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The molecular basis of instability of the *singed*$^{very\ weak}$ mutation in *Drosophila melanogaster*

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Summary

The *singed*$^{very\ weak}$ mutation was created by the sequential addition of two P transposable elements to the *singed* gene. The mutation can be somatically unstable through the action of a dominant maternal effect mutation on the second chromosome. It is also unstable in the germ line in these conditions. Sequencing of the region of the P insertions in the mutation reveals that the two inserted elements have single internal deletions, and the larger of the two is a copy of the KP element. The mutation will generate, at high frequencies, strongly *singed* and pseudo-wild type products by reversions occurred in the germline. These are the result of the precise excision of the smaller and the larger elements respectively. By PCR amplification of dissected thoraces we show that the somatic instability of the mutation, from a weak to a strong *singed* phenotype, is also caused by the excision of the smaller of the two elements.

1. Introduction

The P family of transposable elements in *Drosophila melanogaster* is a well-studied example of a family of transposable genetic elements with inverted terminal repeats and introns in their genes (for review see Engels, 1989). The family are heterogeneous, with the majority of element copies being non-autonomous, being capable of transposition and excision only when in the same genome as an autonomous full-length element, known as the P factor. The non-autonomous elements differ from the intact element by internal deletions, leaving the terminal sequences required in *cis* for transposition (Rio, 1990). The P elements have been of great importance as a tool in *Drosophila* genetics, as transformation vectors and in cloning strategies involving transposon tagging. They now offer further opportunities as tools in gene replacement (Gloor *et al.* 1991). They are responsible for a syndrome of low fertility in interstrain crosses called hybrid dysgenesis, in which a high level of movement of elements occurs in the germ line. This leads to sterility at high temperatures. Transposition of P elements can be restricted by a number of mechanisms (Rio, 1990). Firstly, transposition is germline-specific, which results from the fact that the P factor transcript is only completely spliced in the germ cells, yielding an 87 kD transposase protein. In the soma, the intron between open reading frames 2 and 3 of the transposase message is retained and a 66 kD protein produced. Recent work has studied the mechanism for this process (Kitamura, Kobayashi & Okada, 1993; Kobayashi *et al.* 1993). Transposition in somatic cells can be achieved by transforming flies with P(Δ 2–3), a P element construct in which the open reading frame between 2 and 3 has been spliced out *in vitro*. A second form of repression is called P-cytotype. This is a maternally-inherited repression system, thought to be due to the introduction into the oocyte of repressor proteins derived from certain classes of deleted P element (Nitasaka, Mukai & Yamazaki, 1987; Misra & Rio, 1990). In one strain a naturally-occurring repression system of this kind has been mapped to a pair of P elements at the distal end of the X chromosome (Ronsseray, Lehmann & Anxolabéhère, 1991). Finally, there are repression systems which are biparentally inherited, in which some elements lower the rate of transposition of others, either by competing with them at the DNA level for transposase protein (a system called transposase titration (Simmons & Buchholz, 1985)), or by encoding repressor proteins which interfere with transposition in some way. The operational distinction

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between these last two mechanisms is that transposase titration will lower the rate of reversion of highly unstable P element insertion mutations such as *singed*^{weak}, but will not restore high temperature fertility in conditions of hybrid dysgenesis, a restoration which can only occur through true repression of total transposition. The KP element is a particular type of deleted P element which is abundant in the chromosomes of wild-caught *Drosophila melanogaster* from Eurasia and Africa (Black *et al.* 1987). The element increases rapidly in abundance in some laboratory crosses, but only in the presence of intact P elements (Jackson, Black & Dover, 1988). Some evidence indicates that this element can act as a repressor of transposition. However, some P-element-bearing strains show high levels of chromosomally-inherited repression of gonadal dysgenesis without possessing any KP elements (Heath & Simmons, 1991; Raymond *et al.* 1991). The spread of deleted P elements through wild populations suffering the harmful effects of unrepressed transposition has been modelled and would be expected to occur under a broad range of conditions (Brookfield, 1991).

A mutation has been described in the X-chromosomal *singed* gene, *singed*$^{very\ weak}$ (or *sn*^{vw}) in which the germline specificity of P element movement is incomplete (Brookfield & Lewis, 1989). The mutation has two inserted sequences, which appear, from their restriction maps, to be deleted P elements of around 1150 and 650 base pairs in length in an inverted tail-to-tail orientation (i.e. opposite to that in the *singed*^{weak} mutation). The mutant allele was created in two stages, initially by the addition of the larger element, generating a mutation with a strongly *singed* phenotype (*Loua sn6*). This occurred in a hybrid dysgenic cross using Canton S as an M strain and the Loua 83 strain from Zaire as a P-strain parent (Brookfield & Mitchell, 1985). The Loua *sn6* mutation showed a pattern of somatic instability, in which, in certain crosses, approximately 2% of males hemizygous for this mutation showed areas of wild-type bristles in a strongly *singed* background. A germline revertant of *sn6*, *singed*$^{very\ weak}$ (*sn*^{vw}), arose in these crosses, and had an almost wild-type phenotype. It could revert somatically to strong *singed* patches. *singed*$^{very\ weak}$ differed from *sn6* by the insertion of approximately 650 base pairs of further DNA, the restriction map of which was consistent with it being a second P element, in the reverse orientation to the first. In some crosses mosaicism occurred in around 20% of all males, which allowed the mapping of the *trans*-acting component required for this destabilization. *sn*^{vw} can be induced to revert somatically by the action of a dominant maternal effect mutation that is located towards the middle of the second chromosome. This mutation showed linkage to the loci *black* and *purple* and is not required in the zygote for destabilization. The mutation on the second chromosome, which was called *Mo*, can also produce mosaics with a series of other tested P element insertions in *singed*, but at much lower frequencies. No mosaics were produced using P element insertions in other X-linked genes. The ability of a P element insertion mutation in *singed* to produce mosaics in this system is very poorly predicted by its germline instability in hybrid dysgenesis.

Attempts to clone the *singed*$^{very\ weak}$ allele were unsuccessful, no doubt because of the inverted repeats, which could have interfered with the replication of the lambda 'phage vector L47 utilized (Lewis, 1987). Here we describe experiments to study this mutation using the polymerase chain reaction (PCR) utilizing primers from flanking *singed* sequences in combination with primers from parts of the P sequence themselves. We show the sequence of the *sn*^{vw} allele in the region of the P insertions, and demonstrate that mosaicism results from the somatic excision of one of the two P elements inserted into the locus. We further demonstrate that *sn*^{vw} can be unstable in the germ line, and that the most common events which occur in the germ cells are the precise excision of one or other of the two inserted P elements.

2. Materials and methods

Primers

The experiments utilized the following PCR primers:

(i) *singed* specific

JB3: 5′ tggca acagt gccat ctctg 3′ from base pairs 2281–2300 and JB4: 5′ ggctc tatgc tcttc gctga 3′ from base pairs 2977–2858 from the *singed* sequence as entered by Paterson & O'Hare (1991) in the EMBL Nucleotide sequences database (accession no. X17548). Using the numbering of the manuscript, these are bases 1207–1226 and the complement of bases 1903–1884 respectively in the Section A of the gene.

(ii) P-element specific (from the sequence of O'Hare and Rubin, 1983)

JB7: 5′ tcccg tcggc aagag acatc 3′ which is the complement of a sequence located near the 3′ end of the P-element at base pairs 2884–2865. JB8: 5′ cactg aattt aagtg tatac 3′, the complement of the sequence located from base pairs 70–51 close to the near-terminal *Hind* III site at 39–44, and JB9: 5′ cagct atttg tctcc acacc 3′ which is located at base pairs 701–720 close to the Xho I site at 728–733.

Genomic DNA was extracted from adult male flies using a method adapted from Jowett (1986). DNA was extracted from the dissected thoraces of individual male flies using a scaled-down version of this protocol.

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PCR amplification

This was performed as described in Sambrook, Fritsch & Maniatis (1989), using a Perkin–Elmer Cetus thermocycler. The DNA preparations were subject to 5 min denaturing at 94 °C, followed by 35 cycles of 40 s at 92 °C (denaturing); 1 min at 55 °C (primer annealing); 3 min ramp from 55 to 72 °C and 1–3 min (depending upon expected length of amplified region) at 72 °C (extension).

PCR products were run on 1% agarose gels following Sambrook, Fritsch & Maniatis (1989).

DNA sequencing

In order to prepare template DNA for sequencing, a secondary unbalanced PCR amplification was made using 2 pMoles and 200 pMoles of the two primers for 100 μl reaction. Twenty-five PCR cycles were performed using the above conditions. Excess primers were removed from the reaction mix by passing it through a 1% Nusieve gel and extracting the relevant band by phenol extraction. Small PCR products were sequenced using the Sequenase Version 2 protocol as described. Larger molecules were sequenced using a method described by Winship (1989). Accugel ready-prepared acrylamide solutions were used to make the sequencing gels.

Drosophila strains

The following Drosophila strains were used:

(1) C(1)DX *yf*; Y T(1; Y) y^+; *bw*; *st* females, *y* sn^{vw}; Y T(1; Y) y^+; *bw*, *st* males. This strain was produced as described in Brookfield & Lewis (1989, strain 34). Southern blots have shown that there are many P elements in this strain, which could be on chromosomes 1, 2 and 3, but sn^{vw} is stable in the germ line and the soma.

(2) C23a = In 2R *Cy* sp^2/In 2LR bw^{V1} ds^{33k} *dp*; In 3LR*D CxFruh*/*Sb*. From the University of Birmingham. This is a pure M strain, lacking all P element homology.

(3) Canton S. A wild-type pure M strain.

(4) *sp px c pr b dp al*. The All chromosome supplied in a pure M strain by Dr K. Exley, Nottingham.

(5) C(1)DX *yf*; Y T(1; Y) y^+; *bw*; *st* females: *y* sn^w; Y T(1; Y) y^+; *bw*; *st* males. This strain was produced as described in Brookfield & Lewis (1989, strain 9). It is pure M with the exception of the two P elements present at *singed* in sn^w.

(6) C(1)DX *yf*; Y T(1; Y) y^+; *pr b dp*; *st* females: *y* sn^{vw}; Y T(1; Y) y^+; *pr b dp*; *st* males. This strain was produced by crossing strain 4 females to strain 3 males. F1 females were crossed to strain 2 males, and the F1 of this cross sib-mated. *pr b dp* males and females were selected and sib-mated for three generations to give a strain 6a. 6a was thus pure M, and *pr b dp*. 6a females were crossed to strain 2 males. *Pm D* F1 males from this cross were isolated. These were crossed to strain 5 females. In the F1 Pm^+ *D* males and females were crossed, and *pr b dp st* males and females isolated in the F2. The strain was initially variable for y^+ and wild-type Y chromosomes and y^+ was selected and fixed. The resulting strain was called 6b. This had the Y, the third and the females' X chromosomes derived from strain 5. The second chromosomes were derived from strains 3, 4 and 5, with the visible markers *pr b* and *dp* derived from strain 4. The males' X chromosomes were derived from strains 2, 3 and 4.

Simultaneously, strain 5 females were mated to strain 2 males, and *Pm D* females isolated in the F1. These F1 females were crossed to strain 1 males. From this cross, F1 *Pm D* males were crossed to strain 6b females described above. From this cross, F1 *Pm D* males and females were isolated and sib mated. Pm^+ D^+ males and females were selected in the resulting F1. These were mass mated, and the y^+ Y chromosome, initially segregating, was fixed. The resulting strain was called strain 6. sn^{vw} from strain 1 remained stable during this cross, implying that strain 1 is not a P strain. It was found that *bw* was present at low frequency in the resulting strain, but was eliminated gradually by selection over a number of generations. Strain 6 thus had chromosomes 2 derived from strains 3, 4, and 5, with the *pr*, *b* and *dp* mutations from strain 4. The attached-X chromosome, the third chromosome and the Y chromosome were from strain 5. The X chromosome in males, bearing sn^{vw}, was derived from strain 1. Thus there should have been no P elements on the Y chromosomes, the compound X chromosomes, or chromosomes two or three. The male X chromosome could have many P elements, although few, if any, of these are likely to be active, since the sn^{vw} mutation is completely stable in strains 1 and 6. P elements from strain 1 could also be present on the fourth chromosome of strain 6. However, Southern blotting and hybridization of a cloned P element to Bam H1-digested DNA from females of this strain revealed no P elements. In this strain, *st* was retained since, to our eyes, it eased the scoring of *pr*, particularly in older flies.

(7) C(1)DX *yf*; T(1; Y) y^+; *bw*; *st* females. These were derived from the crosses that produced strain 34 (Brookfield & Lewis, 1989) but selected for mosaicism. They would be expected to bear the *Mo* allele on chromosome 2, although it is not certain that this allele was fixed in the strain.

Crosses

Strain 7 females were crossed to strain 6 males, and F1 females crossed to further strain 6 males. A small number of strain 7 females were initially used, and all of the small number of offspring tested were hetero-

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zygous for *Mo*, a result consistent with *Mo* being homozygous in the parental strain 7 females. In successive crosses $pr^+ b^+ dp^+/b\,pr\,dp$ females were selected and crossed to strain 6 males for a number of generations. The phenotype of somatic instability of sn^{vw}, which requires the maternal effect of the *Mo* mutation, was selected each generation. In other words, females were used whose brothers included flies mosaic for sn^{vw}. The selection would have caused the gradual replacement of the genome of strain 7 with that of strain 6 with the exception of the region between *dp* and *Mo*. This crossing scheme therefore kept the *Mo* mutation heterozygous indefinitely, and was used to map the mutation (Brookfield, Gurd & Ortori, in preparation). From these crosses individual females that were respectively *pr b dp* homozygotes and $pr^+ b^+ dp^+/pr\,b\,dp$ heterozygotes were selected and crossed to strain 6 males. Due to a close linkage between *Mo* and pr^+ (RF = 10% (Brookfield, Gurd & Ortori, in preparation)), the first class of females probably possess *Mo* and the second class probably lack *Mo*. A large number (< 20) of male offspring of each female were examined for mosaicism and dissection of thoraces performed either on mosaic males with pr^+/pr mothers (classified as showing mosaicism), or on males with *pr/pr* mothers, and no visible mosaicism among themselves or their brothers (which were thereby classified as lacking mosaicism).

Strain 6 males were used as a source of sn^{vw} DNA.

A number of germline revertants of sn^{vw} were generated by exposing sn^{vw} to *Mo* either maternally or zygotically, or both. These experiments were performed by taking male offspring of *Mo* or Mo^- mothers, and crossing them to strain 6 females. The presence of *Mo* in the males could be inferred from their genotype at *pr*, and reversions of sn^{vw} in the germline could be observed in the phenotypes of their sons. These had either strongly *singed* or wild-type phenotypes, or, more rarely, intermediate phenotypes that were nevertheless distinguishable from sn^{vw}. These lines were maintained by crossing revertant males to strain 6 females and selecting *pr b dp* homozygotes in the F1. *Mo* was thereby removed and the revertants subsequently remained stable.

Flies were maintained at 22 °C in a 12 h light:12 h dark cycle on a standard oatmeal and molasses mixture.

3. Results

Sequence of singed$^{very\ weak}$

Figure 1 shows the organization of the sites of P element insertion in the $singed^{vw}$ allele. The two P elements are inserted at EMBL access number X17548 (Paterson & O'Hare, 1991) base pairs 2647–54 of the 5′ non-coding exon (this is sequence 1574–81 in section A of Paterson & O'Hare (1991)). The inserted elements have single internal deletions; the larger element corresponds to the KP element of Black *et al.* (1987), with all base pairs between 807 and 2561 removed, and the smaller has a deletion removing base pairs between 204 and 2500 in the sequence of O'Hare & Rubin (1983). The smaller sequence corresponds neither to any of the P elements inserted in *white* in the data of O'Hare & Rubin (1983), nor any of the deleted elements found in the P strain $\pi 2$ (O'Hare *et al.* 1992). It proved impossible to amplify the two P elements simultaneously and the structure presented was derived by amplifying the two P elements individually using primers JB3 and JB7 (which amplify the larger of the two elements), and JB4 and JB7 (which amplify the smaller of the two elements). Sequencing was also performed using these primers. There remains a small piece of DNA, of an expected size of 54 base pairs, which was not sequenced, which is that between the two binding sites for the primers JB7 in Fig. 1. We presume that the 3′ ends of both elements are intact in sn^{vw}. Previous restriction mapping of the allele (Brookfield & Lewis, 1989) is consistent with this interpretation. The 3′ ends of the two elements are normal in the strongly *singed* and pseudo-wild type revertants of the allele. We further presume that the elements are separated by the eight base pair sequence TTCCAGAT, which is duplicated at the outer extremities of the two elements.

Germ-line reversion

The majority of revertants show either a strong *singed* or a wild-type appearance. Figure 2 shows the PCR products using primers JB3 and JB4 generated when a collection of sn^{vw} germline revertants were examined. The sizes of the PCR products are consistent with the strongly *singed* revertants being the result of precise excisions of the smaller of the two P elements, and the wild-type products being the result of precise excision of the larger of the two elements. This has been confirmed by the sequencing of the insertion site of the P elements in seven strongly *singed* revertants and three pseudo-wild type alleles. There are rarer revertant classes of sn^{vw} which differ phenotypically from these, and in which at least two P elements in inverted orientation are retained between primer sites JB3 and JB4, but the molecular characterization of these is not complete.

Somatic reversion

It proved impossible to amplify the sn^{vw} allele with primers JB3 and JB4 derived from flanking *singed* DNA. We hypothesize that this is the result of the long stretch of inverted repeated DNA in the substrate for the amplification forming secondary structures during the PCR amplification and thereby preventing synthesis of a product. Fig. 1 reveals that there should be inverted repeats of 204 base pairs and 347 base pairs generated by the 5′ and 3′ ends respectively of

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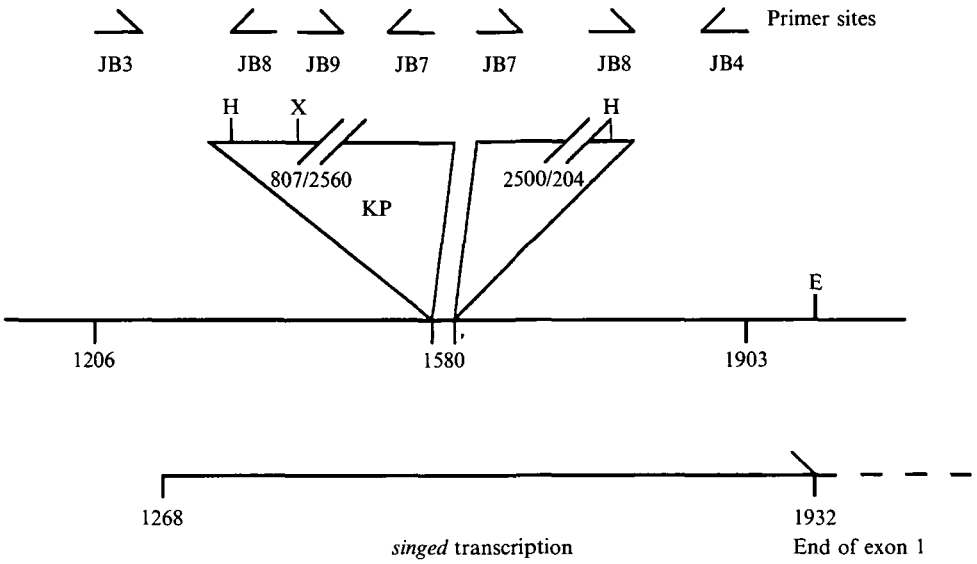

Fig. 1. Structure of the *singed*$^{very\ weak}$ allele (not to scale). The approximate positions of the five PCR primers used in the study are shown above. The positions of the internal deletions within the two P elements are shown within the triangles representing the elements. The lower line represents the structure of *singed* from Paterson & O'Hare (1991), along with the start and end of the (untranslated) first exon. H = *Hin*d III site, E = *Eco* R I site, X = *Xho* I site.

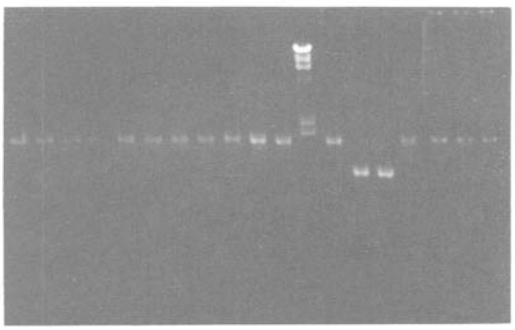

Fig. 2. 1% agarose gel of the DNAs produced by PCR amplification using primers JB3 and JB4 of germline revertants of *singed*$^{very\ weak}$. Lanes 1–3, 5–11, 13 and 16–19 represent independently produced strong *singed* alleles, and lanes 4, 14 and 15 represent independent pseudo-wild type revertants. Lane 12 shows length standards of λ phage digested with *Hin*d III with fragment sizes in kilobases: 23·1, 9·4, 6·7, 4·3, 2·3 and 2·0.

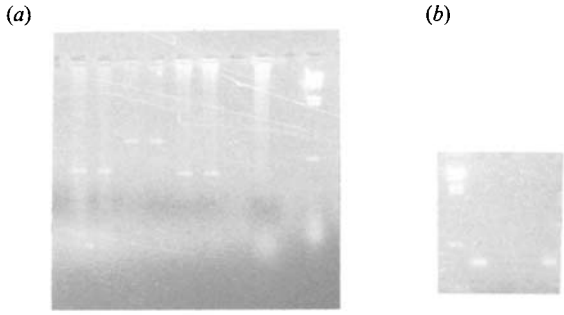

Fig. 3. (*a*) 1% agarose gel showing PCR products from individual thoraces of *sn*^{vw} males with *Mo* mothers. Lanes 1, 3 and 5 are derived from a DNA sample prepared from a single thorax of a mosaic male, and lanes 2, 4 and 6 are derived from a different individual. Lanes 1, 2, 5 and 6 are produced using primers JB4 and JB8 and show the 400 base pair product, whereas lanes 3 and 4 show the 784 base pair product produced by amplification using JB4 and JB9. Lanes 7 and 9 are empty, and lane 8 contains an unsuccessful PCR amplification not relevant to this paper. Lane 10 shows length standards of λ 'phage DNA digested with *Hin*d III. Fragments sizes in kilobases are: 23·1, 9·4, 6·7, 4·3, 2·3, 2·0 and 0·56. (*b*) 1% agarose gel showing the PCR products from DNA from an individual thorax of a *sn*^{vw} male with a *Mo*$^{-}$ mother. Lane 1 shows *Hin*d III-digested λ 'phage DNA, lane 2 the 400 base pair product using primers JB4, JB8 and JB9. Lane 3 shows the absence of a product using JB8 and JB9. Lane 4 shows the absence of a product with JB4 and JB9, and lane 4 the 400 base pair product produced with primers JB4 and JB8.

the two P elements. No problems were found in the amplification of reverted alleles in which only one P element remained between the *singed* primers (Fig. 2). This allowed us to utilize a strategy to search for excisions of the smaller element in mosaic flies. DNA was prepared from the thoraces of males from mothers bearing the *Mo* allele, and, as a negative control, from males whose mothers lacked *Mo*. PCR amplifications using the primers JB4 and JB8, and JB4 and JB9 were performed on this DNA. The amplification using JB4 and JB9 is consistently unsuccessful using *sn*^{vw} DNA as a substrate, due, we hypothesize, to the inverted repeat of 347 base pairs that exists in the target DNA. However, amplification is successful, generating a 784 bp product, using the strongly *singed* germline revertant as a target. Fig. 3(*a*) and (*b*) shows the result of amplification using DNA from thoraces of males with *Mo* and *Mo*$^{-}$ mothers respectively. The former show the 784 base pair band resulting from amplification of DNA from which the smaller P element has been removed. Amplifications with JB4 and JB8 will

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produce a 400 base pair product whenever the smaller of the two elements are retained, and this is seen in Fig. 3(*b*).

4. Discussion

The insertion site of the two P elements lies outside the hotspot for P element insertions described by Roiha, Rubin & O'Hare (1987) but shares 5 of the 8 bases in the consensus sequence derived from P element insertion points. This is significantly different from the null hypothesis of random base sequence at the 5% level. The site of insertion is in the 5′ untranslated exon. However, it is not clear how sn^{vw}, or its strong *singed* or pseudo-wild-type products have their effects on *singed* phenotype. The similar $singed^{weak}$ mutation has its two elements in head-to-head rather than the tail-to-tail orientation of sn^{vw}. Both mutations will revert to strong *singed* and pseudo-wild-type products by precise excision of one or the other of the P elements. The element remaining in the *singed* products is, in each case, the one whose transcription would be in the same direction as *singed*, while, correspondingly, when the element remaining has transcription in the opposite orientation to *singed* the pseudo-wild-type product is generated. However, this is probably coincidental since P element insertions in either orientation can generate strong *singed* mutations (Roiha, Rubin & O'Hare, 1987, Brookfield & Lewis, 1989). Furthermore, while it is easy to imagine how the structure of the strong *singed* product, in which the transcriptional stop signals at the end of the KP element would be expected to stop *singed* transcripts, explains the phenotype, it is hard to see how a weaker phenotype is restored by the addition of the second element downstream in sn^{vw}.

The $singed^{very\ weak}$ allele generates revertants at high frequency by the precise excision of one or other of the P elements that it contains. This process occurs in the germline or occurs somatically under the influence of the maternal *Mo* product. While it could be imagined that the observed phenotypic change in *singed* was an epigenetic phenomenon, in which *singed* is permanently inactivated in a clone of cells despite maintenance of the sn^{vw} structure, the experiments presented here reveal that the phenotypic change is accompanied by an excision of DNA. The mosaicism of sn^{vw} has been described here in terms of the excision of the smaller of the two P elements. The phenotypic difference between sn^{vw} and its pseudo-wild type product is too slight to allow the scoring of flies for mosaicism using this difference. Equally, the choice of primers in our molecular test for mosaicism precludes the demonstration of somatic excision of the larger of the inserted elements. It is possible, however, that the precise excision process of the KP element in sn^{vw} seen in the germ line is being duplicated in somatic cells under the influence of maternal *Mo*. The precise excision could operate via the mechanism discussed by Engels (1989), in which precise excision is the result of the presence of direct repeats of 39 base pairs in the elements. The mechanism for P element excision is now known to be a cut-and-patch DNA repair mechanism, in which transposition of an element is associated with the creation of a double strand break in the donor chromosome. This break can then be repaired using the sister chromatid, the homologue, or an ectopic copy of the same sequence as the donor. If the sister chromatid is used, the repair will regenerate the original sequence, except that the repair process can be incomplete, and can result in deletions, flanked by direct repeats within the element sequence (Gloor *et al.* 1991). If, however, an insertion mutation is heterozygous with a wild-type allele, the repair of the double strand break will substitute the wild type allele for the mutant. This results in a high frequency of precise excisions being observed for single P element insertion mutations in the heterozygous state (Engels *et al.* 1990). In our experiments, the reversion of $singed^{very\ weak}$ is occurring in hemizygous males, and thus the excision of one or the other elements is presumably the result of an incomplete repair of the insertion, in which the repair, using the sister chromatid's $singed^{very\ weak}$ as a donor, is incomplete. The 39-base pair internal repeats of sn^{vw} can thus be used as sites of direct repetition at which the repair can stall. This will result in precise excision of one or other element. That the somatic and germline excision processes produce products that are consistent with those produced by P transposase argues strongly that the *Mo* allele has its effects through P transposase.

The Loua *sn6* mutation, from which the sn^{vw} allele was derived, was generated in a hybrid dysgenic cross in which a strain Loua 83 from Zaire acted as the P-strain parent. The X chromosome into which the P was inserted was from Canton S (Brookfield & Mitchell, 1985). African strains have been shown to contain many copies of KP elements. The presence of the KP element in this hyper-unstable allele argues that this class of elements do not always act as repressors of P element movements. Of course, KP elements are found at diverse chromosomal locations and thus, if these copies are not independently derived from full length sequences, KP elements must be transposable. Indeed, other insertions of the KP element into *singed* have been described (Monastiroti *et al.* 1988). Earlier studies show that the relationship between KP copy number and strength of chromosomally-inherited repression is highly variable and unpredictable (Boussey *et al.* 1988, Heath & Simmons, 1991, Raymond *et al.* 1991, Higuet, Anxolabéhère & Nouaud, 1992, Monastiroti *et al.* 1988). It is possible that the capricious nature of KP's capacity to act as a repressor is the result of repression being conditional upon transcription. This, in turn, will depend upon its chromosomal location. In our experiments the germline excision of the KP element in sn^{vw} has been studied in males in a gene expressed in the female, but

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not the male, germ line. Thus there is no reason to suppose that the level of transcription of KP is any higher than the basal level expected from the weak P element promoter. In females homozygous for sn^{vw} there will be high levels of KP transcription, either from the *singed* promoter or the P promoter stimulated by *singed* enhancers. If KP is a powerful repressor whose activity is transcription-dependent, one would expect that such females would show much lower levels of germline reversion than are seen in males. In addition, we would not expect the mosaicism phenomenon to be repressed in our experiments by a transcription-dependent KP repression. *singed* transcripts are seen in early embryos, and are probably derived from *singed* expression in the ovary (Paterson & O'Hare, 1991). However, the mothers of mosaic males lacked sn^{vw} and thus would not be expected to express KP from the *singed* promoter in their ovaries. Earlier data indicated a lower rate of mosaicism in crosses with normal sex chromosomes than in attached-X crosses (Brookfield & Lewis, 1989), which is consistent with repression through KP expression from the *singed* promoter in the mother. However, recent results in which a KP insertion at 47D on the second chromosome results in repression of P susceptibility indicate that the repression in this case is not the result of KP transcription being stimulated at this site (Higuet, Anxolabéhère & Nouaud, 1992). This is because the effect is recessive, indicating the loss of a chromosomal activity required for high level transposition, and because the level of KP transcripts is reduced in lines showing repression relative to that in KP-bearing lines exhibiting less repression.

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References

Black, D. M., Jackson, M. S., Kidwell, M. G. & Dover, G. A. (1987). KP-elements repress P-induced dysgenesis in *Drosophila melanogaster*. *EMBO Journal* **6**, 4125–4135.

Boussey, I. A., Healy, M. J., Oakeshott, J. G. & Kidwell, M. G. (1988). Molecular analysis of the P-M gonadal dysgenesis cline in Eastern Australian *Drosophila melanogaster*. *Genetics* **119**, 889–902.

Brookfield, J. F. Y. (1991). Models of repression of transposition in P-M hybrid dysgenesis by P cytotype and by zygotically encoded repressor proteins. *Genetics* **128**, 471–486.

Brookfield, J. F. Y. & Lewis, A. P. (1989). Somatic reversion of P transposable element insertion mutations at the *singed* locus of *Drosophila melanogaster* requiring specific P insertions and a *trans*-acting factor. *Genetical Research* **54**, 101–112.

Brookfield, J. F. Y. & Mitchell, S. F. (1985). P-M hybrid dysgenesis using geographically separate P strains of *Drosophila melanogaster*. *Heredity* **55**, 163–165.

Engels, W. R. (1989). P elements in *Drosophila melanogaster*. In *Mobile DNA* (ed. D. E. Berg and M. M. Howe), pp. 437–484. Washington D.C.: American Society for Microbiology.

Engels, W. R., Johnson-Schlitz, D. M. Eggleston, W. B. & Sved, J. (1990). High-frequency P element loss in *Drosophila* is homolog dependent. *Cell* **62**, 515–525.

Gloor, G. B., Nassif, N. A., Johnson-Schlitz, D. M., Preston, C. R. & Engels, W. R. (1991). Targeted gene replacement in *Drosophila* via P-element-induced gap repair. *Science* **253**, 1110–1117.

Heath, E. M. & Simmons, M. J. (1991). Genetic and molecular analysis of repression in the P-M system of hybrid dysgenesis in *Drosophila melanogaster*. *Genetical Research* **57**, 213–226.

Higuet, D., Anxolabéhère, D. & Nouaud, D. (1992). A particular P-element insertion is correlated to the P-induced hybrid dysgenesis repression in *Drosophila melanogaster*. *Genetical Research* **60**, 15–24.

Jackson, M. S., Black, D. M. & Dover, G. A. (1988). Amplification of P elements associated with the repression of hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **120**, 1003–1013.

Jowett, T. (1986). Preparation of nucleic acids. In *Drosophila: a practical approach* (ed. D. B. Roberts), pp. 275–286. Oxford: IRL Press.

Kitamura, T., Kobayashi, S. & Okada, M. (1993). Developmentally-regulated splicing of the third intron of the P element in somatic tissues of *Drosophila* embryos. *Development, Growth and Differentiation* **35**, 67–73.

Kobayashi, S., Kitamura, T., Sasaki, H. & Okada, M. (1993). Two types of pole cells are present in the Drosophila embryo, one with and one without splicing activity for the third P element intron. *Development* **117**, 885–893.

Lewis, A. P. (1987). Ph.D. thesis, University of Leicester.

Misra, S. & Rio, D. C. (1990). Cytotype control of *Drosophila* P element transposition: the 66 kD protein is a repressor of transposase activity. *Cell* **62**, 269–284.

Monastiroti, M., Hatzopoulos, P., Stamatis, N., Yannopoulos, G. & Louis, C. (1988). Cohabitation of KP and full-length P elements in the genome of MR strains inducing P-M-like hybrid dysgenesis in *Drosophila melanogaster*. *Molecular and General Genetics* **215**, 94–99.

Nitasaka, E., Mukai, T. & Yamazaki, T. (1987). Repressor of P elements in *Drosophila melanogaster*: cytotype determination by a defective P element with only open reading frames 0 through 2. *Proceedings of the National Academy of Science U.S.A.* **84**, 7605–7608.

O'Hare, K. & Rubin, G. M. (1983). Structures of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell* **34**, 25–35.

O'Hare, K., Driver, A., McGrath, S. & Johnson-Schlitz, D. M. (1992). Distribution and structure of cloned P elements from the *Drosophila melanogaster* P strain Π2. *Genetical Research* **60**, 33–41.

Paterson, J. & O'Hare, K. (1991). Structure and transcription of the *singed* locus of *Drosophila melanogaster*. *Genetics* **129**, 1073–1084.

Raymond, J. D., Ojala, T. A., White, J. & Simmons, M. J. (1991). Inheritance of P-element regulation in *Drosophila melanogaster*. *Genetical Research* **57**, 227–234.

Rio, D. C. (1990). Molecular mechanisms regulating *Drosophila* P element transposition. *Annual Review of Genetics* **24**, 543–578.

Roiha, H., Rubin, G. M. & O'Hare, K. (1987). P-element insertions and rearrangements at the *singed* locus of *Drosophila melanogaster*. *Genetics* **119**, 75–83.

Ronsseray, S., Lehmann, M. & Anxolabéhère, D. (1991). The maternally inherited regulation of P elements in *Drosophila melanogaster* can be elicited by two P copies at cytological site 1A on the X chromosome. *Genetics* **129**, 501–512.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). Molecular cloning: a laboratory manual. Cold Spring Harbor Press. Cold Spring Harbor, New York.

Simmons, M. J. & Buchholz, L. M. (1985). Transposase titration in *Drosophila melanogaster*: a model of cytotype in the P-M system of hybrid dysgenesis. *Proceedings of the National Academy of Sciences USA* **82**, 8119–8123.

Simmons, M. J., Raymond, J. D., Boedigheimer, M. J. & Zunt, J. R. (1987). The influence of nonautonomous P elements on hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **117**, 671–685.

Simmons, M. J., Raymond, J. D., Rasmusson, K. E., Miller, L. M., McLarnon, C. F. & Zunt, J. R. (1990). Repression of P element-mediated hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **124**, 663–676.

Winship, P. R. (1989). An improved method for directly sequencing PCR-amplified material using DMSO. *Nucleic Acids Research* **17**, 1266.