Prevalence of Localized Rearrangements vs. Transpositions Among Events Induced by Drosophila P Element Transposase on a P Transgene

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ABSTRACT

We have studied P transposase-induced events on a P[w] transgene, P[w\textsuperscript{+/+}], harboring the whole white gene with a 3.44-kb direct duplication of its 5' regulatory sequences (containing the ZESTE-binding region, ZBR). We have recovered mutations leading to an increase or a decrease of zeste' repression, generally as the consequence of modifications of the number of ZBR in close physical proximity and/or jumps to other sites. We describe mutants displaying deletions of the original duplicated sequence or increases in the number of repeats from two to three or four. Internal deletions are more frequent than amplifications. Both require the integrity of P element ends. We have also observed a high frequency of double P elements localized at the original P[w\textsuperscript{+/+}] insertion site. These double P elements are arranged in nonrandom configurations. We discuss the frequencies and the possible mechanisms leading to the various types of derivatives, in light of the current models for P excision and transposition. We propose that the P transposase induces mainly localized events. Some of these could result from frequent changes of template during gap-repair DNA synthesis, and/or from abortive transposition.

P elements are a family of transposable elements responsible for the phenomenon of P-M hybrid dysgenesis in Drosophila melanogaster (reviewed by Engels 1989). Complete P elements are 2907 bp in length and have 31-bp terminal inverted repeats (TIRs) (O'Hare and Rubin 1985). They are autonomously functional for transposition (Spradling and Rubin 1982). The complete P element contains four exons and encodes at least one overlapping genes, one for a 87-kD transposase protein and the other for a 66-kD protein (Rio et al. 1986). The latter is a repressor of P transposition (Robertson and Engels 1989; Coen 1990; Misra and Rio 1990; Lemaitre and Coen 1991). Shorter P elements usually derive from the 2.9-kb element by internal deletions (O'Hare and Rubin 1983). Defective P elements are unable to transpose autonomously but can be mobilized in trans by active elements (Spradling and Rubin 1982; Engels 1984). P elements generate an 8-bp duplication of genomic DNA at the site of insertion.

P elements are thought to transpose by a nonreplicative "cut-and-paste" mechanism (Engels et al. 1990; Kaufman and Rio 1992) starting by an excision of the element that results in a double-strand gap at the donor site. This gap is subsequently repaired by a process similar to gene conversion, using either the sister chromatid, the homologue or any homologous sequences inserted in the genome, as well as oligonucleotides, as a template for repair (Engels et al. 1990; Banga and Boyd 1992; Johnson-Schlitz and Engels 1993; Nassif et al. 1994). The repair process has been used for a gene replacement procedure, using the white gene of D. melanogaster and involving templates with single-base substitutions, deletions and insertions (Gloor et al. 1991; Johnson-Schlitz and Engels 1993; Nassif et al. 1994). Complex conversion events have led Nassif et al. (1994) to postulate that the two broken ends conduct an independent genome search for a template and may use two different templates. They have proposed a synthesis-dependent strand annealing (SDSA) model for the repair of the break. According to their model, each terminus of the break invades a template independently and serves as a primer for DNA synthesis, with the newly synthesized DNA being immediately displaced from the template. These single strands then pair in a region of overlap, and synthesis is completed by each using the other as a template.

We have studied P transposase-induced events on a P transgene, P[w\textsuperscript{+/+9,3}](19DE). This transgene harbors a complete white gene with 3.44 kb of its 5' regulatory sequences duplicated directly in tandem (C. Zucke, personal communication; Coen 1990). The white gene expression is repressed by the zeste' mutation. This effect requires the presence of two or more copies of white regulatory sequences brought into close proximity by homologous chromosome pairing or by tandem duplication (transvection phenomenon, reviewed by Pirrotta 1991). The regulatory sequences that lie 1185–1454 bp upstream of the transcription start site of the white gene contain the eye enhancer element.
and five binding sites for the ZESTE protein (Qian et al. 1992). This ZESTE binding region (ZBR) is necessary for the transvection phenomenon to occur. It is included in the 5′ white duplicated sequence in the P[w9.3] transgene (Figure 1). In a previous report, we have shown that the expression of the white gene carried by this transgene is repressed by the P-encoded repressor(s) (Coen 1990). This P repression occurs only in the presence of the zeste allele and is strongly dependent on the insertion site of this transgene: this effect is very rarely recovered at other sites in the genome (Coen 1990).

In the present work, we describe the recovery and analysis of mutations induced on this transgene by the P transposase. We have used the level of expression of the white gene, influenced by P and zeste repression, to monitor the modifications induced by the P transposase on the P[w9.3] transgene. Our prediction is that a darker phenotype (i.e., less repressed by zeste) would result in most cases, either from the loss of ZBR at the original site or from a jump to another site, where it is insensitive to P repression, and that a lighter phenotype (i.e., more repressed by zeste) would result, in most cases, from an increase of the number of ZBR in close proximity. These predictions were mainly confirmed. Our results show that the P transposase induces deletions and amplifications of the original duplicated sequence and that these two types of events occur at very different rates. We also describe many cases of double P elements at the original site. We observe that these double P elements are arranged in nonrandom configurations. The relative frequencies of all types of events observed, including transposition to other chromosomes, are discussed. Our results show that the P transposase induces preponderantly localized events on the P[w9.3] insertion. The implications of these results for the understanding of P element transposition are examined.

MATERIALS AND METHODS

The P[w9.3] transgene: This transgene (C. Zucker and D. Thierry-Mieg, personal communication) carries an EcoRI-KpnI fragment containing the whole white gene. This fragment includes 5′ and 3′ nontranscribed sequences sufficient for correct temporal and spatial expression of white (Hayashi et al. 1984; Lewis et al. 1985b; Pirrotta et al. 1985). In addition, P[w9.3] (Figure 1) harbors a direct tandem duplication of the 3.44-kb EcoRI-HindIII fragment. This fragment contains the 5′ regulatory sequences and the first exon (Pirrotta and Broccoli 1984), including the sequences responsible for the interaction with the ZESTE protein (Pirrotta 1991) (Figure 1).

Drosophila stocks: y z w118; M stock. w118 is a viable partial deletion of the white locus (Hayashi et al. 1984).

w9.3+ P[w9.3] (19DE): This M strain contains an insertion of the P[w9.3] transposon (Figure 1) at the cytological position 19DE (C. Zucker and D. Thierry-Mieg, personal communication; Rubin et al. 1985; Coen 1990). The inserted transgene and the strain harboring it will be abbreviated 9.3 and z w 9.3, respectively.
**FIGURE 1.**—Structure of the white gene and of the P[w*d1] transgene. (A) Genomic and transcription map of the white+ locus (adapted from HAZELRIGG 1987). Solid boxes, white exons; white ovals, ZBR. The sequences that have been duplicated in P[w*d1] transgene are indicated under the white+ map (diagonally hatched bar). Spotted bars (A—D), white sequence probes used in Southern blot analysis. The A probe is identical to the sequence duplicated in P[w*d1]. (B) The P[w*d1] transgene (C. ZUCKER, personal communication). The two P element termini are indicated by large black arrowheads at the extremities of the construct. Diagonally striped and open boxes represent, respectively, the duplicated and unique white sequences. Spotted bars represent P element specific probes, PS1 and PS3, used in Southern blot analysis (see MATERIALS AND METHODS). Small arrows above the construct (P1, P2, P3, P4 and W) represent the position and direction of primers used for PCR (see MATERIALS AND METHODS). Restriction enzyme sites: B, BamHI; Bg, BglII; E, EcoRI; H, HindIII; K, KpnI; S, Sau; X, XhoI.

**Probes:** Four plasmids containing white' sequences that cover almost the whole gene were used as probes (see Figure 1 for their localization on the white gene map). The pPS1 and pPS3 plasmids, containing respectively the HindIII (+40 to +878) and the SauI (+2411 to +2883) fragments of the original and pPS3 plasmids, containing respectively the HindIII (+40 to +878) and the SauI (+2411 to +2883) fragments of the cover almost the whole gene were used as probes (see Figure 1 for their localization on the white gene map). The pPS1 and pPS3 plasmids, containing respectively the HindIII (+40 to +878) and the SauI (+2411 to +2883) fragments of the cover almost the whole gene were used as probes (see Figure 1 for their localization on the white gene map).

**FIGURE 2.**—Genetic screen used to detect local modifications and transpositions of 9.3. (A) Use of P strains as sources of transposase. Dysgenic F1 males harboring the z w 9.3 X chromosome were individually crossed to compound-X females of the P cytotype. F2 males with eye color that differs from the P-specific brown phenotype were retained for further analysis. (B) Use of Δ2-3(99B) as a source of transposase. Dysgenic F1 males were individually crossed to C(1)DX (M) virgin females. F2 (Sb+) males with either normal red eyes or various nonred phenotypes were then crossed individually to C(1)DX (P) females to test the eye phenotype of their progeny in a P background. After 3 days, the same males were crossed to C(1)DX (M) females, to establish an M stock. Males giving in the first cross (P) progeny not composed exclusively of brown males and white females were retained for further analysis. The same crossing scheme was used following injection of the P helper into z w 9.3 embryos, replacing F1, dysgenic males by G0 males. G0 females were crossed to M5 males, and their G1 male progeny was treated as G0 males.
RESULTS

Instability of $P[w^{9}9.3](19DE)$ in dysgenic conditions: The $z^I w^{1118} P[w^{9}9.3](19DE)$ strain, abbreviated hereafter as $z^I w 9.3$, results from an insertion of the $P[w^{9}11]$ transgene (see Figure 1) into the 19DE locus on the X chromosome as determined by in situ hybridization (COEN 1990). The 9.3 transgene elicits a wild-type eye phenotype when placed in zeste' white individuals (males as well as homozygous females). In a zeste' background, males are almost wild type, but homozygous females display a patterned coloration, with a broken ring of orange spots on an almost white background, constituting an orange halo (Figure 4). A similar but darker phenotype was described for the $w^{9}11$ mutation (PETERSON et al. 1994). zeste' repression of the 9.3 transgene is enhanced by the somatic products of regulatory P elements (COEN 1990). This P repression leads to a brown eye phenotype in hemizygous males (Figure 5) and an almost white phenotype in homozygous females (COEN 1990). P repression has no effect in a zeste' background (COEN 1990).

The 9.3 insertion is very stable in the absence of active transposase: we did not observe any spontaneous mutations in the stocks during several years.

In contrast, in dysgenic crosses the 9.3 transgene is highly mutable. When dysgenic F$_1$ males harboring the $z^I w 9.3$ X chromosome are crossed to compound X females of the P cytotype (Figure 2A), the F$_2$ males are expected to have brown eyes, due to P repression on 9.3 (COEN 1990). Besides these normal brown males, mutants of various phenotypes occur at high rates in the F$_2$ male progeny (e.g., in Table 1).

A fraction of up to 70% of the F$_2$ males are white (Table 1). Mutants of this type are likely to result from the excision of the transgene or at least of a critical part of the white gene, abolishing its expression.

Other mutant males display various kinds of eye pigmentation, either stronger or weaker than that of the nonmutant males. They include spotted or patterned mutants, some of them expressing the same halo-patterned coloration that is seen in homozygous $z w 9.3$ females, despite the fact that they are hemizygous.

Exceptional nonwhite females are also recovered, most of them having wild-type eyes (Table 1). They are due to transposition of the 9.3 transgene to an autosomal location (COEN 1990). The rate of these transposition errors is very low, the $w^{1118} 9.3$ line. The flies were reared at 25°C. Male is on the left and homozygous female on the right.

FIGURE 4.—Phenotype of $z^I w^{1118} 9.3$ line. The flies were reared at 25°C. Male is on the left and homozygous female on the right.

Figure 5.—Phenotypes of $z w 9.3$ males and of $z w X6$ males in M and P backgrounds. The flies were reared at 25°C.
Many P-induced localized rearrangements (PLRs) have been investigated. One of the most frequent with a relative frequency of 81.5% is the P-red phenotype, induced by various sources of transposase. This phenotype will be called "P-red", in contrast to the normal "P-brown" phenotype of z w 9.3 (P) males.

P-red mutants may also be obtained in an M cytotype. This is achieved by using Δ2-3(99B) (Robertson et al. 1988) as a source of transposase (Figure 2B) or by injecting the helper P element π25.7wc (Karefess and Rubin 1984) into z w 9.3 embryos. F2 males with red eyes are selected in the progeny of the dysgenic individuals. They are then crossed individually to C(1)DX (M) females to establish an M stock. Males giving progeny in the first cross (P) composed exclusively of brown males and white females are discarded as nonmutants. Males giving progeny containing at least a fraction of males with red eyes are retained (see below).

Genetic and molecular analysis of the P-red mutants: Genetic analysis of 119 independent mutants of the P-red phenotype, induced by various sources of transposase (P strains, Δ2-3(99B) or injection of the helper) allowed grouping them into three classes: (1) 97 are X-linked; (2) eight are due to transposition to an autosomal location, the original 9.3 insert showing no phenotypically detectable modification; and (3) 14 result from transposition to an autosomal location accompanied by a modification (e.g., excision) of the original insert. The first class (X-linked mutants) is by far the most frequent with a relative frequency of 81.5%. The frequencies of the two other classes (6.7 and 11.7%) are not significantly different from each other.

The X6 derivative was obtained by injection of the helper P element into z w 9.3 embryos. X6 males display the same eye coloration in an M or a P background (Figure 5). This showed that this mutant has indeed become insensitive to P repression. The homozygous X6 females display the same red eye color as hemizygous males (data not shown). Southern analysis of X6 genomic DNA revealed that X6 results from an excision of one of the two copies of the 3.44-kb white5' sequence duplication (Figure 6). This deletion appears to be precise at this level of analysis: the digestion pattern showed the conservation of all the sites tested except those included in the originally duplicated sequence. This Southern analysis showed no difference in the flanking sequences, indicating that the internal deletion event is not accompanied by transposition. PCR amplification and sequencing of the 5' flanking DNA confirm that X6 is at the same site as the original 9.3 insert (Figure 7A).

Southern analysis of genomic DNA was performed on 36 other X-linked P-red mutants. This analysis (results displayed in Figure 6) showed that 31 mutants among 36 are due to the same type of event as X6: an in situ excision of one of the two copies of the 3.44-kb white5' sequence duplication, appearing precise at the level of resolution of Southern analysis. Two derivatives are due to the same type of deletion accompanied by a modification of the flanking sequences (X45 and X42). The last three harbor larger internal deletions (X28, X37 and X43) (Figure 6). All of the X-linked P-red derivatives analyzed are due to modifications involving at least the deletion of one copy of the original 3.44-kb duplicated sequence. Therefore, P transposase appears to induce deletions of an original tandem repeat internal to P ends at a high rate.

P transposase induces phenotypic variants displaying increased zeste' repression: Mutants with a lighter eye pigmentation than the original 9.3 phenotype represent up to 7.7% of the total F2 progeny depending on
the transposase source (Table 1). In all cases, they occur less frequently than P-red mutants.

These mutants exhibit phenotypes ranging from brown to almost white (e.g., in Table 2). They include spotted or halo-patterned colorations. Twenty-nine independent mutants of this class, displaying various phenotypes, were genetically analyzed as described for the P-red mutants. Two classes were observed: 26 (out of 29) are X-linked and three result from a transposition to an autosomal location accompanied by an excision of the original insert in the same gamete. As with the P-red derivatives, the great majority (89.7%) of the lighter derivatives involve X-linked events.

Further analysis of 17 out of the 26 X-linked lines was undertaken. Their phenotypes are described in Table 2. One exceptional mutant line, named X80, displays the same eye phenotype in hemizygous males as well as in homozygous females. This phenotype is identical in all M and a P background (data not shown). For the other 16, the phenotype of homozygous females is lighter than that of hemizygous males, revealing that they remain sensitive to transvection (data not shown). Additionally, unlike X80, all of them remained sensitive to P repression (data not shown).

Genomic DNA from these 17 X-linked mutants was analyzed by Southern blotting; several restriction digests were performed and the blots were sequentially hybridized with probes covering the P[w9.3] transgene and the 5′ flanking sequence of 9.3 (see Figure 1 and MATERIALS AND METHODS). Southern blot analysis of X80 revealed the presence of one complete P[w9.3] element not inserted at the original 9.3 site, but at least 8 kb from it (data not shown). However, in situ hybridization shows that the X80 element lies in 19DE like 9.3 (data not shown). Therefore X80 appears to result from a local transposition event a short distance from the starting site, associated with an apparent excision of the donor element. For the 16 other 9.3 derivatives displaying a lighter phenotype, molecular analysis has shown no modification of flanking sequences, revealing that the inserts are localized at the original 9.3 insertion site. They can be grouped into two general classes: internal amplifications and double P elements.

Amplification of the original duplicated sequence: Three 9.3 derivatives are due to an increase in the number of copies of the previously duplicated 3.44-kb sequence. X7 results from an amplification from two to three repeats, everything else being unmodified (Figure 8). X13 and X87 are the consequence of an amplification from two to four repeats (Figure 8). As for the deletions, the amplifications appear to be precise at this level of analysis. The P transposase can therefore induce amplifications of a tandem repeat internal to a P element, although at a lower frequency than deletions.

Double P elements: The 13 other mutants displaying an increased zestel repression harbor a second element close to the original insert. The molecular characteristics of the different kinds of double P elements are shown in Figure 8. Double P elements can be classified into three categories. (1) The two elements are complete and one element is inside the other. X93 harbors a second P[w9.3] element in the 3′ region of the original insert, in reverse orientation. The two elements are unmodified and have kept the original duplicated 3.44-kb sequence (Figure 8). PCR amplification and direct sequencing of the DNA flanking the internal element

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**Figure 6.**—Molecular structure of P-red derivatives. The P[w9.3] element insertion in 19DE (9.3) with the restriction map of flanking sequences determined by Southern blot analysis. All the P-red derivatives are X-linked, except T20, which results from an autosome insertion (symbolized by a zig-zag line). The 32 9.3 derivatives resulting from an excision of one of the two copies of the original tandem repeat are identical according to Southern blot analysis. Twenty-four out of 32 were generated by P strains or injection of a helper P element; the remaining eight derivatives were induced by Δ2-3(99B). The five lines (X45, X42, X43, X28 and X37) displaying more complex deletion events were obtained with Δ2-3(99B). Dashed lines and dashed boxes, deleted regions; gray boxes, uncertainty on the limits of the deletion. Refer to Figure 1 legends for all other symbols. For more legibility, the restriction sites have been named sequentially by letters in alphabetic order. Restriction enzyme sites: a, BamHI; b, HindIII; c, Xhol; d and e, AvaI; f, BamHI; g, HindIII; h and h′, EcoRI; i and i′, BamHI; j, EcoRI; k, AvaI; l, Xhol; m, AvaI; n, Xhol; o, BamHI; p, SaI; q, AvaI; r, SaI; s, AvaI; t, SaI; u, polylinker (BamHI and HindIII); v, AvaI; w, Xhol; x, BamHI.
**P-Induced Localized Rearrangements**

**A**

**Left**

9.3  
\[
gatcTTGGCAGGATATGAAATAACATAAGGTTGTCGCCGCTGATA
\]

X6  
\[
gatcTTGGCAGGATATGAAATAACATAAGGTTGTCGCCGCTGATA
\]

X13.1  
\[
gatcTTGGCAGGATATGAAATAACATAAGGTTGTCGCCGCTGATA
\]

**B**

**Right**

X6  
\[
TGCCGACGGGACCACCTTATGTATTTTATCATCATGATACAGAAGAGAGCGC...
\]

X13.1  
\[
TGCCGACGGGACCACCTTATGTATTTTATCATCATGATACAGAAGAGAGCGC...
\]

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**FIGURE 7.**—Genomic DNA sequences flanking the 9.3, X6 and X13.1 insertion sites, and TIRs sequence of the elements. The sequences were amplified by the inverse PCR procedure, and the product of the reaction was directly sequenced (see MATERIALS AND METHODS). Bold face characters represent the 8-bp duplicated target site. Sequences underlined or topped with an arrow belong to the 31-bp TIRs of the P element. (A) Sequences at the left side of the elements (i.e., the 5' side of the 9.3 and X6 elements according to their orientation and the 3' side of the X13.1 element that is in reverse orientation). The 3' 31-bp TIR of the X13.1 element contains an additional 26 bp of directly duplicated sequence. Small caps indicate the restriction site of NdeII (gatc), which was used in the inverse PCR procedure on the left side. (B) Sequences at the right side of the elements. The 3' side of the 9.3 insert has not been sequenced. The 5' 31-bp TIR of X13.1 contains a duplication of the first 17 bp of P sequence in a palindromic structure, associated with the deletion of base pair number 18.

in X93 was performed. This showed that the internal element is inserted at the position 14182 (in white sequence coordinates) (O'HARE et al. 1984) at a distance of 1326 bp from the 3' end of the external element. The intact 5' and 3' ends of the internal element are flanked by direct duplication of 8 bp of its insertion site in the primary element, as expected from a normal transposition. (2) The two elements are contiguous and one of them is rearranged. Eight independent mutants were shown to result from the presence of two contiguous elements in the original 9.3 insertion site, associated with a rearrangement (deletion or inversion) of one of the two elements.

X5, X88 and X98 contain a 5' element missing its 3' region and a second complete P[wdr] element arranged in the same orientation (Figure 8). In the case of X98, the two elements harbor an amplification to three copies of the original 3.44-kb tandem repeat.

In the other mutants (X84, X86, X96, X79 and X71), one of the two elements contains an inversion, and the second one is unarranged (Figure 8). In all cases both elements have kept the original 3.44-kb duplication of P[wdr]. The inversion involves either the 3' extremity of the 5'-most element (X84, X86 and X96) or the 5' extremity of the 3'-most element (X71). One last mutant, X79, harbors two contiguous elements associated with complex rearrangements that have not been fully elucidated.

The junction between the two elements present in X93 was sequenced after PCR amplification. The 5'-most element ends at position 14197 in white coordinates (O'HARE et al. 1984): 1371 bp of the 3' end of P[wdr] are missing. The 5'-most element is directly joined to the 5' end of the 3'-most P[wdr] element (data not shown).

The same analysis was performed on X86 genomic DNA. A fragment containing almost all the inverted sequence from the 5'-most element and the junction with the 3'-most element was PCR amplified. Direct sequencing of this junction reveals that the intact 5' P end of the 3'-most element is joined to position 14088 in white coordinates (O'HARE et al. 1984), the inverted sequence therefore consisting of 1420 bp from the 3' end of P[wdr]. As we did not sequence the other junction (5'-most element 3' P end of the inverted sequence) we do not know if the X86 inversion is precise or if some nucleotides are missing or duplicated.

(3) The two elements are arranged in a palindromic structure. After Southern blot analysis, X64, X65, X67 and X75 appeared identical. They display a second ele-
position of method leads us to conclude that there is <50 bp.

PCR amplify and sequence the junctions between the elements have been unsuccessful, presumably because of their palindromic structures.

In conclusion, all the 9.3 derivatives more sensitive to P repression are due either to an internal amplification of the number of ZBR or to the integration of a second element within or very close to the starting insert.

Analysis of autosomal inserts resulting from the transposition of 9.3: Eighty-two independent autosomal insertions generated by the transposition of 9.3 (77 described in COEN 1990; five in this study) were examined. Most of them display a wild-type eye coloration. Only two are sensitive to P repression when in a zeste' background. One, T37, is localized at cytological position 36AB (as determined by in situ hybridization, not shown). The other line, z w; CyO, C4/T(2;3)ap, was recovered in a search for insertions located on the CyO balancer chromosome.

Molecular analysis of 29 of these autosomal insertions, including the two that proved to have a cytotype-dependent expression, was performed by genomic Southern blotting (data not shown).

In 18 cases, the internal structure of the P[w] element appeared unchanged after transposition. These cases include T37, one of the autosomal insertions sensitive to P repression. In nine cases, the insert appeared to have undergone a precise deletion of the 3.44-kb

**Figure 8.** —Molecular structure of P transposase-induced 9.3 lighter derivatives. All these 9.3 derivatives were generated by the use of Δ2-3(99B) as a source of transposase, except X7, X13 and X5, which were induced by injection of the helper P element into z w 9.3 embryos. The phenotypes corresponding to the different X-linked lighter derivatives are given in Table 2. Legends are as in Figure 6. X13 and X87 are strictly identical (by Southern blot analysis). The structures of X5 and X88 are similar but differ only by the size of the 3' missing sequence: 1371 bp in X87 and ~2 kb in X88. The two elements of X98, although harboring an amplification to three copies of the original tandem repeat, are otherwise arranged as in X5. X84, X86 and X96 display a similar double P structure, with a 5'-most element containing an inversion of its 3' extremity and a second unmodified element. The size of the inverted sequence is ~1.4 kb in X86 and X96, and 1.0 kb in X84. The 3'-most element in X71 contains a 6-kb inversion of its 3' extremity, the other element is unmodified. X64, X65, X67 and X75 are strictly identical according to Southern blot results.
duplication, leading to a structure analogous to that of X6. One of these lines contains two insertions of the precisely deleted transgene. In one case, an imprecise deletion had occurred (see T20 in Figure 6). Finally the C4 insertion displays an amplification of the duplicated sequences to four copies, leading to a structure similar to X13 or X87 (data not shown).

Therefore, 38% of the new insertions to autosomal locations are associated with modifications of the transposed insert (internal deletions or amplifications) resembling those recovered at the original insertion site.

Reversion of X6 insensitivity to P repression: X6, which results from a precise deletion of one copy of the original tandem repeat internal to 9.3, is insensitive to P repression (Figure 5). Presuming that this insensitivity was due to a lower sensitivity to zeste' repression, we decided to look for derivatives more sensitive to zeste' repression. To do so, the z w X6 line was put in dysgenic conditions according to the crossing scheme depicted in Figure 3, and we looked for F2 (Sb') females exhibiting an eye color lighter than the normal red of homozygous X6 females. They occurred at a rate of 7.8%. The modified X chromosomes, which they received from their dysgenic fathers, were recovered and 17 lines were established.

Males from these various lines display eye phenotypes ranging from almost wild type to orange, including spotted colorations. Their phenotypes are described in Table 2. All but one (X6.4) show a reversion to sensitivity to P repression: they display a darker eye color in an M background than in a P background (data not shown).

Hemizygous males and homozygous females of the exceptional X6.4 mutant line display the same spotted orange phenotype, in M and P backgrounds. Molecular analysis of X6.4 reveals that the mutation results from a 1.4-kb internal deletion (Figure 9) that removes the eye-specific enhancer and the ZBR (Qian et al. 1992). The 16 other X6 derivatives are double P elements located at the same site as the original X6 insert.

Southern analysis of X6.23 reveals that it contains two nested elements in opposite orientation (Figure 9). This structure is very similar to that of X93 (a 9.3 derivative, see Figure 8). They differ only by the number of the 3.44-kb 5' regulatory regions of white gene and by the insertion site of the second element within the first (insertion at position 14182 in white sequence coordinates in the external element for X93, and ≈800 bp downstream of that position for X6.23). No nested element in direct orientation was recovered.

All the 15 remaining X6 derivatives analyzed contain two very close elements in reverse orientation. Most of them (13) display, at the original site, the same double X6-type element in a 5' reverse configuration (see X6.20 and others in Figure 9). They are identical to one category of 9.3 derivatives described above (X64, X65, X67 and X75, see Figure 8) at the level of Southern analysis.

X6.9 differs from the 13 previous ones only by the fact that both elements contain the same small deletion of ~500 bp of white sequences (Figure 9).

X6.7 is the only mutant displaying a structure not already seen with 9.3 derivatives. X6.7 harbors a double X6-like element, at the original X6 site, the two elements being in reverse orientation (Figure 9). However the two elements are arranged 5' to 3' instead of 5' to 5', like all the other double P elements that we have described before. In conclusion, all the X6 derivatives more sensitive to zeste' repression are due to the presence of two nested or very close elements in reverse orientation.

A derivative of X13 is unable to transpose or to excise: In a test for mobilization of X13 (a 9.3 derivative harboring four copies of the original duplicated sequence) (see Figure 8), a variant appeared that was selected because of its peculiar phenotype. Instead of the X13 light halo patterned coloration, this derivative, named X13.1, exhibits a red crescent-shaped pattern at the posterior part of the eye (data not shown) reminiscent of the w\textsuperscript{asal} allele phenotype (Peterson et al. 1994). X13.1 remains sensitive to P repression (data not shown). However, it seems insensitive to P transposase: it neither transposes nor excises nor gives derivatives with an altered phenotype. A stock harboring the X13.1 element together with the Δ2-3(99B) element was maintained during more than one year with no detectable modification. In situ hybridization on polytene chromosomes showed that the X13.1 insert was at the same site as X13 (19DE, data not shown). This was confirmed by Southern blot analysis. However, X13.1 appeared to be in the opposite orientation, as compared to X13 or 9.3. Besides, the internal structure of the element appeared unmodified: it had kept the four copies of the 5' regulatory sequences present in X13.

Analysis of the ends and flanking sequences of the X13.1 element was undertaken by inverse PCR and sequencing (Figure 7). It revealed that the flanking sequences on both sides are exactly the same as those of the 9.3 insert, the 8-bp duplicated target sequence remaining unchanged, and confirmed the reverse orientation of X13.1 (Figure 7). In addition, it showed that the 31-bp TIRs have been modified at both X13.1 ends. On the 3' side, the 31-bp TIR contains a direct duplication of 26 bp (Figure 7A). At the 5' end, the first 17 bp of P sequence have been duplicated in a reverse orientation, giving a palindromic structure, and base pair number 18 (following the palindrome) is deleted (Figure 7B).

DISCUSSION

The results presented here show that P transposase induces preponderantly localized events on the P\textsuperscript{w\textsuperscript{asal}} derivative. The types of X-linked events that we have recovered are summarized in Table 2 with their esti-
<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Phenotype</th>
<th>ZBR number</th>
<th>Molecular structure</th>
<th>Total of events</th>
<th>Percentage of events</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>9.3 derivatives</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal rearrangements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplification of the 3.44 kb white</td>
<td>X7</td>
<td>Dark orange halo</td>
<td>3</td>
<td>One element containing precise amplification to three copies</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td>duplicated sequence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X13 and X87</td>
<td></td>
<td>Yellow halo</td>
<td>4</td>
<td>One element containing precise amplification to four copies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X98</td>
<td></td>
<td>Quasi white</td>
<td>2 × 3</td>
<td>Two contiguous elements in direct orientation (see below) containing precise amplification to three copies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precise deletion of the duplicated</td>
<td>X6 and 31 others</td>
<td>P-red</td>
<td>1</td>
<td>One element with a precise deletion of 3.44 kb repeat</td>
<td>38</td>
<td>12.8</td>
</tr>
<tr>
<td>sequence</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X42 and X45</td>
<td></td>
<td>P-red</td>
<td>1</td>
<td>One element with a precise deletion of 3.44 kb repeat and a modification of the flanking sequences</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X64, X65, X67 and X75</td>
<td>Brown spotted</td>
<td>2 × 1</td>
<td></td>
<td>Two elements in reverse 5’ configuration (see below) containing a precise deletion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imprecise deletion</td>
<td>X28, X37 and X43</td>
<td>P-red_temperature</td>
<td>1</td>
<td>Unprecise deletion encompassing one ZBR or undetermined deletion</td>
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<tr>
<td><strong>Double P elements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Separated by few bp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse 5’ configuration</td>
<td>X64, X65, X67 and X75</td>
<td>Brown spotted</td>
<td>2 × 1</td>
<td>Both elements are complete</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td>Reverse 3’ configuration</td>
<td></td>
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<td>No double insertion in reverse 3’ configuration</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse orientation</td>
<td>X93</td>
<td>Orange halo</td>
<td>2 × 2</td>
<td>Both elements are complete</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>(see discussion)</td>
<td></td>
<td>Yellow halo</td>
<td>2 × 2</td>
<td>One element with a 1.4 kb deletion of its 3’ end/one complete element</td>
<td>7</td>
<td>2.8</td>
</tr>
<tr>
<td>X88</td>
<td></td>
<td>Yellow halo</td>
<td>2 × 2</td>
<td>One element with a 2.0 kb deletion of its 3’ end/one complete element</td>
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<tr>
<td>X98</td>
<td></td>
<td>Pale yellow</td>
<td>2 × 3</td>
<td>One element with a 1.4 kb deletion of its 3’ end/one complete element</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X71</td>
<td></td>
<td>Brown halo</td>
<td>2 × 2</td>
<td>One complete element/one element with a 6.0 kb inversion of its 5’ end</td>
<td></td>
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<tr>
<td>X84</td>
<td></td>
<td>Orange halo</td>
<td>2 × 2</td>
<td>One element with a 1.0 kb inversion of its 3’ end/one complete element</td>
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<tr>
<td>X86, X96</td>
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<td>Orange halo</td>
<td>2 × 2</td>
<td>One element with a 1.4 kb inversion of its 3’ end/one complete element</td>
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<tr>
<td>Unknown orientation</td>
<td>X79</td>
<td>Brown</td>
<td>2 × 2</td>
<td>Complex rearrangements</td>
<td>1</td>
<td>0.4</td>
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<tr>
<td>Insertion at a distance</td>
<td>X80</td>
<td>Brown spots on an almost white background</td>
<td>1 × 2</td>
<td>Excision of the original element and insertion of an unmodified element at ≥8 kb (but still in 19DE)</td>
<td>1</td>
<td>0.4</td>
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<tr>
<td><strong>X6 derivatives:</strong></td>
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<td></td>
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<tr>
<td>Internal rearrangements</td>
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<td></td>
</tr>
<tr>
<td>Imprecise deletion</td>
<td>X6.4</td>
<td>Orange spotted</td>
<td>0</td>
<td>One element containing a 1.4 kb internal deletion that removes the ZBR</td>
<td>2</td>
<td>0.9</td>
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<td>X6.9</td>
<td>Brown spotted</td>
<td>2 × 1</td>
<td></td>
<td>Two elements in reverse 5’ configuration (see below) containing the same internal deletion of ~500 bp</td>
<td></td>
<td></td>
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</tbody>
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TABLE 2
Continued

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Phenotype</th>
<th>ZBR number</th>
<th>Molecular structure</th>
<th>Total</th>
<th>Percentage of events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double P elements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Separated by few bp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse 5' configuration</td>
<td>X6.20 and 12 others</td>
<td>Brown spotted</td>
<td>2 x 1</td>
<td>Both elements are complete</td>
<td>14</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>X6.9</td>
<td>Brown spotted</td>
<td>2 x 1</td>
<td>Both element have the same internal deletion of ~500 bp</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Reverse 5' configuration</td>
<td>X6.7</td>
<td>Quasi-red</td>
<td>2 x 1</td>
<td>Both elements are complete</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Nested</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse orientation</td>
<td>X6.23</td>
<td>Red</td>
<td>2 x 1</td>
<td>Both elements are complete</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Direct orientation</td>
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<td></td>
<td></td>
<td>(No double element in direct orientation)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Eye phenotype of hemizygous males at 25°.

* All these elements are localized at the original 9.3 insertion site except X80.

* Some double P derivatives have been considered twice: once as a double P element and once as an internal rearrangement (X98, X64, X63, X67, X73 and X6.9).

* Estimated percentage of each type of event among the gametes of 9.3 or X6 males bearing Δ2.3(99B) as source of transposase (based on the data from Table 1 and text).

* See Figure 6 for an example.

estimated frequencies. The frequencies of the different events vary depending on the sources of transposase (Table 1). As most of the derivatives studied were obtained with Δ2.3(99B), their frequencies of occurrence will be referred to in the following discussion and used to estimate the relative frequencies of each type of derivatives (Table 2). We observe high rates of deletions or amplifications of internal repeats and/or occurrence of double P elements in various configurations. Recently, other authors have reported results similar to ours: transposition at close proximity (EGGLESTON 1990; DANIELS and CHOYNIK 1993; TOWER et al. 1993; ZHANG and SPRADLING 1993; DORER and HENIKOFF 1994) and internal amplification or deletion of repeated sequences (PAQUES and WECNEZ 1993; KURKULOS et al. 1994). Our experimental design allows us to recover X-linked events as well as transposition to other chromosomes in the progeny of the same cross and therefore

![Figure 9](image-url)

**Figure 9.**—Molecular structure of P transposase-induced X6 derivatives. Mutants were recovered according to the mating scheme shown in Figure 3. Dashed boxes, deleted regions. Other legends are as in Figure 6. X6.4 contains an internal deletion that removes the ZBR and the eye-specific enhancer. The 13 X6 derivatives that display double P element in 5' reverse configuration are identical at the level of Southern blot analysis. X6.9 differs from them only by a small deletion of ~500 bp, contained in both elements. This deletion appears included in the first large intron of the gene (see Figure 1) and therefore does not dramatically influence the white expression.
to assess their relative frequencies. Localized events appear to occur at a high rate (18.7%) in the progeny of dysgenic 9.3 males. The first conclusion that can be drawn is that their frequency is by far greater than the frequency of transposition to autosomal sites (4%). If all localized events are the consequence of the repair of the break caused by the excision of the target P element (ENGELS et al. 1990), one should conclude that all excisions do not lead to an insertion event as expected from a conservative model for transposition. Either most of the excised elements are lost after excision (abortive transposition) or most of the events do not intrinsically give rise to a transposition intermediate (see below). Alternatively, it could be possible that most of the transposed inserts have lost the white gene expression and are therefore undetectable. This could be due either to internal rearrangements or to position effect associated with the new insertion site (e.g., heterochromatic sites).

**P transposase induces deletions and amplifications of directly repeated sequences at high frequency:** In all cases, the X-linked P-red derivatives result from the loss of one of the ZBR. Most of them have undergone a precise (at this level of resolution) deletion of one copy of the original tandem repeat contained in \( P[w^{9}]] \) [34/37 (91.8%), including the two cases where it was accompanied by another event]. This type of event is also recovered among double P elements localized at the original site (X64, X65, X67 and X75) and among insertions to autosomal sites (9/29). The deletion of one copy of the original tandem repeat is therefore present in up to 14% of all the gametes of dysgenic 9.3 males.

In contrast, some mutants that display a higher sensitivity to zeste repression are due to an increase to three or four copies of the 3.44-kb tandemly arranged repeat in the \( P[w^{9}] \) transgene. Internal amplification occurs at the original site within simple (X7, X13, X87) or double insertions (X98) and is also found within a transposed insert. This type of event represents 1.7% of all the gametes of dysgenic 9.3 males.

The kind of rearrangements that we have observed here have also been reported by other authors. PAQUES and WEGNEZ (1993) have described frequent deletions and amplifications of tandemly repeated ribosomal 5S genes located within a P element. KURKULOS et al. (1994) have observed 2% deletions between direct repeats separated by several kilobases of unique sequences. More recently, THOMPSON-STEWART et al. (1994) have described transposase-induced deletions and amplifications of repeated sequences flanking a P element. Hence, it appears that \( P \) transposase frequently induces deletions and amplifications between direct repeats contained within a \( P \) transgene or outside it.

**Internal deletions and amplifications require the integrity of \( P \) element ends:** The exceptional derivative \( X13.1 \) is insensitive to any transposase-induced rearrangement: in dysgenic conditions, the \( X13.1 \) insert appears unable to excise, to transpose or to undergo internal deletion or amplification, although it contains four tandem repeats of the 3.44-kb white 5' sequence. Sequence analysis reveals that both \( X13.1 \) TIRs are modified (Figure 7). The addition of 26 bp directly repeated at one \( P \) end has already been described and has been shown not to prevent transposition (TAKASU-ISHIKAWA et al. 1992). Thus the immobility of \( X13.1 \) is probably not due to the 26-bp direct duplication at the 3' TIR but rather to the presence of the palindromic repeat of 17 bp associated with a 1-bp deletion at the other terminus (Figure 7). This is in agreement with the study of MULLINS et al. (1989) that showed that the integrity of the 31-bp inverted repeat is absolutely required for transposition.

This result clearly shows that, as with transposition, internal deletions and amplifications of direct repeats require, at least, an intact 5' TIR. They are therefore a direct or indirect consequence of the process of \( P \) transposase-induced excision and/or transposition.

**Mechanism for excisions and amplifications of direct repeats:** According to the SDSA (synthesis-dependent strand annealing) model (NASSIF et al. 1994), \( P \) element excision results in a double-strand break (Figure 10A) that can be extended by degradation of the ends. Each 3' end then invades independently a homologous sequence and acts as a primer for the initiation of DNA repair synthesis (Figure 10B). The growing single strands are released while the replication proceeds, displacing only a local loop of DNA (bubble migration). When overlapping sequences have been replicated, i.e., when the growing strands contain homologous sequences, the two extended termini can anneal. The process is then terminated as in the single strand annealing (SSA) model (LIN et al. 1984; HABER 1992; SUGAWARA and HABER 1992) by removal of any nonhomologous sequences (that may have been synthesized beyond the overlap), DNA repair synthesis (each strand serving as a template for the other), and ligation.

The SSA model has been proposed to explain deletions between direct repeats in yeast (HABER 1992; SUGAWARA and HABER 1992). KURKULOS et al. (1994) have used this model to interpret the occurrence of deletions between internal direct repeats included in a \( P \) element: when homologous sequences are encountered on both growing strands, annealing between them is possible leading therefore to premature termination of the repair process. To explain amplifications from two to four repeats, one needs to add some features to this model.

1. The invading strand could change template during the repair process, making a "copy-choice" possible (Figure 10). The work of GONZY-TRÉBOUL et al. (1995) strongly supports the hypothesis of template change: they described chimerical elements that contain sequences from two \( P \) elements used successively as templates for the repair process. When repeats are encoun-
DNA synthesis could proceed further, allowing amplifications to occur (see below and Figure 10). In our results, the ratio of deletion vs. amplification is highly biased toward deletions (14% deletions compared to 1.7% amplifications in the whole progeny of 9.3Δ2-3(99B) males). Jumping between repeats should occur at similar frequencies forward and backward, leading to approximately equal frequencies of deletions and amplifications. This high excess of deletions can be explained if we consider that the growing single strands are in constant search for homology. Therefore, in most but not in all cases, the repair process would be terminated as soon as homologous sequences were encountered.

In the P[w] transgene, the size of the P 5' end + two duplicated regions (7470 bp) is approximately equivalent to the size of the unique region of white + P 3' end (7481 bp). Therefore, if the extension proceeds at a similar speed from both 3' ends, they will reach homologous sequences approximately in the middle of the P[w] element (Figure 10C). The right-side strand will be able to pair with the left-side strand, with equal frequencies, to repeat 2 (restoring the duplication) or 1 (leading to a deletion). If forward jumping occurs (Figure 10D), the result will be a deletion in most if not all cases. If backward jumping occurs, in most cases only two repeats would have been copied on the left-side strand when the right-side strand will reach the repeated region (Figure 10E). This will result again in deletion or restoration of the duplication with equal probabilities, depending on which repeat the right-side strand will anneal to (1 or 2, respectively). The recovery of an amplification will require that the repair process is not interrupted as soon as homology is encountered. In this case (Figure 10F), it is possible to obtain a deletion, restoration of the duplication, or tri- or quadruplication, depending on which repeat of the left-side strand (1, 2 or 3) will anneal to which repeat of the right-side strand (1 or 2). The overall result will be a strong excess of deletions or restorations of the duplication vs. amplifications. One prediction of this model is that the ratio of the different types of events will strongly depend on the size and position of repeats but also on their sequence, which could have an effect on the speed of the replication and its stalling at some positions (see Chedin et al. 1994 and references therein).

In the P elements assayed by Kurukolos et al. (1994), each repeat is close to one end, allowing only (or mostly) deletions. This will hold also for the large excess of external deletions vs. amplifications observed by Thompson-Stewart et al. (1994). The element assayed by Piques and Wegnez (1993) contains eight short repeats (350 bp) located at one end and a unique region (the nos' gene, 7.2 kb) much longer than the repeated region as a whole (2.8 kb). Therefore, before both growing strands reach an homologous sequence, there will be enough time for multiple occurrence of

---

**Figure 10.**—Mechanism of generation of deletions and amplifications. Thin curved arrows indicate the position and direction of the jump between templates (see text). Other symbols are as in Figure 1. Although the repeats are identical, for the sake of clarity the diagonally hatched patterns symbolizing them are different. (A) Top: double-strand DNA break generated by P excision. Bottom: the unmodified sister chromatid. (B) Invasion of the template by the broken DNA ends, according to the SDS model (Nassif et al. 1994). (C–F) Resolution of the repair process: the moment just before the annealing between the homologous sequences present on both nascent strands is represented. Various possibilities are shown, depending on whether or not the invading strand will change templates (D–F or C), whether the jump of the invading end from one repeat to the other occurs forward (D) or backward (E and F) and whether or not the replication proceeds after the encounter of homologous sequences on both growing strands (F or E). In the cases where more than one repeat has been copied on one of the strands, the resolution will depend on the homologous sequences that will anneal in the last step of the repair (not represented) (see text for explanation).
jumps between repeats, by changing template. This will lead to an almost equal rate of deletions and amplifications of various number of repeats.

**Double P elements occur at high frequency:** Besides internal amplifications, the great majority of 9.3 derivatives (13 out of 16) and all X6 derivatives (16) that display a higher sensitivity to zeste' repression are due to the presence of a second element in close association with the donor element. These double insertion events occur at up to 5.2% in the progeny of dysgenic 9.3 males and at up to 7.4% in the progeny of dysgenic X6 males. In all the cases of double insertions analyzed (29), the second element is adjacent to or integrated into the original insert.

A number of previous reports have described mutant alleles containing two or more closely integrated P elements (NITASAKA et al. 1987; HAWLEY et al. 1988; ROHSA et al. 1988; EGGLESTON 1990; O'HARE et al. 1992; DANIELS and CHOYNIK 1993; TOWER et al. 1993; ZHANG and SPRALDING 1993; DORER and HENIKOFF 1994). Therefore, the high frequency of multiple insertions appears to be a general property of the P element. This has been interpreted as resulting from a great tendency of the P element to transpose in the proximity of a preexisting element (EGGLESTON 1990).

**Nested P elements:** Among the 29 double P elements described in our study, two events can be clearly interpreted as insertions inside the starting element: one out of 13 9.3 double P derivatives (X93) and one out of 16 X6 double P derivatives (X6.23). In both cases the two elements are in opposite orientation. The fact that sequences flanking the internal element in X93 harbor an 8-bp duplication, strongly argues in favor of a classical P insertion.

In addition, eight contiguous double P elements were observed among 9.3 derivatives, but none among X6 derivatives. All of them are associated with rearrangements (inversion or deletion) modifying the extremity of one of the elements. One interpretation for the origin of this type of elements could be that they result from multiple nested insertions followed by excision and gap-repair events, according to the model of ENGELS et al. (1990) (see above). Deletion and inversion would result from the choice of the homologous sequence used as a template for repair on the sister chromatid. Mechanisms for the origin of nested rearranged double P elements that we have observed are proposed in Figure 11.

Regardless of the orientation, the second element seems to integrate frequently into the 3' region of the white transgene rather than into its 5' side. Eight out of the nine nested P elements that we have fully analyzed can be interpreted as resulting from a second insertion into the 3' region of white transgene. Five out of eight appear to be clustered in a region between 14088 and 14182 of white coordinates (O'HARE et al. 1984), as deduced from the three sequenced junctions of X5, X86

**Figure 11.—Model for the origin of nested rearranged double P elements.** Nested insertions (---) are followed by excision of the internal element(s). This leaves a gap that is repaired by the invasion of the unmodified sister chromatid by the broken 3' ends (half-arrows). We have represented the choice of templates that accounts for the observed structures. Refer to Figure 1 for all other symbols. (A) The structure of X5 (similar to X88 and X98) could result from three successive events: insertion of a second element within the 3' side of 9.3 in direct orientation, excision of the internal element, and repair of the gap. The homologous template chosen for this repair would be the internal 3' end, leading to the loss of one of the duplicated 3' extremities in the resulting element. The same types of events, starting with two new insertions within the 5' side or 3' side of 9.3, would lead respectively to X71 (B), and X84, X86 and X96 (C).
and X93 (Southern analysis of two other derivatives, X96 and X98, gave similar results). This region is close to, but does not include, the previously described hot spot for P insertions (RUBIN et al. 1982) that lies at position 13221 of white coordinates (O'HARE and RUBIN 1983). The three other cases of insertion into the white 3' region (X84, X88 and X6.23) are dispersed in sites 500–1000 bp from the cluster region. Only X6.23 could result from an insertion in the conventional white hot spot, according to Southern blot results. This suggests that the specificity of P element insertion does not depend only on the sequences immediately flanking the insertion site. It can be influenced by sequences located at least 1 kb away from the insertion point in the white gene, as this specificity is modified in the P[w\(^d\)] environment.

However it should be noted that all the nested elements arranged in direct orientation share two features. (1) In all cases, both elements are identical in the sense that all have either retained the duplication (X5, X88, X71, X79, X84, X86 and X96) or have undergone a triplication of the direct repeats (X98). (2) In all of them, one element has one rearranged end. This does not happen when the two nested elements are in opposite orientation (X93 and X6.23): in this case, the two elements are both complete and unrearranged (besides the insertion of one element inside the other). Consequently, as discussed further, the possibility remains that the observed structures do not result from successive and independent actual events of transposition, excision and gap-repair, but rather from the involvement of one single element in an aberrant and complex gap-repair event, leading to its partial or rearranged duplication.

**Double P elements in close proximity:** All of the remaining (not nested) double insertions (19/29) contain two very close copies of the P element. These results are consistent with those of EGGLESTON (1990) who found that the vast majority of secondary insertions at the yellow and singed loci occurred within 50 bp of the primary element or into the inverted repeats. Of the 13 double-insertion alleles described by DANIELS and CHOVNICK (1993), eight contained a second element integrated into or immediately adjacent to the starting element. Such very closely integrated P elements have also been described by many other authors (HAWLEY et al. 1988; ROHHA et al. 1988; EGGLESTON 1990; DANIELS and CHOVNICK 1993; TOWER et al. 1993; ZHANG and SPRADLING 1993).

However, all these authors have also described double elements separated by intermediate or long distances (0.2–200 kb). ZHANG and SPRADLING (1993) showed that this type of event is more frequently observed in the progeny of females than those of males. However, they observed in males that 30% (4/13) of new insertions occurred at >6 kb from the donor element. In our study we have not recovered this type of double element structure. Such events would have been detected as they would have predominantly resulted in a P-red phenotype like the autosomal insertions. This observation suggests that in our study the nature of the target element and/or of the flanking sequences (position effect) may have strongly influenced the type of events resulting from transposase action, in favor of transpositions at very short range.

Double P elements have been thought to result from the tendency of P elements to transpose inside or near other P elements (EGGLESTON 1990). This implies an affinity between the P intermediate of transposition and inserted P elements. P elements containing a fragment of engrailed (HAMA et al. 1990; KASSIS et al. 1992) or polyheterotic (FAUVARQUE and DURA 1993) are subjected to transposition to specific targets (homing). The same authors have proposed that this homing may result from an affinity between proteins bound to the sequences included in the P element and to the target site. Double P elements may result from the same type of homing: for instance, the transposase that has affinity for P elements may remain attached to the excised P element and target it to other P elements inserted anywhere in the genome.

However, this mechanism cannot account for the very strong bias that we observed in favor of insertions in the 5' side rather than in the 3' side of an inserted element (18 out of 19 not nested double P elements). This strong preference for the 5' side was also observed by EGGLESTON (1990), TOWER et al. (1993) and ZHANG and SPRADLING (1993).

Besides the 5' side preference, we observed a very strong preference for reverse orientation. All the double P elements in close proximity that we have found (19) are arranged in reverse orientation (opposite orientation relative to the donor insert). Most (18) are 5' to 5', and in only one (X6.7) the two elements are 3' to 3'. The same observation can be made for the multiple insertions described by other authors (HAWLEY et al. 1988; ROHHA et al. 1988; EGGLESTON 1990; DANIELS and CHOVNICK 1993; TOWER et al. 1993; ZHANG and SPRADLING 1993; DORER and HENIKOFF 1994).

To account for this strong bias, ZHANG and SPRADLING (1993) and TOWER et al. (1993) have postulated that proteins would maintain the excised element in a fixed orientation and tightly associated to the donor site. This would lead the excised element to transpose preferentially at close proximity, with the orientation preference observed.

However, if double P elements result from transposition of an excised element near another one, it is expected that the two elements may differ in certain occasions, given the high frequency of internal modifications that we observe. All the double P elements in close proximity that we have observed contain two identical elements. The two elements are unmodified (X6.20 and 12 others), have undergone the same precise deletion of one copy of the originally duplicated elements.
sequence (X64, X65, X67, X75) or display the same little internal deletion (X6.9).

To explain the identity of both members of a double P element in reverse orientation, we propose that, in most cases, they do not result from a local transposition event. They may rather result from a reverse duplication of the original element, subsequent to an abortive transposition, according to the model described in Figure 12. This model shares some features with the models proposed for the excision of plant transposable elements (Sædler and Nevers 1985; Coen and Carpenter 1988).

First, the P transposase would induce staggered cuts at only one P element end. It has recently been shown (Haniford and Kleckner 1994) that cleavages at the two ends of the bacterial transposable element Tn10 can occur at observably distinct times before transposition. Similarly, the two P termini may be not simultaneously cleaved by the transposase. Then, ligation of the free ends would occur to form a hairpin structure. For the DNA molecule containing the P element, the hairpin structure may be resolved by a single-strand cut at the 3′ end of the element (Figure 12). The two DNA molecules are then ligated and repaired. Replication would proceed through the hairpin, giving rise to an inverted duplication of the starting P element.

The nature of the junction sequence between the two elements would be of utmost interest for an understanding of the mechanism generating these structures. Unfortunately, our attempts to clone, PCR amplify or sequence these regions have so far been unsuccessful, as for other authors (Hawley et al. 1988; Roha et al. 1988; Eggleston 1990).

The preponderance of 5′ to 5′ over 3′ to 3′ double P elements could be due to the preferential occurrence of the first transposase cut at the 5′ end of the P element. Daniels and Chovnick (1993) observed small deletions (50–100 bp) in the adjacent 5′ genomic DNA of several derivatives, but never in the 3′ flanking DNA. These deletions may result from the extension by exonucleases of the first cut made by the transposase, preferentially at the 5′ side of P elements.

In conclusion, at least three mechanisms may be involved in P element “transposition” for very short, medium and long distances. First, the model in Figure 12 could explain the production of double inserts in reverse orientation integrated into the same target site. Second, the recovery of insertions at nearby sites or into the original P element without additional rearrangements could imply an intermediate of transposition that has a tendency to stay nearby the donor site. Finally, jumps to distant sites could arise by disassociation of the excised P element from the donor site. The potential existence of different mechanisms for localized rearrangements and transposition to unlinked sites is reminiscent of the finding that intermolecular and intramolecular events of the IS911 transposable element are mutually exclusive (Polard et al. 1992). As with recombination mechanisms, the initial break(s) made by the P transposase may be resolved by using different pathways, most of them failing to engender genuine transposition.

P repression on 9.3 and its derivatives necessitates at least two ZBR in close proximity: P repression on 9.3 acts through the enhancement of the zeste′ repression on this transgene and is strongly position dependent (Coen 1990). Here we show that 9.3 derivatives insensitive to P repression are due to in situ modifications of the original insert (Xlinked P-red mutants) or, less frequently, to transposition to a new genomic position. All the in situ modified P-red mutants have lost one copy of the originally duplicated 5′ regulatory sequences.

This shows that the presence of at least two ZBR is a

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**Figure 12.**—Model for the origin of double P element in reverse orientation. Small arrows, endonucleolytic cuts; dashed arrows, progression of the repair DNA synthesis. Other symbols are as in Figure 1. (A) Staggered cuts at the 5′ end of P element. (B) Intramolecular ligation of the 5′ and 3′ broken ends to form a hairpin structure at the P 5′ end. This hairpin structure will be resolved by a single-strand cut at the 3′ end of the element. On the other side, the 3′ free end could invade the sister-chromatid and begin the repair process, regenerating an apparently unmodified flanking sequence (as suggested by Southern blot data). Alternatively, the free ends of this side could also be ligated to form a second hairpin structure. Resolution on this side would then occur by a cut in the single-stranded region of the hairpin. (C) Repair synthesis begins from the 3′ ends. (D) The free ends are joined by ligation on the left side (curved line) and repair DNA synthesis proceeds through the hairpin. (E) This results in two closely integrated P elements in 5′ reverse configuration. The same process starting with staggered cuts at the 3′ end of the P element will lead to a double P element in 3′ reverse configuration.
necessary condition for P repression. It confirms our previous conclusions showing that P repression involves zeste repression on 9.3 and acts by enhancing it (COEN 1990). Amplifications leading to three or four copies of the regulatory regions in a direct tandem array do not prevent P repression and may even enhance it (data not shown). The same is true for secondary insertion at a short distance. The insensitivity to P repression due to the loss of one ZBR can even be reverted by the insertion of the same element at a short distance (e.g., X6 and its revertants).

The two autosomal insertions that are sensitive to P repression have either retained the original duplication of white regulatory sequences or harbor an amplification to four copies of these sequences. Among the 27 autosomal insertions insensitive to P repression that have been molecularly analyzed, 10 have been modified upon transposition and lost one of the ZBR. The remaining 17 appear to have retained the duplication of the regulatory sequences and thus two ZBR. They are nevertheless insensitive to P repression. This is also the case for X80, which has transposed at a nearby site. This confirms that, besides the presence of two ZBR, the flanking chromosomal sequences are important for the occurrence of the P repression that appears extremely sensitive to position effects, like the zeste-white interaction (LEVIS et al. 1985a).

Our allelic series of mutants, harboring various numbers of regulatory regions of the same gene inserted exactly at the same localization, will be useful to study these interactions and the phenomenon of transvection as it alleviates the problem of position effects that often complicate these kinds of studies.

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