vector, creating $pP[w^{pch}, ry^+]$, which was used to transform *D. melanogaster* (Garza *et al.* 1991). The $P[w^{pch}, ry^+]$ transgene is inserted into the *X* chromosome at map position 27.0 between *singed* and *lozenge*; we designate this insert as the *wpch* transgene to distinguish it from the original w^{pch} allele, which is at the *white* locus. In the experiments reported here, the genetic background of all stocks contained the w^{118} deletion at the *white* locus. In the absence of *mariner* transposase, flies of genotype $w^{118} wpch$ have peach-colored eyes; in the presence of transposase, the eyes are heavily mosaic owing to the excision of *peach* from multiple pigment-cell lineages, restoring wild-type pigmentation (Garza *et al.* 1991). Typical mosaics have >200 red spots in each eye, in an otherwise peach-colored background.

To isolate excision-defective mutations in *wpch*, we used either of two transposase-producing transgenes. The *Mr182* transgene (Lohe *et al.* 1995) is a transposase-producing $P[hsp70::Mos1, ry^+]-182$ transgene inserted in chromosome 2. *Mr182* includes the *heat shock 70* promoter (*hsp70*) fused to the *Mos1* promoter at nucleotide position 58–59 [numbering as in Jacobson *et al.* (1986)] and all of the transposase open reading frame. The *Mos1* element is an autonomous *mariner* element, but it cannot be mobilized in this transgene owing to the absence of the 5' ITR in $P[hsp70::Mos1, ry^+]-182$. The promoter in the *hsp70::Mos1* transgene has high activity even in the absence of heat shock (Lohe *et al.* 1995). The *MrX* transgene, located at map position 30.2 on the *X* chromosome, was derived from *Mr182* by *P*-element mobilization of $P[hsp70::Mos1, ry^+]-182$ and selection for an *X* chromosome encoding high transposase activity (data not shown).

The *sn* (*singed*) and *lz* (*lozenge*) alleles employed as markers in some of the experiments were sn^3 and lz^{7a7} . Genetic crosses were carried out at 25° on standard cornmeal-molasses medium.

Isolation of mutants: Excision-defective derivatives of *wpch* arise in stocks containing both *wpch* and a source of functional transposase. Such mutants are recognized by absent, or markedly reduced, levels of somatic mosaicism. Some of the mutants also have altered eye color, but the primary basis of the screen was degree of somatic mosaicism. Approximately half of the mutant *wpch* alleles reported here were obtained from exposure of *wpch* to the transposase source *Mr182* on chromosome 2, which was crossed out of each excision-defective stock immediately after isolation. The rest were obtained from exposure to the transposase source *MrX* in *X* chromosomes carrying *wpch* *MrX*. Flies containing excision-defective mutations were crossed for one generation to replicate the *X* chromosome and the progeny were frozen at -70° for subsequent analysis by PCR and sequencing. The same kinds of *wpch* mutations were found, at statistically indistinguishable frequencies, from both sources of transposase; hence the mutations are grouped by type rather than by origin.

In the continued presence of transposase, excision-defective *wpch* alleles are more stable genetically than *wpch*, probably owing to impaired transposase binding to the mutant allele. We were, however, able to obtain secondary mutations in some *wpch* *MrX* stocks by selecting for derivatives in which somatic mosaicism was absent or markedly reduced relative to the level found in the original mutant allele. These secondary

derivatives are denoted by appending the letter *S* to the original mutant allele designation.

Molecular analysis: The *peach* element present in each mutant *wpch* allele was characterized by PCR amplification of genomic DNA from single flies using two pairs of primer oligonucleotides, each pair consisting of one primer that anneals to flanking *white* DNA and one primer that anneals to an internal region of *peach*. Amplified mutant *peach* elements were cloned with a TA cloning kit (Invitrogen, Carlsbad, CA) or sequenced directly (Lohe *et al.* 1996). The 5' half of *peach* was amplified with the primer pair 5'-GCCCATGCGGGATTTTCA-3' and 5'-AGTCATAGTAAATGACACCG-3', which also amplifies 134 bp of flanking *white* sequence. The 3' half of *peach* was amplified with the primer pair 5'-TTGCGAGAGATGGGAAAGATT-3' and 5'-CTGTTTGCCTCCTTCTCTGTC-3', which also amplifies 155 bp of flanking *white* sequence. DNA sequencing was carried out first to examine the extreme 5' and 3' ends of *peach*, including the ITRs. The complete mutant element was sequenced in all cases in which the ~350 bp at each end was found to be intact.

Verification of template-directed gap repair: We used the *peach* mutant *41R* because of its characteristic rosy-like phenotype, reduced excision rate, and unique CAGTA sequence at the 5' end (see Table 2 below). Putative *wpch* alleles that had been converted to *41R* by TDGR were isolated and analyzed by PCR using primers 5'-TACTTGACACCTGCCAGTA-3' and 5'-GCCCATGCGGGATTTTCA-3'. These primers are specific to the lesion in *41R*, and they amplify a characteristic 149-bp fragment only from the *41R* mutant allele.

RESULTS

Preliminary experiments indicated that, in the absence of *mariner* transposase, the *wpch* transgene containing the nonautonomous element *peach* is genetically stable. Although no attempt was made to estimate the spontaneous mutation rate of *wpch* to forms that exhibit reduced mosaicism when exposed to functional transposase, the spontaneous mutation rate is certainly less than 10^{-4} and perhaps much less (Lohe *et al.* 1997). However, we did note that in the presence of *mariner* transposase, derivatives of *wpch* showing absent or reduced somatic mosaicism were recovered at appreciable frequencies. We call the lesions in these derivatives self-inflicted wounds because their production appears to be the result of aberrant excision, initiated by at least one transposase-induced scission of one or both DNA strands, followed by repair using host functions. Accordingly, systematic experiments were carried out to recover a large set of such excision-defective *wpch* transgenes in order to characterize the molecular nature of the lesions. To isolate such mutations, we used either the *Mr182* transgene in chromosome 2 or the *MrX* transgene in the *X* chromosome, both of which produce high levels of transposase even in the absence of heat shock. (These transgenes have a dual *hsp70::Mos1* promoter.)

Two eye-color phenotypes predominate in progeny of flies that carry both the *wpch* transgene and a source of functional transposase. Most progeny have a strong mosaic eye color like their parents, but some progeny have a uniform bright red eye color that results from germline excision of *peach*. In addition, males with an

exceptional phenotype appeared with a frequency of ~1/500 per generation. In these exceptions, the *peach* element showed either no somatic excision or a much reduced level of somatic excision. In these flies, the number of red spots per eye was usually less than ~10, substantially smaller than the >200 spots observed in mosaic siblings. Some of the exceptions lacking mosaicism also showed various shades of eye color including white, yellow, orange, or rosy. Each of the mutant lines was retested in the presence of either *Mr182* or *MrX* to confirm the mutant phenotype of the excision-defective *wpch* allele. Subsequent PCR analysis and DNA sequencing confirmed that, in each of the mutant alleles, either the *peach* element was missing from its normal position near *wpch*, or else there were molecular lesions in the elements to which the low rate of excision could be attributed.

We report in detail two classes of mutations that are recovered frequently. They demonstrate not only some of the sequence requirements for efficient excision, but they also appear to illuminate the first steps in the *mariner* excision reaction. These classes are: (1) aberrations in or near the 5' end of *peach* (40 alleles) and (2) aberrations in or near the 3' end of *peach* (11 alleles). We also examine six secondary mutations derived from mutant alleles with 5' lesions, since these serve as important controls against bias in the phenotypic selection based on absent or much reduced somatic mosaicism.

Other types of mutations were also found that warrant a brief description, although they will not be discussed in detail in this report. The most numerous classes had either an excision of *peach* accompanied by an unusual footprint (11 alleles) or an excision or deletion of *peach* accompanied by a deletion of flanking *white* DNA (18 alleles). As might be expected, the latter class had no somatic mosaicism and a bleached-white eye-color phenotype. These kinds of lesions have been reported previously (Bryan *et al.* 1990). Together with the aberrations at either the 5' or the 3' end of *peach* mentioned above, these classes account for 80% of the mutant alleles analyzed. The remaining types of lesions included a variety of insertions, inversions, apparent local hops into nearby *white* sequences, and internal deletions. None of these classes was sufficiently frequent to infer general patterns.

Alteration in or near the 5' end of *peach*: A total of 40 alleles in this class were recovered (Table 1). Most of the phenotypes were weakly mosaic in the presence of transposase, as shown by the counts of number of mosaic spots per eye. Nucleotide sequencing revealed alterations in or adjacent to the 5' ITR of the *peach* element. The 3' ITR and flanking DNA were sequenced and were found to be unaltered. The 5' lesions consisted of either a simple deletion (21 alleles) or of a deletion accompanied by the insertion of additional DNA between the deletion endpoints (19 alleles). In mutant alleles with a simple deletion, the deletion was 1–14 bp in length and it always included nucleotides 2 or 3 of

TABLE 1
Lesions at the 5' end of the *wpch* insertion

| Allele | 5' <i>white</i> sequence | <i>mariner</i> sequence | Spots/eye | Deletion Δ (bp) | Insertion ∇ |
|------------------------|--|-------------------------|-----------|--------------------|---------------------------------------|
| <i>w^{pcH}</i> | 5'...CGTGTTTAATTGATGGCGTACCAGGTGTACAAGTAGGGAA... | | >200 | | |
| 110R | 5'...CGTGTTTAATTGATGGCGTAC-AGGTGTACAAGTAGGGAA... | | ~50 | Δ1 | |
| 22R, 40R, 46R, 48R | 5'...CGTGTTTAATTGATGGCGTACC-GGTGTACAAGTAGGGAA... | | 10-30 | Δ1 | |
| 64R | 5'...CGTGTTTAATTGATGGCGTAC--GGTGTACAAGTAGGGAA... | | <10 | Δ2 | |
| 53R | 5'...CGTGTTTAATTGATGGCGTA--GGTGTACAAGTAGGGAA... | | <10 | Δ3 | |
| 69R | 5'...CGTGTTTAATTGATGGCG----GGTGTACAAGTAGGGAA... | | 0 | Δ5 | |
| 18R, 79R | 5'...CGTGTTTAATTGATGGC-----AGGTGTACAAGTAGGGAA... | | <10 | Δ5 | |
| 17R | 5'...CGTGTTTAATTGATG-----AGGTGTACAAGTAGGGAA... | | 5-10 | Δ7 | |
| 86R | 5'...CGTGTTTAATTGA-----AGGTGTACAAGTAGGGAA... | | <10 | Δ9 | |
| 1R, 45R, 68R, 105R | 5'...CGTGTTTAATTGAT-----GGTGTACAAGTAGGGAA... | | 1-2 | Δ9 | |
| 2R, 33R | 5'...CGTGTTTAATTGATGGC-----GTACAAGTAGGGAA... | | 0-1 | Δ9 | |
| 28R, 71R | 5'...CGTGTTTAATTGAT-----GTACAAGTAGGGAA... | | 1-2 | Δ12 | |
| 36R | 5'...CGTGTTTAA-----GGTGTACAAGTAGGGAA... | | <10 | Δ14 | |
| 11R | 5'...CGTGTTTAATTGAT-----TACAAGTAGGGAA... | | 1-2 | Δ13 | ∇A |
| 21R | 5'...CGTGTTTAATTGATGGC---CAGGTGTACAAGTAGGGAA... | | 5-10 | Δ4 | ∇ACAAGGTCATCAGGTGT |
| 23R | 5'...CGTGTTTAATTGATGGCGTACCA-GTGTACAAGTAGGGAA... | | 0 | Δ1 | ∇TCCGCC + 143 bp of <i>white</i> gene |
| 30R | 5'...CGTGTTT-----TGTACAAGTAGGGAA... | | 0 | Δ18 | ∇AAGTAGG |
| 32R | 5'...CGTGTTTAATTGATGGCG---AGGTGTACAAGTAGGGAA... | | 0-1 | Δ4 | ∇TTAAACAGCCCA |
| 38R | 5'...CGT-----AGGTGTACAAGTAGGGAA... | | <10 | Δ19 | ∇TTG |
| 41R | 5'...CGTGTTTAATTGATGGCGT-CCAGGTGTACAAGTAGGGAA... | | 10-30 | Δ1 | ∇TACTGG |
| 54R | 5'...CGTGTTTAATTGATGGC--CCAGGTGTACAAGTAGGGAA... | | 10-30 | Δ3 | ∇GTACAAGTGT |
| 61R | 5'...CGTGTTTAATTGATGGCG-----TGTACAAGTAGGGAA... | | <10 | Δ7 | ∇TGTACAAGTAGGG |
| 65R | 5'...CGTGTTTAATTGATGGCGTA-----AA... | | 0 | Δ18 | ∇ATTAAATTGTAATTGTAATTGTAATT |
| 66R | 5'...CGTGTTTAATTGATGGCGTA TTTGGCGCAAATTGAGCGT... | | 0 | Δ1-123 | ∇CAAATTTAA |
| 75R | 5'...CGTGTTTAATTGATGGC-----GGTGTACAAGTAGGGAA... | | <10 | Δ6 | ∇GGGTACA |
| 77R | 5'...CGTGTTTAATTGA-----AGGTGTACAAGTAGGGAA... | | <10 | Δ9 | ∇ACACCCTAC |
| 89R | 5'...CGTGTTTAATTGATGGC-----GGTGTACAAGTAGGGAA... | | <10 | Δ6 | ∇GTGTA |
| 96R | 5'...CGTGTTTAATTGA-----CCAGGTGTACAAGTAGGGAA... | | 10-30 | Δ7 | ∇GGTGATNTTTAATC |
| 100R | 5'...CGTGTTTAATTGATGGC-----GGTGTACAAGTAGGGAA... | | <10 | Δ6 | ∇GTGTACA |
| 104R | 5'...CGTGT-----GTACAAGTAGGGAA... | | 0 | Δ20 | ∇GTGTT |
| 114R | 5'...CGTGTTTAATTGATGGCGT---GGTGTACAAGTAGGGAA... | | ~50 | Δ4 | ∇GGGTACAGGTGTACA |
| 115R | 5'...CGTGTTTAATTGATGGCGTAC-AGGTGTACAAGTAGGGAA... | | ~50 | Δ1 | ∇CAAGTAGGTGGTAAAGTACA |

the 5' ITR. The most common mutation was a 1-bp deletion of nucleotide 3 (alleles *22R*, *40R*, *46R*, and *48R*). Although 4 other alleles contained an identical 9-bp deletion (*1R*, *45R*, *68R*, and *105R*), this deletion could have been generated in three different ways, depending on how one aligns the sequences.

The 21 alleles grouped at the top of Table 1 are all simple deletions. The 7 alleles at the very top retain the 5' TA duplication but they have a small deletion at or near the end of the 5' ITR. A deletion of even 1 bp in the 5' ITR is sufficient to reduce somatic excision by a substantial amount (alleles *110R*, *22R*, *40R*, *46R*, and *48R*), with a greater effect observed for 2–3-bp deletions at the 5' end of the ITR (alleles *64R* and *53R*). The alleles *69R* through *36R* imply that some ability to be excised is retained even by *wpch* alleles that are missing up to 6 bp of the 5' ITR along with the 5' TA and some flanking *white* sequences.

The 19 mutant alleles grouped at the bottom of Table 1 all have a deletion and an accompanying insertion. Except for allele *66R*, the deletions range in size from 1 to 20 bp, and, except for allele *23R*, the insertions range in size from 1 to 25 bp. The largest insertion (149 bp in allele *23R*) accompanies the smallest deletion (1-bp deletion of nucleotide 4 in *peach*); at least 143 bp of the inserted DNA in this allele is derived from nucleotides 14,151–14,293 of the *white* gene, ~7 kb upstream from the insertion point of the *peach* element in *white*. In contrast, the smallest insertion (1 bp in allele *11R*) accompanies a relatively large deletion of 13 bp. In the remaining 17 mutant alleles, a sequence 3–25 bp in length was present in the region that had been deleted. In general, the sequence of the inserted DNA bears no relationship to the sequence of the deleted DNA.

In virtually all of the alleles with a deletion and insertion, some or all of the inserted DNA appears to originate by replication of either *white* or *peach* sequences flanking the deletion endpoint, usually at a distance of 0–10 bp. The underlined sequences in Table 1 are identical between the inserted DNA and either *white* or *peach* flanking DNA. The replicated sequence can be derived from either strand, which is indicated in Table 1 by roman font when it is derived from the strand shown and by italics when it is derived from the complementary strand. In the insertions in alleles *65R* and *114R*, a sequence appears to have been copied more than once to generate tandem repeats. Such “filler” sequences have been observed in *P*-element derivatives and are presumed to originate by replication slippage (O'Hare and Rubin 1983; Kurkulos *et al.* 1994; Staveley *et al.* 1995).

In three mutant alleles (*41R*, *54R*, and *96R*), the repair process by chance restored the exact sequence of the 5' ITR, but the TA dinucleotide was altered to either **GG** (allele *41R*), **GT** (*54R*), or **TC** (*96R*). The phenotype of each of these mutants was a weak mosaic of 10–30 spots per eye. Therefore, the TA dinucleotide

immediately adjacent to the 5' ITR is not essential for excision of *peach*, but the efficiency of excision is greatly enhanced if the TA dinucleotide is present.

The allele *54R* was studied further to determine the actual site of strand scission. To excise the *peach* element in this allele, the transposase must recognize either the GT dinucleotide or a TA dinucleotide, of which the nearest is 7 bp upstream in *white* or 7 bp downstream in *peach*. Germline revertants had the footprint 5'-GTAC AAGTGTXXXTAACGC-3', where XXX indicates the extreme 3 bp from either the 5' or 3' end of *peach* (data not shown). The 5' flanking sequence is the insertion in *54R*, and the 3' flanking sequence is that of the *white* gene at the 3' end of *peach*. This result indicates that, in allele *54R*, the transposase initiates strand scission at the GT dinucleotide adjacent to the 5' ITR rather than at either of the nearby TA dinucleotides.

Among the mutations with a deletion plus insertion, the alleles yielding the greatest levels of somatic excision (*114R* and *115R*) have a sequence resembling the 5' ITR as well as the adjacent TA dinucleotide. In particular, both *114R* and *115R* have a repaired sequence at the 5' end of *peach* that reads 5'-TACAAGG...-3', which is equivalent to a C → A transversion at position 2 of the 5' ITR with the 5' TA dinucleotide left intact.

Only six alleles in Table 1 had nonmosaic eyes in the presence of transposase. This result would be expected of alleles *23R* and *66R*, both of which have a substantial part of the 5' ITR replaced with unrelated sequence. The lesions in *65R* and *104R* are also substantial: *65R* differs in 8 of the first 10 nucleotides in the 5' ITR, *104R* differs in 4 of the first 6 nucleotides in the 5' ITR, and both alleles are flanked by a GT dinucleotide. Comparing *wpch* with *30R* and *69R* reveals smaller differences. In the *wpch* allele, the 5' dinucleotide and ITR are 5'-TACCAGG-3'. In allele *30R* this sequence reads 5'-AAGTAGG-3' and in allele *69R* it reads 5'-TGGC GGG-3'. Evidently the altered flanking dinucleotide, along with the multiple substitutions in the first few nucleotides of the 5' ITR, is enough to prevent excision, although this finding does not identify whether the defect is in transposase recognition or strand scission.

Alteration in or near the 3' end of *peach*: In 11 alleles there was no alteration in the 5' ITR or flanking *white* DNA, but there was a deletion at the 3' end of the element (Table 2). With the exception of allele *58R*, in which very weak somatic mosaicism was observed, none of the alleles evidenced somatic mosaicism in the presence of transposase. Allele *58R* was also exceptional in having a small 3' deletion (8 bp), in contrast to the other alleles. In relative position with respect to the nearest end of *peach*, overall size, and response to transposase, allele *58R* more nearly resembles the 5' deletions *2R* and *33R* in Table 1 than the other 3' deletions recovered.

The rest of the 3' deletions were large (73–1069 bp) and extended internally into the element from the 3'

TABLE 2
Lesions at the 3' end of the *wpch* insertion

| Allele | <i>mariner</i> sequence | <i>white</i> sequence | Deletion Δ (bp)/Insertion ∇ |
|----------------------------|--------------------------------------|-----------------------|---|
| <i>wpch</i> | 5'...TTTCATACTTGTACACCTGATAAACCGC... | | |
| <i>58R</i> | 5'...TTTCATACTTGTAC-----AACCGC... | | $\Delta 8$ (1281-1286 + TA) |
| <i>99R</i> | 5'-----CCTGATAAACCGC... | | $\Delta 705$ (577-1281) |
| <i>29R</i> | 5'-----CTGATAAACCGC... | | $\Delta 956$ (327-1282) |
| <i>101R</i> | 5'-----TGATAAACCGC... | | $\Delta 73$ (1211-1283) |
| <i>5R</i> | 5'-----TGATAAACCGC... | | $\Delta 1030$ (254-1283) |
| <i>7R</i> | 5'-----GATAAACCGC... | | $\Delta 375$ (910-1284) |
| <i>27R</i> | 5'-----TAAACCGC... | | $\Delta 971$ (316-1286) |
| <i>83R</i> | 5'-----TAAACCGC... | | $\Delta 385$ (902-1286) |
| <i>10R</i> | 5'-----TAAATAAACCGC... | | $\Delta 740$ (543-1286) ∇ TAAA |
| <i>15R</i> | 5'-----AAACCGC... | | $\Delta 421$ (867-1287) |
| <i>24R</i> | 5'-----CCGC... | | $\Delta 1069$ (222-1290) |
| Secondary mutations | | | |
| <i>86RS</i> | 5'...TTTCATACTTGTACACC-GATAAACCGC... | | $\Delta 1$ |
| <i>18RS</i> | 5'...TTTCATACTTGTACACCT--TAAACCGC... | | $\Delta 2$ |
| <i>48RS</i> | 5'...TTTCATACTTGTACACC-----GC... | | $\Delta 9$ |
| <i>100RS</i> | 5'...TTTCATACTTGTACACCTGA---ACCGC... | | $\Delta 3$ |
| <i>22RS</i> | 5'...TTTCATACTTGTACACCTGA---ACCGC... | | $\Delta 3 \nabla C$ |
| <i>1RS</i> | 5'...TT-----ACGAAACCG... | | |

ITR terminus. The deletions rarely included the 3' TA dinucleotide and flanking *white* sequences. Allele *10R* was the only allele with a deletion accompanied by an insertion; in this case the 4-bp sequence TAAA was inserted at the site of deletion, which may have been copied from the flanking DNA to generate a 4-bp tandem duplication.

Although the alleles in Table 2 were isolated based on reduced or absent somatic mosaicism, their eye colors also differed from *wpch*, in general according to the length of the deletion. Thus, the smallest deletion was 73 bp in mutant *101R*, and the phenotype was dull red. The next smallest deletion was 375 bp in *7R*, and this phenotype was orange. All other deletions ranged from 385 to 1069 bp in length and the eye phenotype was pale yellow.

Secondary mutations at the 3' end: The dramatic difference between the 5' and 3' lesions was quite unexpected. One possibility is that there is a bias in the identification or recovery of 3' lesions, such that primarily large 3' deletions are isolated. To assess this possibility, we reexposed some alleles with 5' lesions and weak somatic mosaicism to active transposase from either *Mr182* or *MrX* and selected from each a derivative in which somatic mosaicism was reduced even more or was absent altogether. The alleles chosen were *86R*, *18R*, *48R*, *100R*, *22R*, and *1R* (Table 2). Secondary mutations were recovered in each of the lines. DNA sequencing showed that the original aberration within the 5' ITR remained in each of the secondary mutations, but that each had an additional deletion at or near the 3' ITR. The alleles with secondary mutations are denoted *86RS*, *18RS*, and so forth, and the 3' lesions are shown in the

bottom part of Table 2. All secondary alleles except *22RS* show no somatic mosaicism.

Unexpectedly, five of the six secondary mutations at the 3' end were small deletions of 1-9 bp. They were quite different from the large primary deletions at the 3' end shown in the upper part of Table 2. In fact, the secondary 3' deletions were similar in size and position to the deletions in the 5' ITRs in Table 1, except that they occurred at the 3' end instead of at the 5' end. For example, in the secondary allele *86RS*, nucleotide 1284 is deleted. This nucleotide is 3 bp from the 3' terminus of the element, and the deletion is similar in pattern to alleles *22R*, *40R*, *46R*, and *48R* in Table 1, in which nucleotide 3 is deleted at the 5' terminus. Similarly, the secondary allele *22RS*, which has a very weak mosaic phenotype of one to two spots per eye, has a 3-bp deletion at the 3' terminus accompanied by insertion of the single nucleotide C; the overall result is to change the dinucleotide flanking the 3' ITR from TA to CA, explaining the lower level of mosaicism. The secondary allele *1RS* was exceptional in including a relatively large 39-bp deletion spanning 18 bp of the 3' ITR, the TA dinucleotide, and 19 bp of flanking DNA of the *white* locus.

Template-directed gap repair: Many of the aberrant repair products in Table 1 have sequences duplicated from elsewhere in the *white* gene or the *peach* element, as would be expected of TDGR. To quantify how frequently TDGR accompanies transposase scission of *peach*, we made use of the unusual sequence of the *41R* allele in Table 1. This allele has a 1-bp deletion of the A in the 5' TA dinucleotide, which is replaced with a 6-bp insertion TACTGG. The net effect is that *41R* has

TABLE 3
Transposase-induced recombination hotspot

| Genotype | Recombinant | Nonrecombinant | <i>N</i> | <i>r</i> | χ^2 |
|--|-------------|----------------|----------|---------------|----------|
| $\frac{sn \quad wpch \quad lz \quad \Delta}{+ \quad wpch \quad + \quad MrX}$ | 590 | 8,511 | 9,101 | 0.065 ± 0.002 | |
| $\frac{sn \quad wpch \quad lz \quad \Delta}{+ \quad wpch \quad + \quad \Delta}$ | 479 | 8,182 | 8,661 | 0.055 ± 0.002 | 7.1 |
| $\frac{sn \quad \Delta \quad lz \quad \Delta}{+ \quad \Delta \quad + \quad MrX}$ | 673 | 13,064 | 13,737 | 0.049 ± 0.002 | 26.3 |

a 4-bp insertion at the 5' end of *peach*, which results in reduced mosaicism and a characteristic rosy-like phenotype. In a *wpch/41R* heterozygote, any *peach* scission events in *wpch* that are accompanied by TDGR from the *41R*-bearing homolog would yield the *41R* type of inverted repeat and the rosy-like phenotype. The unique 5' sequence of the *41R* element also enables molecular verification of the repair event (equivalent to a gene conversion), since the CCAGTA sequence at the 5' end of *41R* (Table 1) can be used to design *41R*-specific PCR primers that will not amplify *peach* or any other *mariner* elements.

Accordingly, crosses were carried out between females of genotype *sn*⁺ *wpch* *lz*⁺ *MrX/sn 41R lz* and males of genotype *w*⁻. The markers *sn*, *wpch*, *lz*, and *MrX* are at map positions 21.0, 27.0, 27.7, and 30.2, respectively (data not shown), and across the region *sn-wpch-lz* there is complete interference (data not shown). Among the male progeny of the cross were 225 *sn*⁺ *w*⁺ *lz*⁺, resulting from excision of *peach*, and 93 *sn*⁺ *lz*⁺ with an eye-color phenotype indistinguishable from that of *41R*. Genomic DNA from all 93 *41R*-like males or their progeny was tested by PCR with *41R*-specific primers, and all yielded the expected *41R*-specific amplification product. Consistent with the occurrence of TDGR, we had also observed occasional *sn wpch lz* male progeny in the original cross, in which the *41R* allele had become converted into an allele indistinguishable from *wpch*.

Transposase-induced hotspot of recombination: To test whether the *wpch* transgene becomes a recombinational hotspot in the presence of transposase, we carried out the experiments reported in Table 3. The symbol Δ , depending on its location in the genotypic formula, indicates absence of either the *wpch* or the *MrX* transgene. The experimental cross is in the top row. One type of control is the same as the experimental genotype but lacking a source of active transposase. The recombination fraction in the *sn-lz* interval in the control was 1.0 map unit smaller than that observed in the experimental crosses, which is statistically highly significant ($P <$

0.01). The other control lacked the *wpch* transgene as well as a source of active transposase. In this case the map distance was 1.6 map units smaller than that in the experimental genotypes, and this result has even greater statistical significance ($P \ll 0.01$). On the other hand, the map distances observed in the control crosses do not differ significantly from each other. Taken together, these results imply that *wpch* does act as a hotspot of recombination, but only in the presence of active transposase.

DISCUSSION

We have exploited the somatic activity of the *Mos1 mariner* transposase to screen for mutations in a nonautonomous target element *peach* that have an impaired ability to be excised. In the absence of transposase, the *wpch* transgene, which contains a *peach* insertion into the *white* gene, is genetically stable and the eyes are a uniform peach color. In the presence of transposase, the eyes are mottled red due to somatic excision of *peach*, but mutant *wpch* alleles with reduced mosaicism are recovered at a frequency of $\sim 1/500$ per generation. In the absence of transposase, mutations of this type are not recovered at any appreciable frequency. The screen is apparently very sensitive, since even 1-bp deletions in the 5' ITR were recovered. Altogether we isolated 100 mutant alleles and characterized them by DNA sequencing. Among these, 40 had aberrations in or near the 5' end of *peach*, 11 had aberrations in or near the 3' end of *peach*, and 29 had either an excision of *peach* leaving an unusual footprint or an excision or deletion of *peach* accompanied by deletion of flanking *white* DNA. The remaining 20 alleles were an eclectic collection of insertions, inversions, apparent local hops into nearby *white* sequences, and internal deletions. Most of the mutations appear to represent aberrant products from host-mediated repair of intermediates formed in the excision reaction.

In about half of the 5' mutations, the only alteration

was a small deletion, averaging ~ 6 bp, that always included the beginning of the 5' ITR (Table 1). Most of the other mutant alleles had a small deletion (8 bp average size) accompanied by the insertion of DNA sequences. The inserted DNA appears to have been copied from a template flanking the deletion endpoints, typically 0–10 bp distant from the site of insertion, and often shows evidence of replication slippage leading to two or more direct repeats of a short sequence (O'Hare and Rubin 1983; Staveley *et al.* 1995). A single-stranded overhang long enough to promote such events could result from host exonuclease acting on one or both ends of the transposase-induced scission. However, in one allele (*23R*), 143 bp had been copied from genomic DNA ~ 7 kb upstream from the *peach* element.

Deletions were also recovered in the 3' ITR (Table 2), but they were much less frequent (ratio $\approx 1:4$) and larger than deletions in the 5' ITR (average 669 vs. 6 bp). One possible explanation of this finding is that small deletions at the 3' end are not recovered because they have a negligible effect on the level of somatic mosaicism. To test this possibility, we chose six mutant alleles with small 5' deletions, each of which still exhibited somatic mosaicism in the presence of transposase, and selected a secondary mutation in which the mosaicism was reduced still further. In this case, most of the 3' deletions were typical in size and location to the 5' deletions (average size 9.5 bp; see Table 2), showing that, in fact, small 3' deletions can be detected when they occur.

The asymmetry of the 5' and 3' deletions therefore suggests a polarity in recognition of the ends of *peach* by the *Mos1* transposase, whereby the 5' end is recognized and cleaved first and usually somewhat protected during the repair process to minimize long deletions. When the 5' end is not recognized efficiently due to a small deletion in the 5' end, the 3' end is chosen occasionally as the substrate, and then typical small deletions are formed at the 3' end. The ITRs of *peach* are 28 bp in length and differ at positions 1, 16, 18, and 26; but perhaps internal recognition sites rather than (or in addition to) the differences in the ITRs account for the polarity. For the six alleles in which secondary 3' deletions were recovered (alleles *1R*, *18R*, *22R*, *48R*, *86R*, and *100R* in Table 1), the primary 5' deletion had an altered TA dinucleotide and one to three changes in the first three nucleotides of the 5' ITR. Certainly, secondary mutations at the 5' end resulting in even less mosaicism would have been detected had they occurred, but no mutations of this type were found. The absence of secondary 5' mutations suggests that the lesion in the 5' end prevented efficient recognition or strand scission by the transposase, and that the intact 3' ITR was targeted instead. For example, the allele *18R* (Table 1) has a 5' deletion that resulted in only two changes in *peach*: the TA dinucleotide is altered to TG, and the C at nucleotide 1 is changed to G. The location of the

secondary deletion at the 3' end in allele *18RS* (Table 2) suggests that, compared with an intact 5' end, the mutated 5' end in *18R* is a poorer substrate for the transposase than the intact 3' end, which in this case has only one change in the corresponding region (nucleotide 1286). These results further suggest that the region including the TA dinucleotide and nucleotides 1–3 of the 5' ITR are critical to enable the transposase to distinguish between the 5' and 3' ITRs.

The mutations with alterations in the 5' ITR often resulted in changes to the flanking TA dinucleotide and/or the extreme 5' end of the ITR. The altered response of these alleles to transposase provides information about the sequences required for transposase recognition. The TA dinucleotide adjacent to the 5' ITR is important, but not essential, for *peach* excision. This finding contrasts with those obtained for *Tc1*, in which the 5' TA appears to be essential for excision (Vos *et al.* 1996), and for *Tc3*, in which mutation of the 5' TA has no effect on excision (van Luenen *et al.* 1994). In the *mariners* system, one change in the 5' dinucleotide from TA to TC (allele *96R*) reduces somatic mosaicism significantly, and two changes (to GG in allele *41R* or to GT in allele *54R*) also reduce it (Table 1). However, in none of these cases was somatic mosaicism abolished. In the mutant *54R* allele, the footprint of germline revertants after *peach* excision was GTXXXTA, showing that transposase was able to recognize the 5' GT dinucleotide, despite the presence of TA dinucleotides in close proximity. Similarly, if the 5' TA dinucleotide is intact, but nucleotides 1, 2, or 3 of the ITR are deleted (or if there is a C to A transversion at nucleotide 2) somatic mosaicism is reduced but not abolished (Table 1). These results demonstrate that the TA dinucleotide and the first three nucleotides of *peach* are required for efficient excision. They also reveal the robustness of the *Mos1* transposase, because most mutant *peach* elements with 5' lesions can be attacked by the transposase, although with reduced efficiency.

The mutations observed in this screen differ significantly from those found with the *P* element (Staveley *et al.* 1995). These authors studied a *P*-element insertion in the 5' end of the *vestigial* gene in the allele *vg*²¹. Selection for phenotypic revertants, for a stronger *vestigial* phenotype, or for altered PCR fragment length from the *P* element yielded internal deletions in *P* in which the last 15–17 bp of the termini were retained. The deletions recovered from the phenotypic screens were smaller than those recovered from the PCR screen and were biased by the selection for stronger or weaker *vestigial* expression, but the PCR screen yielded almost complete deletions that retained only the terminal 15–17 bp on each end (occasionally as small as 10 bp). The terminal 15–17 bp bind with the inverted-repeat binding protein (IRBP), and the authors proposed that the IRBP acts to protect the *P*-element ends when transposition creates a double-strand gap.

In the case of *mariner*, the termini of the inverted repeats are not as strongly protected. Among the 5' lesions, we find deletions extending through the terminal 18 bp of the inverted repeat of 28 bp (allele *65R* in Table 1), and the 3' deletions often include the entire 3' inverted repeat (Table 2). In contrast, the very different lengths of the 5' deletions and the 3' deletions (average 6–8 vs. 669 bp) suggest that there may be some protection of the 5' end, perhaps by the transposase itself. In proposing such a model, one must be careful about possible biases introduced by phenotypic selection. Our selection was based on reduced somatic mosaicism, and it is conceivable that any *peach* element that retains the terminal few base pairs of the inverted repeats remains an efficient target for excision; therefore, only those lesions affecting these base pairs would be recovered. However, this possibility can be excluded based on two lines of evidence. The first is that, in three mutant alleles with 5' lesions (*41R*, *54R*, and *96R* in Table 1), the repair process by chance restored the exact sequence of the 5' inverted repeat, but the adjacent TA dinucleotide was altered to GG (allele *41R*), GT (*54R*), or TC (*96R*). Yet all three of these alleles show at least a 10-fold decrease in the number of mosaic spots per eye (Table 1). The second line of evidence is that, among the 20 mutant *peach* elements not discussed in detail in this article, 6 were found to have deletions completely internal to the inverted repeats, but they nevertheless reduced somatic excision significantly (data not shown).

Template-directed gap repair: The allele *41R* with its characteristic phenotype and 5' end afforded a unique opportunity to test for the presence of TDGR in *mariner* excision. This process is well established for the *P* element (Gloor *et al.* 1991), but has not been demonstrated previously for the *mariner/Tc1* superfamily. Transposable elements that move via a cut-and-paste mechanism cannot multiply easily in a population. Since the chromosome from which the element is “cut” loses a copy, whereas the chromosome with the “paste” gains a copy, there is no net gain in copy number due to transposition. TDGR using the element present in the sister chromatid, or in the homologous chromosome, as a template tips the balance, because each transposition accompanied by TDGR results in a net gain of one copy in the genome.

To estimate the rate of homolog-dependent gap repair, we tested the heteroallelic combination *sn*⁺ *wpch* *lz*⁺ / *sn* *41R* *lz* in the presence of transposase, where the *41R* allele was flanked by the closely linked markers *sn* and *lz* to rule out double recombination. Excision accompanied by *41R*-directed gap repair would lead to *sn*⁺ *lz*⁺ progeny with the characteristic rosy-like eye color of *41R* and the diagnostic 5-bp insertion at the end of the 5' ITR. Excision not accompanied by *41R*-directed gap repair would lead to *sn*⁺ *lz*⁺ progeny with the characteristic wild-type eye color. The data indicate that homolog-dependent gap repair of *mariner* is very efficient. For

wpch excision, the ratio of *peach* deletions to gap repair using a *41R* allele present in the homologous chromosome as a template is ~2.4:1. This value does not include use of the sister chromatid as a template, which might be expected to be at least equally frequent, yielding an overall frequency of TDGR of perhaps 50%. This fraction could be even higher to the extent that the sister chromatid is favored over the homolog as the repair template. The high frequency of TDGR explains why the maximum frequency of *wpch* excision observed as revertants to a wild-type eye-color phenotype is only ~15% (Lohe *et al.* 1997), since these are the (perhaps minority of) excision events not accompanied by TDGR. Under similar experimental conditions, a rate of ~15% is also observed for *P*-element precise excision, probably for the same reason (Johnson-Schlitz and Engels 1993).

Hotspot of recombination: Effects of transposable element mobilization on recombination have been examined closely in the case of the *P* element in *Drosophila* (Svoboda *et al.* 1995; Gray *et al.* 1996; Tanaka *et al.* 1997). Effects of *mariner* mobilization have not been examined previously. The studies reported here were motivated by the finding that two *mariner*-like elements in human chromosome region *17p11.2-p12* are hotspots of unequal recombination (Kiyosawa and Chance 1996; Reiter *et al.* 1996; Robertson *et al.* 1996). These elements are separated by about 1.5 Mb and flank the gene for peripheral myelin protein 22. Mismatching and unequal crossing over between the *mariner*-like elements result in reciprocal duplication and deletion products, the former associated with Charcot-Marie-Tooth disease type 1A (CMT1A) and the latter with hereditary neuropathy with liability to pressure palsies (HNPP).

In the case of *Drosophila*, we found that presence of a homozygous *wpch* element increased recombination by ~1 map unit in the *sn-lz* region in the presence of active transposase, but that neither *wpch* alone nor the transposase alone had any statistically significant effect (Table 3). The rate of recombination in the *sn-lz* region is ~1 map unit/250 kb (Heino *et al.* 1994). Hence, recombination is stimulated ~200-fold by the 1.3-kb *peach* element in the presence of active transposase. The increase is even greater to the extent that the target of the transposase is smaller than 1.3 kb. If the target consisted only of the ITRs, for example, the increase in recombination would be by a factor of ~4500, which makes it a very hot hotspot indeed.

We are grateful to the suggestion from John Sved that the demonstration of template-directed gap repair would be important in understanding the multiplication and persistence of *mariner* elements in natural populations. This caused us to be on the lookout for any *wpch* mutants with a structure that would allow this question to be addressed. We are also grateful to Nathan Blow for contributing some of the DNA sequencing. This work was supported by a grant from the Australian Federal Government (A.R.L.) and by National Institutes of Health grant GM-33741 (D.L.H.).

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