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P Element Insertions and Rearrangements at the *singed* Locus of *Drosophila melanogaster*

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ABSTRACT

DNA from the *singed* gene of *Drosophila melanogaster* was isolated using an inversion between a previously cloned *P* element at cytological location 17C and the hypermutable allele *singed-weak*. Five out of nine *singed* mutants examined have alterations in their DNA maps in this region. The *singed* locus is a hotspot for mutation during P-M hybrid dysgenesis, and we have analyzed 22 mutations induced by P-M hybrid dysgenesis. All 22 have a *P* element inserted within a 700-bp region. The precise positions of 10 *P* element insertions were determined and they define 4 sites within a 100-bp interval. During P-M hybrid dysgenesis, the *singed-weak* allele is destabilized, producing two classes of phenotypically altered derivatives at high frequency. In *singed-weak*, two defective *P* elements are present in a "head-to-head" or inverse tandem arrangement. Excision of one element results in a more extreme singed bristle phenotype while excision of the other leads to a wild-type bristle phenotype.

TRANSPOSABLE elements (TEs) are discrete segments of DNA that can move from site to site in the genome. They have been identified in a wide variety of bacteria, fungi, plants and animals, and have a wide range of internal structures (reviewed by SHAPIRO 1983). The genome of *Drosophila melanogaster* contains at least 30 families of TE that can be divided into at least four structural classes (reviewed by FINNEGAN and FAWCETT 1986).

The *P* element is a member of the class of TE which has small inverse terminal repeats (reviewed by ENGELS 1983). There are strains with no *P* elements (M strains) and strains (P strains) with many *P* elements, at least some of which are functionally intact. When a P male is crossed with an M female, P transposase is produced in the developing germ cells of the F_1 progeny. This results in mobilization of *P* elements and leads to new mutations, chromosomal rearrangements and other abnormalities which are collectively known as P-M hybrid dysgenesis. Transposition of *P* elements is suppressed in P strains, nor does it occur when P females are mated with M males. This is thought to be due to maternally contributed regulatory factors which ultimately depend on the *P* elements themselves. This control is referred to as cytotype and P strains are said to have the P cytotype.

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Thus for mobilisation to occur, functional *P* elements must be introduced into the M cytotype.

P strains appear to have 30–50 *P* elements dispersed throughout the chromosomes (BINGHAM, KIDWELL and RUBIN 1982). The *P* elements from one P strain, π_2, have been analyzed in detail (O'HARE and RUBIN 1983). About one-third of the elements are 2.9 kb in size and have been shown to produce P transposase when injected into M strain embryos (SPRADLING and RUBIN 1982). The structures of each of the remaining *P* elements appear to be unique and can be derived from that of the 2.9-kb element by different internal deletions which conserve at least 150 bp from each end. They cannot themselves encode transposase, but they retain the sequences required in *cis* to be mobilized by transposase provided in *trans* by the 2.9-kb elements.

P elements show considerable specificity in their insertion sites. The frequency of mutation of different loci varies enormously, from greater than 10^{-3} for the *singed* locus to less than 10^{-6} for the *alcohol dehydrogenase* gene (reviewed by ENGELS 1983; KIDWELL 1986). For the 14 visible loci studied by GREEN (1977) and the 15 lethal loci studied by SIMMONS and LIM (1980), the rates were between these extremes figures.

Within a locus there is specificity for the site of *P* element insertion. Three of four insertions studied at the *white* locus were at precisely the same nucleotide (O'HARE and RUBIN 1983) and studies of mutations of the *rudimentary* (TSUBOTA, ASHBURNER and SCHEDL 1985), *RpII* (SEARLES *et al.* 1986), *yellow* (CHIA *et al.*

1986) and *Notch* (KELLEY *et al.* 1987) loci show clustering of *P* element insertions. Examination of 18 of the 8-bp target site duplications found bounding *P* elements in the π_2 genome indicated only a weak consensus sequence (O'HARE and RUBIN 1983). Therefore, the features which constitute an attractive site for insertion may be complex.

Mutations caused by insertion of different classes of TE in *Drosophila* differ in the frequency with which they revert or mutate further. Insertions of retroviral-like elements such as copia in *white-apricot*, and insertions of F-like elements such as Doc in *white-one* are generally stable, while insertions of foldback elements as in *white-crimson* are highly mutable (for references, see the review by FINNEGAN and FAWCETT 1986). Insertions of *P* elements are stable in the P cytotype, but in the presence of transposase in the M cytotype, many of them revert at frequencies between 10^{-2} and 10^{-3} (RUBIN, KIDWELL and BINGHAM 1982). ENGELS (1979) has described an unusual mutation of the *singed* (*sn*) locus which was induced by P-M hybrid dysgenesis, *singed-weak* (sn^w). The sn^w allele is hypermutable in the M cytotype, mutating to either a more extreme allele (sn^e) or reverting to a wild-type bristle phenotype at a rate which can exceed 50%, the two derivatives being generated at approximately equal rates (ENGELS 1979). The two classes of derivative are themselves unstable, but only at the rate seen for other mutations generated at *sn* or other loci by P-M hybrid dysgenesis.

In this paper we address two main questions. First, why is *sn* a hotspot for P element insertions? Second, why is the sn^w mutation 50 times more mutable than other *sn* mutations induced by P-M hybrid dysgenesis? In addition, we describe the structure of two *P* element induced chromosomal rearrangements and the isolation of at least part of the wild-type *sn* locus.

MATERIALS AND METHODS

Drosophila strains, mutants and genetics: The sn^w M cytotype strain and the rearrangement strains B332.1 and B337.2 have been described by ENGELS and PRESTON (1981). All other *sn* mutants (Table 1) were from stock centers.

Derivatives of sn^w which differed only in their *sn* genotype were generated by mating females of the sn^w M strain with males from the P strain π_2. F_1 male progeny were mated individually to compound *X* M strain females and stocks established from individual F_2 male progeny showing the weak, extreme and wild-type bristle phenotypes. In this way, sets of stocks carrying *sn* alleles which arose in the germline of a single dysgenic male were established.

The *P* element insertion mutants (Table 2) were provided by M. M. GREEN, C. OSGOOD and W. R. ENGELS. The derivation of those from M. M. GREEN has been described (GREEN 1986). The KH, KHL and CFL series are from C. OSGOOD, and the P strains used were Kerbiniou (KH and KHL series) or Luminy (CFL series) males. The P strain used by ENGELS was π_2.

Recombinant DNA techniques: Phage libraries were constructed using λ charon 28, λ charon 35 and λ EMBL4 as vectors and screened as described in RUBIN, KIDWELL and BINGHAM (1982). Mapping and subcloning of the cloned regions was by standard techniques (MANIATIS, FRITSCH and SAMBROOK 1982). Restriction enzyme fragments were cloned into M13 vectors and the sequence of their inserts determined by dideoxynucleotide sequencing as described by O'HARE and RUBIN (1983). For the analysis of *P* element insertion sites, if the λ clone contained the left end of the *P* element, then a *Hin*dIII or *Hin*dIII-*Eco*RI fragment was subcloned into appropriate M13 vectors and the universal M13 primer was used to determine the DNA sequence of the *P* element-*sn* junction. If the λ clone contained the right end of the *P* element, then a *Sal*I or *Eco*RI-*Xba*I fragment was subcloned into M13 and the sequence determined using an oligonucleotide corresponding to positions 2847–2865 of the 2.9-kb *P* element as primer.

RESULTS

Attempts to clone *sn* by transposon tagging: Our initial attempts to clone the *sn* locus made use of the sn^w M strain described by ENGELS and PRESTON (1981). The sn^w mutation arose during P-M hybrid dysgenesis (ENGELS 1979), and the properties of this sn^w M strain indicate that any *P* elements present cannot produce either transposase or any factors which might regulate transposition (ENGELS 1984). Preliminary DNA blotting experiments (data not shown) showed that the sn^w strain had very few *P* elements. Preliminary *in situ* hybridization experiments (A. SPRADLING, personal communication) showed that, as expected, the only region of *P* element homology was at cytological position 7D, the location of the *sn* locus (LINDSLEY and GRELL 1968).

Genomic DNA from the sn^w M strain was partially digested by *Sau*3AI and a library of 12–14-kb fragments was made using the λ vector charon 28. A total of 6×10^5 recombinants were screened using as probe a mixture of fragments containing the *P* elements inserted into the *white* locus in mutants $w^{\#6}$ and $w^{\#12}$ (RUBIN, KIDWELL and BINGHAM 1982). Forty-four positively hybridizing phage were purified and counterscreened with probes from the wild-type *white* locus. This eliminated 16 phage, and 6 of the remaining phage with P homology were grown up and their inserts mapped. The maps were overlapping, indicating that all 6 were derived from the same genomic location where a 0.5-kb *P* element was inserted. The corresponding regions from both π_2 and Canton S were cloned and shown to differ by the absence of this *P* element. Hybridization of flanking probes to the remaining 22 phage showed that all the phage purified from the library which had *P* element homology were derived from this region.

However, a number of experiments indicate that this cloned region does not correspond to the *sn* locus, but is a closely linked site where a *P* element is inserted. DNA blotting experiments showed that phenotypic changes from sn^w to sn^e and to the phe-

notypically wild-type derivative were not correlated with changes in the restriction enzyme map of this region. This apparent discrepancy was resolved by the observation that *in situ* hybridization experiments on well stretched chromosomes of the sn^w M strain resolve two sites of *P* element homology, at 7D1-2 and 7D4-5 (A. SPRADLING, personal comminication; W. R. ENGELS, personal communication). The site described above corresponds to 7D4-5 and the *P* element insertion is not associated with any discernible phenotype. DNA from this site has been used to initiate a chromosomal walk in this region (DIGAN *et al.* 1986).

When unique probes became available for the sn^+ region (see below), repeated unsuccessful attempts were made to clone the sn^w allele directly in λ libraries. Other regions of the genome were appropriately represented in the libraries, indicating that this failure was due to some particular feature of the DNA sequences of the *sn* region from sn^w. We estimate that these sequences were underrepresented, even in unamplified libraries, at least 50-fold.

Cloning of *sn* by chromosome jumping: ENGELS and PRESTON (1981) identified a chromosomal inversion present in the strain B337.2 between sn^w at cytological position 7D1-1 and a *P* element at 17C, the location of the *held up b* (*hdp-b*) locus. This inversion was generated in P-M hybrid dysgenesis and was associated with simultaneous mutation from sn^w hdp-b^+ to sn^e hdp-b^-. The 17C region, including this *P* element had already been cloned (O'HARE and RUBIN 1983), so it was possible to use probes from this region to investigate the structure of the rearrangement. The site of insertion of the *P* element at 17C is approximately in the center of a 1.8-kb *Bam*HI fragment cloned in the plasmid pS25.1. When this plasmid was used to probe a DNA blot of B337.2 genomic DNA digested with *Bam*HI, two fragments of 8 and 12 kb were detected (data not shown). These novel fragments presumably represent fusions of sequences from 17C to sequences from *sn*. A bacteriophage λ library of *Bam*HI digested B337.2 DNA was constructed using the vector charon 28, and 6 phage (out of 10^5 screened) which hybridized to pS25.1 were purified and analyzed. Five contained the 8-kb fragment (often inserted with other small *Bam*HI fragments) and one contained the 12-kb fragment. The structures of the 8- and 12-kb fragments, as well as those of similar junction fragments cloned from another, cytologically identical inversion, B332.1, also induced by P-M hybrid dysgenesis (ENGELS and PRESTON 1981) are shown in Figure 1. Each fragment contained, in addition to *P* element sequences and flanking sequences from 17C, sequences which are presumably derived from the other end of the inversion. Fragments from the presumptive *sn* region of B337.2 were used as probes to isolate

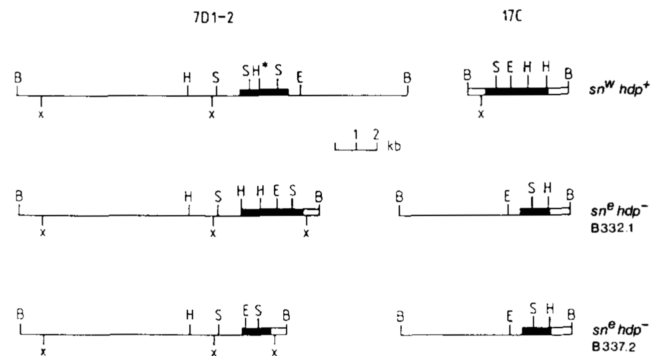

FIGURE 1.—The arrangement of DNA sequences at cytological locations 7D1-2 and 17C in the sn^w hdp-b^+ strain and its two inversion derivatives, B332.1 and B337.2. The *solid bars* represent *P* element sequences, the *open bars* are sequence from 17C and the thin lines are from 7D1-2. The maps of the fragments from B332.1 and B337.2 were constructed from restriction enzyme mapping of the cloned fragments in conjunction with DNA blotting using both *P* element and 17C probes. The fragment from 17C in the sn^w hdp-b^+ strain has been described (O'HARE and RUBIN 1983). The fragments are orientated as they are in the *X* chromosome with distal to proximal being from left to right. The analysis that led to the proposed structure for the 7D1-2 region of the sn^w hdp-b^+ strain is presented in the text. Restriction enzyme cleavage sites are abbreviated as follows: *Bam*HI (B), *Eco*RI (E), *Sal*I (S), *Xba*I (X) and *Hin*dIII (H). H* represents a small region that we believe contains two *Hin*dIII sites separated by 88 bp (see text).

homologous phage from a wild-type library (MANIATIS *et al.* 1978). The inserts of the phage were overlapping and a map (Figure 2) of some 35 kb was constructed which should include at least a part of the *sn* locus. *In situ* hybridization experiments (A. SPRADLING, personal communication; W. R. ENGELS, personal communication) showed that this region corresponds to 7D1-2. The map showed no similarities to that for 7D4-5, nor did the two regions cloned from the Canton S library show any homology in DNA blotting experiments. DNA blots showed that there was an insertion in this region in sn^w, and that phenotypic changes to sn^e and to the phenotypically wild-type derivative were correlated with changes at the DNA level (see below). We conclude that at least a part of the *sn* locus is contained within this genomic region.

Analysis of *sn* mutations: Nine *sn* mutants obtained from stock centers were examined by DNA blotting for changes in the cloned interval using the plasmid subclones sn1, sn4, sn9 and sn12 (see Figure 2) as probes. The results of this analysis and our conclusions about the nature of the changes are shown in Table 1. The locations of those changes detected are shown in Figure 2. Out of 9 mutants analyzed, 5 had changes in this region, clustered between coordinates −1.9 and +4.3. This supports our conclusion that at least part of the *sn* locus is within the cloned interval.

A fine structure genetic map has been constructed from data on recombination between *sn* alleles (HEX-

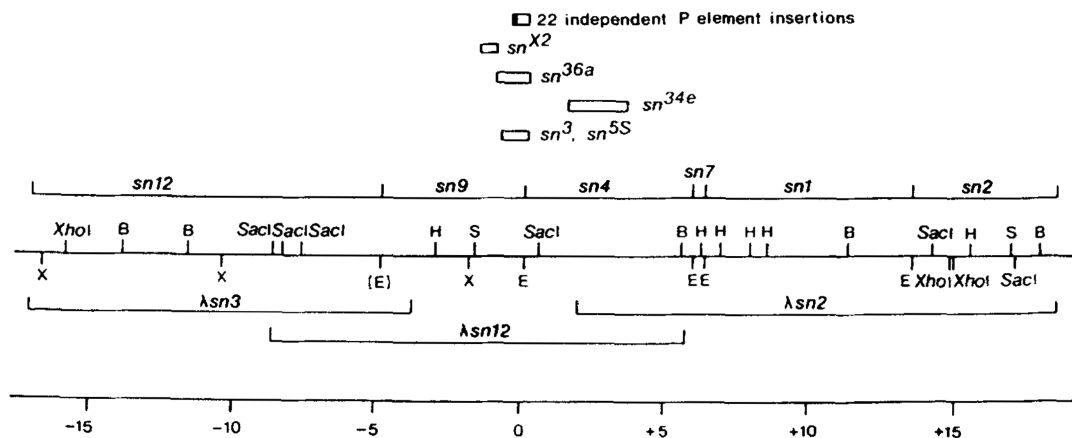

FIGURE 2.—DNA map of the *singed* region. The map was derived by restriction enzyme mapping of overlapping phage containing inserts from Canton S (λsn3, λsn12 and λsn2 are shown as examples) and of the *Eco*RI fragments subcloned into pBR322 (sn12, sn9, sn4, sn7, sn1 and sn2). The coordinate system is in kilobases and uses the *Eco*RI site closest to the breakpoint in the inversions B332.1 and B337.2 as origin. The abbreviations for restriction enzyme sites are as in the legend to Figure 1. The site shown as (E) is a polymorphic *Eco*RI site found only in Canton S. The intervals within which different *sn* mutants show changes in their DNA map are indicated (see Table 1). In the interval where the *P* element insertions lie, the *shaded area* is the 100 bp which contains the sites of insertion of those 10 whose precise position was determined by DNA sequencing.

TABLE 1

***sn* mutants**

Allele	Origin	Phenotype: Bristles	Phenotype: Female fertility	Change	Position
sn^1	Spontaneous	Extreme	Sterile		
sn^2	Spontaneous	Weak	Fertile		
sn^3	Spontaneous	Extreme	Fertile	0.3-kb deletion	−0.9 to 0.0
sn^4	Spontaneous	Weak	Fertile		
sn^5	Spontaneous	Extreme	Sterile		
sn^{34e}	Spontaneous[a]	Weak	Fertile	Complex[b]	+2.4 to +4.3
sn^{36a}	Spontaneous	Extreme	Sterile	5.5-kb insertion	−1.2 to 0.0
sn^{5S}	Spontaneous	Extreme	Sterile	2-kb insertion	−0.9 to 0.0
sn^{X2}	X-ray	Extreme	Sterile	0.1-kb insertion	−1.9 to −1.2

[a] sn^{34e} probably arose spontaneously (see LINDSLEY and GRELL 1968).
[b] The change in sn^{34e} could be either a large insertion or an inversion.

TER 1957; BENDER 1960). Of the 7 mutants mapped (sn^1, sn^2, sn^3, sn^4, sn^5, sn^{36a} and sn^{50k}), we have examined 6 (see Table 1). Changes were detected in the DNA maps of sn^3 and sn^{36a}, and both these alleles define one end of the genetic map. The other mutations could be point changes within the region analyzed, or be gross changes in a large gene which extends beyond the region analyzed. However, the gene is not likely to be unusually large. If figures from other cloned genes such as *white* (LEVIS, BINGHAM and RUBIN 1982) and *Notch* (ARTAVANIS-TSAKONAS, MUSKAVITCH and YEDVOBNICK 1983) are used to provide a conversion factor from map units to kb of DNA, then the frequency of intra-allelic recombination observed in the genetic studies suggests that the *sn* gene is perhaps 10 kb long.

***P* element insertions at *sn*:** Although new mutations occur at *sn* during P-M hybrid dysgenesis at up to 1%, *sn* is not a hotspot for other mutagenic agents. To investigate the nature of *sn* as a hotspot for *P* element insertions, we have examined the distribution of *P* element insertion sites in 22 independent P-induced *sn* mutations generated in three laboratories (see MATERIALS AND METHODS). The *P* elements came from several P and MR (male recombination) strains, and several different M strains were used (Table 2). All 22 had insertions between coordinates −0.7 and 0.0 (Figure 2) as determined by DNA blotting experiments. This represents a smaller interval within the region where changes are found in other *sn* mutants (see above).

To determine precisely the positions of insertion of the *P* elements, we isolated a DNA fragment spanning at least one junction of the inserted *P* element with *sn* DNA for each of 10 mutations. DNA from the mutants was digested with *Sac*I and a λ library of these fragments made using charon 35 as vector. Phage which hybridized to the sn9 subclone were purified and analyzed. For insertions of *P* elements which lack *Sac*I sites, the cloned fragment

TABLE 2

***P* element insertion *sn* mutations**

sn allele	Source[a]	Size and orientation of insertion		Position
79h2(26)	1	1.0 kb	←	−667 to −660
79b10(2)	1	1.0 kb	←	
79f22	1	1.0 kb	←	
83i7(1)	1	2.9 kb	− − →	−667 to −660
83i7(15)	1	2.9 kb	− − →	
83i7(2)	1	2.9 kb	← − −	−667 to −660
83i7(3)	1	2.9 kb	− − →	−667 to −660
83i7(17)	1	2.9 kb	← − −	−667 to −660
CFL3	2	2.9 kb	← − −	
CFL5	2	1.3 kb	→	−645 to −638
KH36	2	0.4 kb	→	
KH40	2	1.0 kb	←	
KHL1	2	0.4 kb	←	
KHL3	2	0.4 kb	←	
KHL4	2	0.6 kb	→	
KHL5	2	0.4 kb	←	
MJ19.3	3	0.5 kb		
MK7	3	2.9 kb	← − −	−589 to −582
MH18.2	3	1.0 kb		
MI17	3	0.5 kb		
MH26.5	3	2.9 kb	← − −	−667 to −660
A2.4	3	1.2 kb	→	−684 to −677

[a] 1 - M. M. GREEN, 2 - C. OSGOOD, and 3 - W. R. ENGELS.

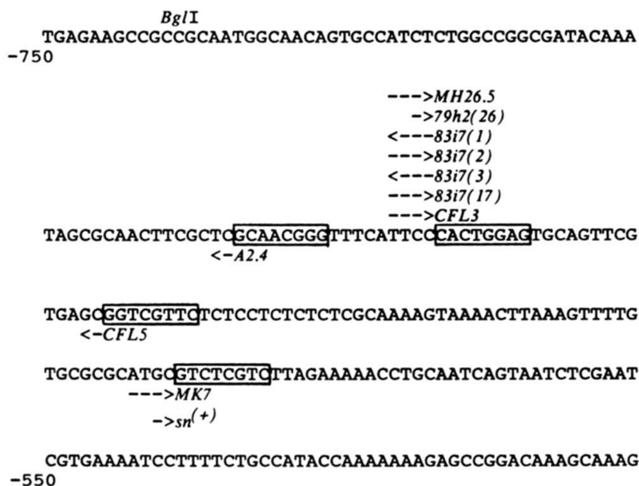

FIGURE 3.—*P* element insertions at the *singed* locus. The boxed 8-bp sequences are "target" sites which are found as direct duplications flanking inserted P elements. The orientation and an indication of the size of the P element associated with each mutation is shown. ← − − represents a 2.9-kb element while ← represents a deleted element. The *arrowhead* corresponds to the right end of the *P* element, which is 3′ with respect to *P* element transcription. The numbers, −500 and −750, indicate the distance in nucleotides from the *Eco*RI site used as origin in the coordinate system.

contained the entire *P* element and the *sn* sequences between the *Sac*I sites at coordinates −7.9 and +0.5. For insertions of *P* elements with a *Sac*I site (such as 2.9-kb *P* elements), the cloned fragment extended from this internal site to that in *sn* at −7.9. The DNA sequence of the junction between the *P* element and *sn* was determined and compared with that of the corresponding region from Canton S. The results of

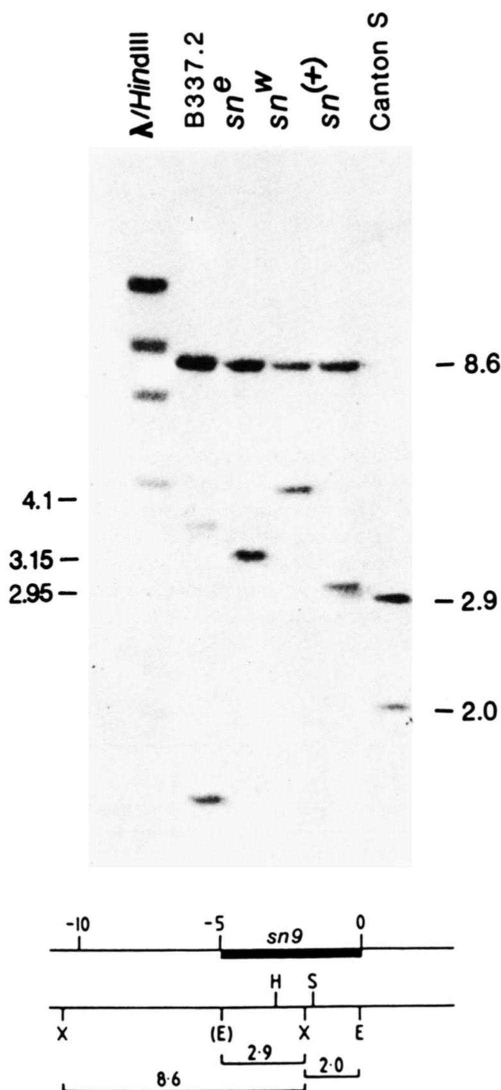

FIGURE 4.—Insertions in sn^w and its derivatives, sn^e and $sn^{(+)}$. Genomic DNA digested with *Eco*RI and *Xba*I was probed with the sn9 plasmid subclone. A portion of the map shows that fragment corresponding to the 2.0-kb *Eco*RI-*Xba*I fragment in Canton S is increased in size to 4.1 kb in sn^w, to 3.15 kb in sn^e and to 2.95 kb in $sn^{(+)}$. This same interval is that disrupted by the inversion in B337.2. The restriction enzyme sites are as in Figure 2, where the site shown as (E) is a polymorphic *Eco*RI site present only in Canton S.

this analysis are shown in Figure 3. Four sites of insertion were found within a 100-bp interval.

The *singed-weak* allele: Sets of sibling stocks whose *sn* alleles arose from sn^w in the germline of individual dysgenic males were established (see MATERIALS AND METHODS). The structures of sn^w and its derivatives were investigated using plasmid subclones which span the 35-kb cloned interval to probe DNA blots of restriction enzyme digests of DNA isolated from these flies. Only the sn9 subclone detected changes in the DNA associated with changes in phenotype.

DNA blot analysis of one set of sibling stocks is shown in Figure 4. In the sn^w allele, an insertion of 2.1 kb is present in the 2.0-kb *Eco*RI-*Xba*I fragment (coordinates −2.0 to 0.0). When sn^w mutates to sn^e

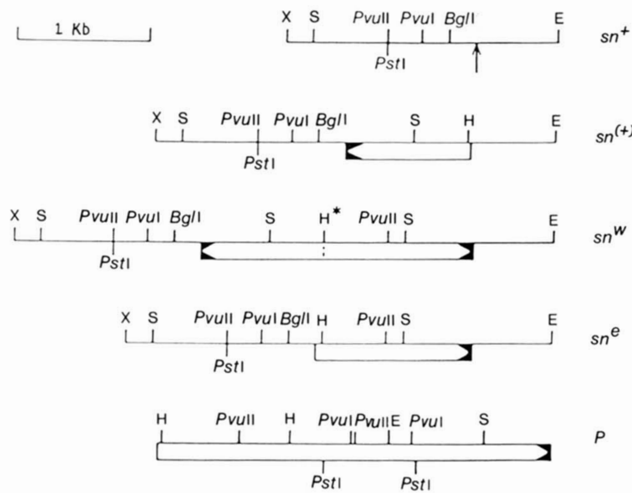

FIGURE 5.—Restriction enzyme maps of sn^{w} and its derivatives sn^{e} and $sn^{(+)}$. Detailed maps of the *Eco*RI-*Xba*I fragments of sn^{w}, sn^{e} and $sn^{(+)}$ that have *P* element insertions (see Figure 4) are shown. The maps were established by probing single and double digests of genomic DNA with the sn9 subclone. For sn^{+} (Canton S) and $sn^{(+)}$, these were confirmed by mapping cloned DNA. A map of the 2.9-kb *P* element is shown for comparison. Abbreviations of restriction enzymes are as in Figure 1. The extent, position and orientation of the inserted *P* elements are shown where the *arrowhead* indicates the right end, which is 3′ with respect to transcription of the *P* element. We believe that in sn^{w}, the terminal sequences of both *P* elements are present, separated by just 8 bp of *sn* sequences (see text). H* thus represents a small region that we believe contains two *Hin*dIII sites separated by 88 bp (see text). ↑ is the position in sn^{+} where *P* elements are inserted in sn^{w}, sn^{e} and $sn^{(+)}$.

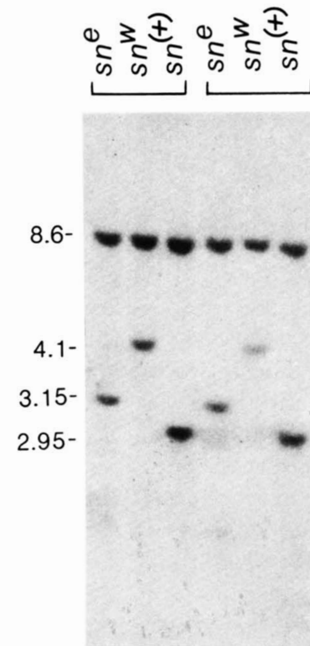

FIGURE 6.—Destabilization of sn^{w}. Sets of stocks which differed only in their *sn* phenotype and which were derived by destabilization of sn^{w} during P-M hybrid dysgenesis in the germline of a single male were analyzed by DNA blotting. The digest and probe were as in Figure 4.

or to a wild-type bristle phenotype, the size of this apparent insertion decreases to 1.15 kb or 0.95 kb, respectively. As the phenotypically wild-type flies still have an insert, we refer to them as $sn^{(+)}$ in order to distinguish them from true wild type, sn^{+}. Additional DNA blotting experiments elucidated the structural change at *sn* in these mutations. Single and double digests of genomic DNA were probed with the sn9 subclone. The maps constructed from this analysis are in Figure 5.

The changes in size are highly reproducible; identical changes were seen in the 15 independent mutations to sn^{e} and in the 11 independent mutations to $sn^{(+)}$ which were examined. Figure 6 shows two more examples of comparisons between sn^{w}, sn^{e} and $sn^{(+)}$ siblings. Changes in the size of the fragments homologous to sn9 are also seen in the chromosomal inversion strain B337.2 (Figure 4), as would be expected if the 7D1-2 breakpoint occurred within or at the site of the insertion in sn^{w}.

A λ recombinant carrying the *sn* region of $sn^{(+)}$ was recovered from a library of *Sac*I fragments as described above. The DNA sequence of the junction of this element with the flanking *sn* sequences was determined and the site of insertion is shown in Figure 3. The *P* element associated with sn^{e} appears to be present in the fragments from the rearrangements B332.1 and B337.2. The DNA sequence of the junction of this element with the flanking *sn* sequences suggests that it is inserted at the same site as the *P* element in $sn^{(+)}$. We conclude that the sn^{w} allele contains two *P* elements of 1.15 and 0.95 kb inserted at the same site in inverted orientation, with only the 8 bp duplication usually found bounding inserted *P* elements (in this case GTCTCGTC) between them.

The structures deduced for the sn^{e} and $sn^{(+)}$ alleles and the reproducibility of the molecular events by which they are generated lend strong support to the hypothesis that the sn^{w} mutation results from the insertion of two *P* elements in inverted orientation. Excision of the 0.95-kb element gives rise to sn^{e} while excision of the 1.15-kb element produces $sn^{(+)}$. The continued instability of the derivatives, albeit at rates typical for other *P* element insertions at *sn*, is presumably due to the presence of the remaining single *P* element.

DISCUSSION

Inversion structures: The inversions between 17C and 7D1-2 created two fusion fragments carrying sequences derived originally from both cytological locations. The proximal fragment is apparently identical in both inversions, and has one of the defective *P* elements from sn^{w} bounded distally by 7D1-2 sequences and proximally by 17C sequences. The distal fragment is somewhat different between the two inversions. In B332.1, the 2.9-kb *P* element from

17C is bounded distally by sequences from 7D1-2 and proximally by sequences from 17C. In B337.2, the flanking sequences are apparently the same as in B332.1, but part of the *P* element from 17C has been lost. In both inversions, the smaller of the two defective *P* elements from sn^w is no longer present. These structures agree with the work by ENGELS and PRESTON (1984) which showed that rearrangements generated during P-M hybrid dysgensis were between sites where *P* elements were inserted and that there was a tendency to lose *P* element sequences upon rearrangement. These two inversions were between *P* element sites, and there was a net loss of *P* element sequences. The flanking sequences appear to have been entirely conserved.

ENGELS and PRESTON (1984) have proposed that during P-M hybrid dysgenesis, a number of chromosomal breaks are made at *P* element sites (presumably by P transposase at the ends of *P* elements) and that the chromosomal fragments so produced reassort to give rearrangements. However, other mechanisms cannot be excluded. In particular, the B332.1 inversion could have been generated by precise excision of the smaller *P* element from sn^w as occurs in the formation of the sn^e allele, followed by homologous recombination between the remaining *P* element and the 2.9-kb element at 17C. In this case, the only role for P transposase would have been to excise a *P* element. However, these inversions occurred in males (ENGELS and PRESTON 1981), and homologous recombination does not normally occur in *Drosophila melanogaster* males. Furthermore, the loss of sequences from the end of the 2.9-kb *P* element in B337.2 is more consistent with the active involvement of transposase, perhaps by making a chromosomal break at this end of this *P* element. Errors during replication of the *P* element sequences from the end, as might occur during transposition (O'HARE and RUBIN 1983), could contribute to the generation of the observed structures.

Why is *sn* a hotspot? Many studies have shown that *sn* is a hotspot for apparent insertion mutations generated in crosses involving strains from the wild (*e.g.*, GOLUBOVSKY, IVANOV and GREEN 1977). The mutagenic agent responsible for many of these events has been determined to be a strain-specific transposable element, the *P* element (BINGHAM, KIDWELL and RUBIN 1982; RUBIN, KIDWELL and BINGHAM 1982). This study confirms that mutations generated in these crosses at *sn* are indeed due to *P* element insertions.

The fact that all *P* element insertions occur in such a small region (less than 700 bp and possibly only 100 bp) indicates that *sn* is not a hotspot merely because it is a very large gene. Our results do confirm the sequence-specific nature of *P* element insertions previously seen at *white* (O'HARE and RUBIN 1983). Elements insert at the same nucleotide position in independent events, but not necessarily in the same orientation. We have previously suggested a consensus sequence for the site of insertion of *P* elements. While this region of *sn* has sequences which do resemble this consensus, not all have been found to be sites of insertion in this study. Furthermore, of the observed sites, the most frequently used is not the best match with the consensus. It seems unlikely therefore that *sn* is a hotspot for insertion of *P* elements merely because the gene has many good matches with this 8-bp consensus. A similar conclusion has been reached from analysis of *P* element insertion sites in the *Notch* gene (KELLEY *et al.* 1987).

It may be relevant that expression of *sn* in the female germline is required for normal oogenesis in Drosophila (PERRIMON and GANS 1983), and *P* element mobilization occurs only in the germ cells. As male *sn* mutants are fertile, *sn* is unlikely to be expressed during male germline development. However, the frequency of *sn* mutation in the male and female germlines is similar (W. R. ENGELS, personal communication). Thus, the basis for the high frequency of *P* element insertion at *sn* remains obscure.

The region where the *P* elements insert is close to the 5′ end of the *sn* gene (K. O'HARE, A. DRIVER and J. PATERSON, unpublished observations). This clustering of insertion sites at the 5′ end has been seen in other genes (TSUBOTA, ASHBURNER and SCHEDL 1985; CHIA *et al.* 1986; SEARLES *et al.* 1986; KELLEY *et al.* 1987), perhaps reflecting chromatin structure(s) which would allow P transposase to interact with these regions. Although counter examples are known, notably at *white* (RUBIN, KIDWELL and BINGHAM 1982), this may explain the distribution of insertion sites within *sn*.

Hypermutability of sn^w: The unusual structure of the sn^w allele is correlated with an unusually high sensitivity to transposase (ENGELS 1979). Each of the two classes of derivative of sn^w generated during P-M hybrid dysgenesis appear to be precise excisions of one of the two *P* elements at a rate of 10^{-1} or higher. We detected no obviously imprecise excisions from sn^w. The sn^e allele reverts to wild-type in P-M hybrid dysgenesis at a significantly lower frequency, between 10^{-2} and 10^{-3} (ENGELS 1979). Other simple *P* element insertions at *sn* revert at similar low rates, and imprecise excisions have been documented (W. R. ENGELS and W. EGGLESTON, personal communication). This suggests that it is the association of two elements in sn^w which is particularly sensitive to transposase and not insertions at *sn* generally or even single *P* elements at the sn^w site. Other hypermutable alleles of *sn* have been described (GOLUBOVSKY 1978a, b; W. EGGLESTON and W. R. ENGELS, personal communication). Preliminary analysis of the structures of some suggests that they may have double *P* element insertions, as in sn^w (W. EGGLESTON and W. R. ENGELS, personal communication).

Perhaps the major difference between a single *P*

element inserted in *sn* and the double *P* element insertion in sn^w, is the close juxtaposition of two *P* element ends. It is possible that during excision of a single *P* element, transposase binds independently to both termini, and that there has to be some interaction between the bound proteins before excision is initiated. The structure of the insertion at sn^w may ensure that this interaction occurs more frequently here than at a single *P* element insertion. This suggests that other sorts of double *P* element insertions might also be hypermutable. As yet, no such structures have been described. However, there is evidence that the left and right ends of a *P* element are not equivalent for transposition (M. MULLINS and G. M. RUBIN, unpublished observations). It therefore remains possible that "head to head" insertions (where two left ends are together), "tail to tail" insertions (where two right ends are together) and "head to tail" insertions (where a left and a right end are together) have different properties.

The sn^e and $sn^{(+)}$ derivatives of sn^w appear to be generated by precise excision of one or the other *P* element. However, other mechanisms could be imagined which would generate their structures. In sn^w, there are two sets of direct 39-bp repeats (made up of 31-bp terminal inverse repeat of the *P* element and the 8-bp target site duplication of *sn* sequences). Homologous recombination or slippage of a replication fork between these repeats, perhaps during attempted transposition, would also produce the observed structures.

***sn* phenotypes:** There is no simple explanation for the phenotypes seen in the *P* element insertion mutations analysed here. Products of the *sn* locus are required for the production of normal bristles and hairs in adult flies, and for female fertility (BENDER 1960). At the most frequently used site (position −667 to −660) all insertions, irrespective of size and orientation, result in strong mutant bristle phenotypes and are female sterile. This suggests that the region disrupted is important for both somatic and germline *sn* functions. Conversely, sn^e and $sn^{(+)}$ appear to have different defective elements inserted in opposite orientation at the same position (−589 to −582) and have quite different bristle phenotypes. That an insertion may have no bristle phenotype suggests that this site is not in the coding sequences of the protein(s) required for the production of normal bristles. The DNA sequence of this region shows no long open reading frames. Peliminary RNA mapping and analysis of cDNAs (K. O'HARE, A. DRIVER and J. PATERSON, unpublished observations) shows that transcription of the *sn* locus proceeds from left to right in Figure 2 and suggests that it is initiated upstream of the sn^w insertion sites. This site may therefore be in an intron or perhaps in the 5′ untranslated region.

An added complication is the recent observation that the female fertility phenotype of at least some *P* element insertion mutants is affected by cytotype (W. R. ENGELS and H. ROBERTSON, personal communication). The basis for this is unclear, and an understanding of this and many of the other questions discussed in this paper will clearly require more information about the structure of the *sn* gene and its pattern of expression.

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