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A MOLECULAR ANALYSIS OF THREE UNSTABLE ALLELES IN DROSOPHILA

(transposable elements, mutable alleles, white locus)

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SUMMARY

We have determined the structure of several unstable mutant alleles of the white *locus in* Drosophila melanogaster. *The* white ivory (w^i) *allele is a moderately unstable allele, which gave rise to the highly unstable* white-crimson (w^c) *allele. We have determined that the* w^i *mutation is due to the duplication of 2.9 kilobases (kb) of DNA within the* white *locus, and that reversion of* w^i *to wild type usually occurs by simple loss of one copy of the duplication. We have also analyzed two highly unstable alleles of the* white *locus,* w^c *and* white dominant zeste-like (w^{DZL}) *and have shown that both are insertion mutations. The* w^c *mutation results from the insertion of 10 kb of DNA into the* w^i *duplication, and the* w^{DZL} *mutation results from the insertion of 13 kb of DNA at or near the right end of the* white *locus. The* w^c *and* w^{DZL} *insertions are structurally related, but not identical, and are related to a previously characterized family of transposable elements, the foldback (FB) elements. The* w^c *insertion consists of a single FB element with a low copy number sequence between the moderately repetitive terminal inverted repeats. The* w^{DZL} *insertion contains two FB elements which flank a single copy sequence in the middle of the insertion. Reversion of* w^c *to* w^i *is mediated by an apparently precise excision event, while reversion of* w^{DZL} *to wild type occurs by an imprecise excision of the insertion. We suggest that structural differences in the two insertions may account for these different modes of reversion.*

INTRODUCTION

Several mutations have been characterized in *Drosophila* with genetic properties which suggest that they arose by the insertion of a transposable element. These alleles are characterized by genetic instability, they mutate to new phenotypes

at high frequencies, and generate chromosomal rearrangements with one breakpoint at the mutant locus (GREEN 1977; BINGHAM 1981). We have been particularly interested in determining the molecular structure of two highly mutable alleles of the *white* eye color locus, *white-crimson* (w^c) and *white-dominant zeste-like* (w^{DZL}), and of the moderately unstable *white-ivory* (w^i) allele which gave rise to the w^c allele.

The *white* locus, located on the X chromosome, is required for the normal brick red eye color of *Drosophila*; deletions of this locus result in a bleached white eye color. The w^i mutation results in a very light yellowish pink eye color indicating a decrease in function of this locus. The w^i alelle is unstable and reverts to wild type at a frequency of 1 in 5 x 10^5 x chromosomes in females, and at a frequency of 1 in 5 x 10^6 x chromosomes in males (BOWMAN 1965).

The w^c mutation was isolated as a partial revertant of the mutant w^i allele, and results in a light reddish-orange eye color. The w^c allele has been well characterized genetically, and a summary of the genetic properties of w^c is found in GREEN (1967). w^c reverts to a phenotype indistinguishable from the original w^i at a frequency of 1 in 10^3 x chromosomes, and mutants to a wild type phenotype at a similar frequency. w^c also mutates to a bleached white eye phenotype at a frequency of about 1 in 10^3 x chromosomes. These white eyed derivatives can be either phenotypically stable, or mutable themselves. The stable derivatives include deletions with one end point at the *white* locus, and extend either to the left or right. The mutable white derivatives are capable of mutation to w^c, w^i, and wild type, and can also generate deletions. Transpositions of w^c from the X chromosome to an autosome have also been detected (GREEN 1969b).

w^{DZL} was isolated as a yellow eyed female from a wild type Oregon R strain (BINGHAM 1980). This mutation partially represses the function of the *white* locus in cis and trans, but apparently only when two copies of the *white* locus are present on chromosome homologues which are able to synaptically pair in the *white* locus region. Thus, females of the genotype w^{DZL}/w^{DZL} or $w^{DZL}/+$ have yellow eyes, while w^{DZL}/Y males have reddish-brown eyes which are almost wild type in color. w^{DZL} is highly unstable, and reverts to a wild type phenotype at a frequency of about 1 in 10^3 x chromosomes (BINGHAM 1981). Other derivatives of w^{DZL} include deletions and inversions with one endpoints at the *white* locus.

Derivatives of both w^c and w^{DZL} appear to arise premeiotically in the germ line since mutations are recovered as clusters of progeny (GREEN 1967; BINGHAM 1981). Furthermore, these mutations can occur in males, where homologous recombination during meiosis is apparently absent, and mutation in females is not accompanied by recombination of flanking markers. These observations indicate that these mutation events cannot be accounted for by normal homologous meiotic recombination. Mutations are almost entirely limited to the germ line, as somatic events are only rarely detected.

We have been interested in analyzing the structure of the w^i, w^c and w^{DZL} alleles in order to determine if these mutations result from the insertion of transposable elements into the white locus. This would allow an analysis of the effects of independent transposable element insertions in a particular genetic locus. We can also analyze the structure of derivatives of these alleles to see how transposable elements can mediate genotypic and phenotypic changes in a eucaryote.

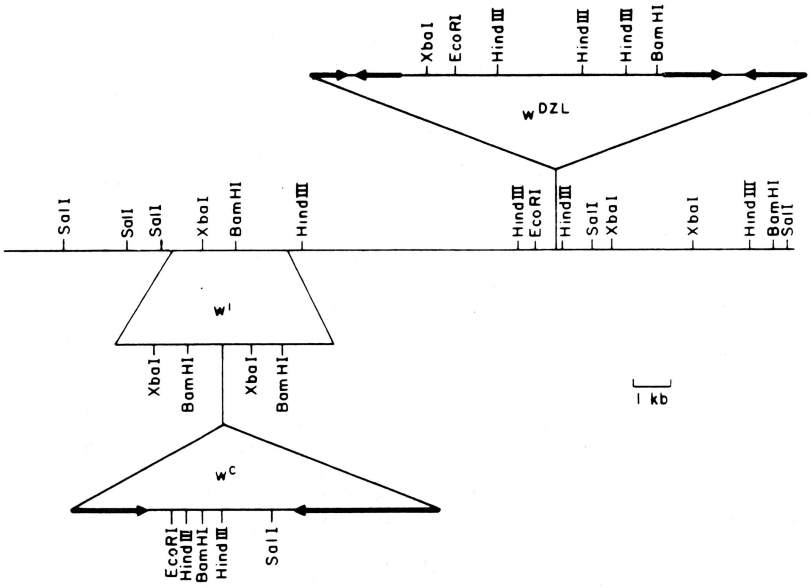

Figure 1. Restriction enzyme maps of the *white* locus region in wild type, w^i, w^c and w^{DZL}. The w^i mutation results from a duplication of sequences within the white locus, as indicated below the wild type restriction map. The w^c mutation results from the insertion of 10 kb of DNA in the w^i duplication. The w^{DZL} mutation results from the insertion of 13 kb of DNA at or near the right end of the white locus. The arrows on the maps of the w^c and w^{DZL} insertions indicate the size and location of the inverted repeats within the insertions.

THE w^i ALLELE CONTAINS TANDEM DUPLICATION WITHIN THE *white* LOCUS

The recent molecular isolation of sequences from the wild type *white* locus (BINGHAM et al. 1981; LEVIS et al. 1982a; GOLDBERG et al. 1982) has made a structural analysis of mutant alleles of the *white* locus possible. The structure of the w^i allele has been determined by a combination of whole genome Southern blot analyses and molecular cloning, using sequences

derived from the wild type *white* locus as probes. Using these techniques, it has been shown that the moderately unstable w^i mutation results from a tandem duplication of 2.9 kb near the center of the *white* locus (KARESS and RUBIN 1982). Restriction maps of sequences from the w^i allele and the wild type *white* locus are given in figure 1.

The structures of five independent wild type revertants of w^i and of one partial revertant of w^i have also been determined (KARESS and RUBIN 1982). Four of the five reversions of w^i to wild type are simple deletions of the duplicated sequence, formally proving that the duplication is responsible for the w^i phenotype. One of the wild type revertants, w^{i+A}, and the partial revertant, w^{ip}, have more complex structures (KARESS and RUBIN 1982). Both alleles have resulted from the insertion of new sequence information into the site of the w^i lesion, with the concomitant loss of sequences within the duplication. In both cases the insertion contains middle repetitive DNA, although phenotypic instability has not been detected in either stock. Simple reversion of w^i to wild type has been postulated to occur by intrachromosomal recombination between the two copies of the w^i duplication. Unequal crossing over between homologues is probably not involved, since reversion is usually not accompanied by the exchange of outside markers (BOWMAN 1965).

w^c CONTAINS A 10 kb INSERTION IN THE w^i DUPLICATION

A comparison of the structures of the w^i and w^c alleles by whole genome DNA blotting experiments has indicated that w^c contains an insertion of 10 kb of DNA in the w^i duplication (COLLINS and RUBIN 1982). This was confirmed by molecular cloning of sequences from the *white* locus of homozygous w^c flies (COLLINS and RUBIN 1982). Sequences to the left and right of the insertion within the *white* locus have been isolated by molecular cloning in the lambda phage vector Charon 28 (RIMM et al. 1980), and restriction mapping has indicated that these regions are conserved in the w^i and w^c alleles. We have initially attempted to isolate sequences from the w^c insertion in this vector as well, but sequences within the insertion were deleted during phage propagation. However, we have isolated cloned sequences from the w^c insertion which accurately represent the genomic structure by using the plasmid vector pBR322 (BOLIVAR et al. 1977) in the recombination deficient (*recA*) host, *HB101* (BOYER and ROULLAND-DUSSOIX 1969). Restriction mapping of these clones has allowed us to obtain an accurate map of the w^c insertion sequence, as illustrated in figure 1 (COLLINS and RUBIN 1982). By comparing the restriction maps of the w^c and w^i alleles we have determined that the w^c insertion did not occur exactly in the center of the w^i duplication, but rather occurred several hundred bases pairs to the right of center.

One of the more interesting consequences of the w^c insertion is an increase in expression of the *white* locus as judged by phenotype. The w^i duplication reduces *white* locus function dramatically, resulting in a very lightly pigmented eye. The insertion of 10 kb of DNA into this duplication results in a partial reversion of the w^i phenotype, indicating that *white*

locus function has been partially restored. It is possible that the w^c insertion contains a promoter which restores partial function by allowing transcription of *white* locus sequences. Alternatively the w^c insertion may function simply by disrupting the w^i duplication.

w^{DZL} CONTAINS A 13 kb INSERTION

The structure of the w^{DZL} allele has been determined by comparing the restriction maps of cloned sequences from the *white* locus in homozygous w^{DZL} flies with the restriction map of the wild type *white* locus determined by LEVIS et al. (1982a). The primary difference between these maps was the presence of a 13 kb insertion in the w^{DZL} map as illustrated in figure 1 (LEVIS and RUBIN 1982). Sequences in the wild type locus which extend no more than 13.4 kb to the left and 0.4 kb to the right of the site of the w^{DZL} insertion are known to contain all of the information necessary for a wild type phenotype (LEVIS et al. 1982a). This places the w^{DZL} insertion at or near the right end of the *white* locus.

Sequences from the w^{DZL} allele were cloned in the lambda phage vector Charon 28 (RIMM et al. 1980). Sequences from within the w^{DZL} insertion, like sequences from the w^c insertion, were susceptible to deletions during phage propagation. However, somephage were recovered with restriction enzyme maps which were consistent with restriction enzyme maps of w^{DZL} genomic DNA as determined by DNA blotting experiments (LEVIS and RUBIN 1982). Subcloning of these sequences into the plasmid vector pBR322 (BOLIVAR et al. 1977) using the recombination deficient (*recA*) host, *HB101*, (BOYER and ROULLAND-DUSSOIX 1969) suppressed deletion formation. The restriction map of the w^{DZL} allele obtained from clonsed sequences was verified by a series of genomic DNA blotting experiments (LEVIS and RUBIN 1982).

The w^{DZL} insertion results in a dominant mutant phenotype. It is difficult to account for the dominance of the w^{DZL} phenotype, since dominance is not predicted for an insertion mutation. One possibility is that an insertion of this size at this position leads to a perturbation of the *white* locus region which is propagated to the homologue lacking the insertion. Alternatively, the insertion may actively interfere with *white* locus expression, and sequences within the insertion may be important in determination of the w^{DZL} phenotype (BINGHAM 1980; LEVIS and RUBIN 1982).

REVERSION OF w^c AND w^{DZL}

w^c reverts to a w^i phenotype at a frequency of about $1/10^3$ X chromosomes. An analysis of the structure of five independently isolated w^i derivatives of w^c by whole genome blotting experiments has indicated that all five arose by an apparently precise excision of the w^c insertion, restoring the w^i duplication (COLLINS and RUBIN 1982). This excision event is precise within the experimental limits of about 50 base pairs. Figure 2 shows a DNA blotting experiment which illustrates the loss of sequences from within the w^c insertion, and the restoration of

the w^i tandem duplication in these revertants.

Excision of the w^e insertion also occurs in reversions of w^e to wild type. An analysis by whole genome DNA blotting experiments of six independently isolated phenotypically wild type derivatives of w^e has indicated that all six occurred by excision of both the w^e insertion and one copy of the w^i duplication (COLLINS and RUBIN 1982). This reversion event could occur by recombination between the two copies of the w^i duplication, removing the insertion and one copy of the duplication.

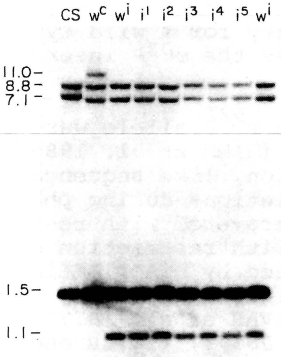

Figure 2. Blot hybridization of Xba I/Xho I digests of DNA from Canton S wild type (CS), w^e, w^i and five independently isolated w^i revertants of w^e (i1-i5). 2 ug of DNA from each strain were digested to completion with Xba I and Xho I, separated on a 0.6% agarose gel and transferred to nitrocellulose by the method of SOUTHERN (1975). The blots were hybridized with a 32P-labelled fragment which is complementary to sequences within the w^i duplication. Fragment sizes were determined by comparison to a Hind III digest of lambda DNA, and are indicated to the left of the autoradiograph. The 8.8 kb and 7.1 kb fragments are derived from the sequences to the left and right of the w^i duplication; these sequences are conserved in wild type, w^e, w^i and the w^i revertants of w^e. The 1.5 kb fragment is duplicated in w^i and w^e, but is present only one in the wild type strain. The 1.1 kb fragment in w^i is also derived from within the w^i duplication. The 11.0 kb insertion fragment interrupts the duplication in w^e, resulting in the loss of the 1.1 kb fragment from the w^i duplication. Reversion of w^e to a w^i phenotype results in the loss of the 11 kb insertion fragment, and restoration of the 1.1 kb fragment which is derived from the w^i duplication.

Reversion of w^i to wild type has been proposed to occur by similar mechanism (BOWMAN 1965; KARESS and RUBIN 1982). However, w^e reverts to wild type nearly two orders of magnitude more frequently w^i reverts to wild type. Thus, the w^e insertion must play some role in this reversion event. The w^{DZL}

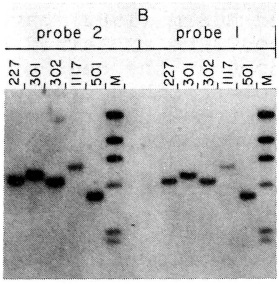

Figure 3. Blot hybridization analysis of the residual insertions in phenotypic revertants of w^{DZL}. Two identical sets of HpaI/ Sal I digests of DNA from Oregon R wild type (Ore R), w^{DZL}, and four independently isolated revertants of w^{DZL} (1,2,5,226) were separated on a 0.5% agarose gel and transferred to nitrocellulose by the method of SOUTHERN (1975). The resulting blot was cut in half and hybridized with 32P-labelled probes containing sequences just to the left (probe 1) or just to the right (probe 2) of the w^{DZL} insertion. Since neither Hpa I nor Sal I cut within the w^{DZL} insertion, the resulting fragment in w^{DZL} or revertants of w^{DZL}, is larger than the wild type fragment (Ore R) by the size of the insertion or residual insertion. Fragment sizes were determined by comparison with a Hind III digest of lambda DNA run on the same gel (lane M).

mutation also reverts to a wild type phenotype at a frequency of about 1 in 10^3 X chromosomes. However, these revertants differ from the wild type revertants of w^e in that many, if not all, are phenotypically unstable (LEVIS and RUBIN 1982). The phenotypic classes of the exceptional progeny of w^{DZL} revertants are generally similar to those reported for w^{DZL} (BINGHAM 1981), although novel phenotypes have been recovered.

Whole genome DNA blotting experiments have been done to compare the structure of the *white* locus in revertants of w^{DZL} with that of w^{DZL} and wild type flies. In eight out of twelve revertants examined, reversion has been accompanied by the simple loss of part, but not all, of the w^{DZL} insertion (LEVIS and RUBIN 1982). As illustrated by the genomic blotting experiment in figure 3, the size of the residual insertion varies from revertant to revertant. Four revertants have been examined

which show a more complex pattern. One interpretation which is consistent with the data for these four revertants is that the initial reversion event, mediated by loss of part of the insertion, was followed by subsequent deletions or rearrangements of the remainder of the insertion during propagation of the stock, resulting in a genotypic heterogeneity within the stock. Further mapping experiments (LEVIS and RUBIN 1982) have indicated that the central 6 kb of the insertion between the leftmost Xba I site and the right Bam HI site (see figure 1) has been deleted in all twelve of the revertants. In most cases, including the eight simple revertants, the residual insertion is between 1.9 kb and 4.1 kb. LEWIS and RUBIN (1982) have suggested that these reversion events could be explained by a recombination event between the sequences at the ends of the w^{DZL} insertion; differences in the site of recombination within the ends of the insertion would account for the heterogeneity in size of the insertion left in w^{DZL} revertants.

THE ENDS OF THE w^{e} AND w^{DZL} INSERTIONS ARE HOMOLOGOUS TO FB ELEMENTS

Cross hybridization experiments between closed sequences have revealed that the sequences at the ends of the w^{e} insertion are homologous to each other. The ends of the w^{DZL} insertion are also homologous, and furthermore, are homologous to the ends of the w^{e} insertion (LEVIS et al. 1982b). However, sequences from the middle of the two insertions, containing the clusters of restriction sites, are not homologous. Thus, the two insertions are structurally related, but are not identical.

We have also discovered that the ends of the two insertions are homologous to a previously characterized family of transposable elements, the foldback (FB) elements, first identified by POTTER et al. (1980). These elements are characterized by long terminal, imperfect, inverted repeats (POTTER et al 1980; Truett et al. 1981). These inverted repeats are themselves made up of a complex arrangement of short, tandem, imperfectly repeated sequences. Most restriction enzymes fail to cut within the inverted repeats of the FB elements due to this simple tandemly repeated subunit structure. However, one enzyme, Taq I, cuts frequently within this sequence, at intervals which average approximately 155 base pairs (TRUETT et al. 1981). As illustrated in figure 4, the ends of the w^{e} and w^{DZL} insertions share this structural property with FB elements. A partial digest of end-labelled fragments from the ends of both insertions generated a ladder of fragments, indicating a regularly spaced series of Taq I cleavages. At the left and right ends of the w^{e} insertion, 13 and 22 Taq I sites with an average spacing of 160 bp can be resolved, respectively. The left end of the w^{DZL} insertion displays 10 regularly spaced Taq I sites at an average distance of 145 bp, and the right end of the w^{DZL} insertion has two sets of 8 Taq I sites, an average of 135 bp apart. Thus, the w^{e} and w^{DZL} insertions share structural similarities, as well as homology, with FB sequences.

THE w^c INSERTION IS A REPETITIVE FOLDBACK ELEMENT CONTAINING A LOW COPY NUMBER SEQUENCE IN THE MIDDLE

We have determined that the ends of the w^c insertion are inverted with respect to one another by analyzing heteroduplexes formed between cloned fragments containing the ends of the insertion in the electron microscope (LEVIS et al. 1982b). The position and size of these inverted repeats at the end of

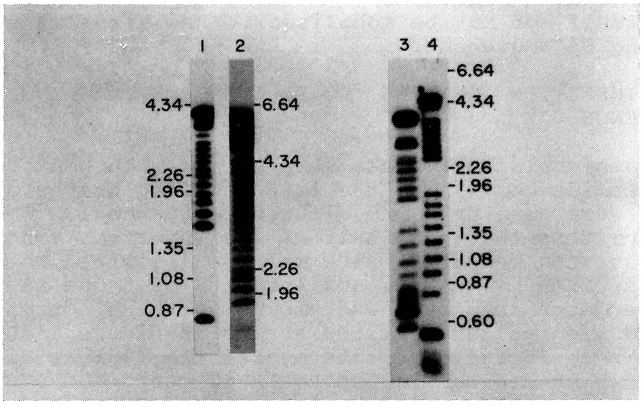

Figure 4. Autoradiographs of partial TaqI digests of end-labelled fragments derived from the left and right ends of the w^c and w^{DZL} insertions. Lanes 1 and 2 contain end-labelled fragments derived from the left and right ends of the w^c insertion, respectively. Lanes 3 and 4 contain end-labelled fragments derived from the left and right ends of the w^{DZL} I insertion, respectively. The positions of these Taq I "ladders" in the w^c and w^{DZL} insertions are indicated by the arrows in figure 1.

the w^c insertion are indicated by the arrows in figure 1. The inverted repeats correspond to the regions of the w^c insertion which show the "Taq I ladders" characteristic of FB sequences. The central portion of the w^c insertion is not included in this inverted repeat. Thus, the w^c insertion is an FB element which contains a non-FB segment between the inverted repeats.

Like other FB elements (TRUETT et al. 1981), the w^c insertion is homologous to sequences which are present many times in the *Drosophila* genome. Using a probe derived from the FB end of the w^c insertion, we have done in situ hybridizations to the polytene chromosomes of our w^c stock, and have detected hybridization to 29 dispersed sites on the chromosome arms and to the chromocenter (LEVIS et al. 1982b). However, when we used a probe derived from the "middle" of the w^c insertion (the region between the inverted repeats), a very different distribution pattern was observed (LEVIS et al. 1982b). We have

detected only a few sites of hybridization to the polytene chromosomes of our w^c stock using such a probe; four strongly hybridizing sites have been observed along with a fifth weaker hybridization site. This result has been confirmed by genomic blotting experiments. Thus, the w^c insertion is a single fold-back element which contains a low copy number sequence between the inverted repeats. We do not know at this time whether all of the other copies of the w^c "middle" are associated with flanking FB sequences. One observation we have made is that the w^c "middle" is polymorphic in its location both within and between strains. Thus, the "middle" may be a transposable element itself, or may be mobilized to new sites in the genome by flanking FB sequences.

THE w^{DZL} INSERTION CONTAINS TWO FB ELEMENTS FLANKING A SINGLE COPY SEQUENCE

Intramolecular snapbacks were formed when DNA from either of two plasmids containing the left or right halves of the w^{DZL} insertion were denatured and allowed to reanneal. We have concluded from this that each half of the insertion contains a pair of inverted repeats, which we have localized to the ends of the insertion. The size and location of these inverted repeats were determined by measurements in the electron microscope, and are indicated by the arrows in figure 1 (LEVIS et al. 1982b). These inverted repeats map to the regions of the w^{DZL} insertion which are homologous to FB elements and w^c, and which show the "Taq I ladders" characteristic of FB elements. Again, the central region of the insertion, which is not homologous to FB, is not included in either pair of inverted repeats.

As expected from our knowledge of FB elements, sequences homologous to the inverted repeats at the ends of the w^{DZL} insertion are present many times in the *Drosophila* genome (LEVIS et al. 1982b). We have done in situ hybridizations to the polytene chromosomes of our w^{DZL} stock using a radioactively labelled probe from the right inverted repeat of the w^{DZL} insertion, and have deteced 49 sites of hybridization of the chromosome arms in addition to labelling of the chromocenter. Similarly, hybridization to the polytene chromosomes of a wild type Oregon R strain resulted in many sites of labelling.

Strikingly different results were seen when a probe from the central portion of the w^{DZL} insertion was used. A probe from the region of the w^{DZL} insertion between the pairs of inverted repeats labelled only 2 sites on the polytene chromosomtes of the w^{DZL} stock (LEVIS et al. 1982b). These two sites are at 3C on the X chromosome, the site of the *white* locus, and at 21D near the tip of chromosome arm 2L. In situ hybridizations to the wild type strain Oregon R using the same probe resulted in only one site of hybridization at 21D. In fact, this site, and no other, hybridized in each of 4 wild type *D. melanogaster* strains and in a *D. simulans* wild type strain. Thus, the center of the w^{DZL} insertion appears to be a single copy DNA sequence which is flanked by a pair of FB elements on either side in the w^{DZL} insertion. This interpretation was confirmed by genomic blotting experiments; these experiments

also indicated that restriction enzyme sites are highly conserved in the w^{DZL} insertion as compared with the sequence at 21D (LEVIS et al. 1982b).

The fact that the center of the w^{DZL} insertion is located at 21D in several strains of *Drosophila* indicated that this sequence is not normally transposable. Therefore it appears that this segment was mobilized by flanking FB elements from its normal position on chromosome 2 and transposed to the *white* locus on the X chromosome as a one time event. We do not know at this time whether this sequence is flanked by FB elements at 21D. However, in situ hybridization of the w^{DZL} inverted repeat to several wild type strains resulted in labelling of this site, indicating that FB sequences could be present. The resolution of in situ hybridization is not sufficient to determine if the sequence at 21D is actually flanked by FB elements.

FB INSERTIONS AND GENETIC INSTABILITY

Given the large number of families of transposable elements in *Drosophila*, it is significant that both of the insertions causing the highly unstable w^{c} and w^{DZL} mutations contain FB sequences. This suggests that FB sequences are responsible for this genetic instability, a hypothesis which is further supported by the structure of the phenotypically wild type revertants of w^{DZL}. These revertants have deletions in the central region between the FB elements of the w^{DZL} insertion, yet retain the property of genetic instability. FB elements are also associated with a number of the unstable insertions of the large transposable segment TE (unpublished results of PARO, GOLDBERG and GEHRING, cited in GOLDBERG et al. 1982). TE's usually contain the entire *white* and adjacent *roughest* loci and have been shown to insert at a large number of chromosomal sites, where they sometimes promote deletions of adjacent sequences and can undergo further transpositions (ISING and RAMEL 1976; ISING and BLOCK 1981).

The instability of the w^{c} and w^{DZL} alleles is extraordinary among alleles of the *white* locus, including several others associated with insertions. Three *white* mutations caused by the insertion of a copia element, w^{a} (GEHRING and PARO 1980; BINGHAM et al. 1981; BINGHAM and JUDD 1981; LEVIS et al. 1982a; GOLDBERG et al. 1982), and *whd81b11* and *whd81b25* (RUBIN et al. 1982) are relatively stable. Several *bithorax* alleles which contain insertions of the copia-like element, gypsy, (W. BENDER, unpublished results cited in SPRADLING and RUBIN 1981), have never been noted as being highly mutable. This suggests that the genetic instability of the highly unstable w^{c} and w^{DZL} alleles may be largely an intrinsic property of FB element insertions.

EXCISION AND THE STRUCTURE OF THE w^{c} AND w^{DZL} INSERTIONS

w^{c} and w^{DZL} appear to undergo phenotypic reversion by different pathways; w^{c} reverts to w^{i} by an apparently precise excision of the insertion, whereas w^{DZL} reverts to wild type by imprecise excision. We believe that the difference in the

two classes of revertants examined is significant, since precise excision of the w^{DZL} insertion would certainly restore a wild type phenotype. If the w^{DZL} insertion is capable of precise excision, this must occur at a much lower frequency than precise excision of the w^{c} insertion. We cannot rule out the possibility of imprecise excision of the w^{c} insertion, since we cannot predict the phenotype of these flies. Thus, the apparent absence of imprecise excisions among the w^{i} revertants of w^{c} does not exclude their occurrence in another phenotypic class.

Precise excision of the w^{c} insertion may be mediated by mechanisms similar to those proposed for procaryotic transposable elements with long inverted terminal repeats, like Tn 10 and Tn5 (FOSTER et al. 1981; BERG et al. 1981). If, like other transposable elements, the w^{c} insertion event was accompanied by a short duplication of the target sequence, pairing of the inverted repeats could serve to align these duplicated sequences and facilitate recombination between them, resulting in a precise excision. In contrast to w^{c}, the w^{DZL} insertion contains a pair of inverted repeats at each end of the element, and pairing is most likely to occur between sequences within each terminal repeat, rather than between the two terminal repeats. Thus, recise excision events would be predicted to occur less frequently.

Imprecise excisions of the w^{DZL} insertion may be mediated by recombination between sequences at either end of the w^{DZL} insertion (LEVIS and RUBIN 1982; LEVIS et al. 1982b). The w^{DZL} insertion has FB elements repeated in direct orientation at its termini. Imprecise excisions could occur by recombination between these direct repeats. The presence of short tandem subrepeats within the w^{DZL} terminal FB element repeats provides an explanation for the heterogeneity in the lengths of the residual insertions remaining in simple eye color revertants of w^{DZL}. Recombination between any two directly repeated subunits at either end of the insertion would result in deletion of the sequences from the center of the w^{DZL} insertion, as well as a variable amount of the terminal repeats. Based on our estimates of the lengths of these subunit arrays, we could account for imprecise excision events which generate residual insertions between 1.3 and 4.1 kb in length. In fact, most of the residual insertions examined to date fall into this size class (LEVIS and RUBIN 1982).

CONCLUSIONS

We have examined the structure of two highly unstable alleles of the *white* locus, w^{c} and w^{DZL}. Both of these mutations were found to contain insertions of DNA which are structurally related. The w^{c} insertion is a foldback element with an unrelated low copy number sequence between its inverted repeats. The w^{DZL} insertion contains two foldback elements which flank a single copy DNA sequence. Based on these observations, and on the observation that mutability is retained in revertants of w^{DZL} which have undergone deletions of the sequence between the flanking foldback elements, we postulate that fold-

back sequences are primarily responsible for the instability of w^c and w^{DZL}. In contrast to w^c and w^{DZL}, a moderately unstable allele of the *white* locus, w^i, was found to be a small duplication of sequences within the *white* locus. We are currently beginning to analyze more derivatives of w^c and w^{DZL}, and hope to be able to draw further correlations between molecular structure and mutability. In this way we hope to be able to define those regions of the insertions which are required for the genetic instability of w^c and w^{DZL}.

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