

A *P* Element Containing *suppressor of Hairy-wing* Binding Regions Has Novel Properties for Mutagenesis in *Drosophila melanogaster*

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ABSTRACT

P elements are widely used as insertional mutagens to tag genes, facilitating molecular cloning and analyses. We modified a *P* element so that it carried two copies of the *suppressor of Hairy-wing* [*su(Hw)*] binding regions isolated from the *gypsy* transposable element. This transposon was mobilized, and the genetic consequences of its insertion were analyzed. Gene expression can be altered by the *su(Hw)* protein as a result of blocking the interaction between enhancer/silencer elements and their promoter. These effects can occur over long distances and are general. Therefore, a composite transposon (*SUPor-P* for *suppressor-P* element) combines the mutagenic efficacy of the *gypsy* element with the controllable transposition of *P* elements. We show that, compared to standard *P* elements, this composite transposon causes an expanded repertoire of mutations and produces alleles that are suppressed by *su(Hw)* mutations. The large number of heterochromatic insertions obtained is unusual compared to other insertional mutagenesis procedures, indicating that the *SUPor-P* transposon may be useful for studying the structural and functional properties of heterochromatin.

ALL organisms carry a wide variety of transposable elements in their genomes. In *Drosophila*, several classes of transposable elements have been identified which collectively constitute 10% of the genome (RUBIN 1983). One of the best characterized of these is the *P* element. Complete (autonomous) *P* elements are 2.9 kb in length and contain 31 bp inverted repeats that flank a unique region, which encodes a transposase activity (O'HARE and RUBIN 1983).

A great deal of research has elucidated both the *cis* and *trans* requirements for *P* element mobilization. This research has allowed investigators to exploit *P* elements for several purposes, including germ line transformation (RUBIN and SPRADLING 1982) and isolation of genes by *P* element tagging (reviewed in ENGELS 1988). *P* element insertions into or near a gene can be recognized in one of two ways. Integration of a *P* element may disrupt gene activity resulting in a mutant phenotype, or the regulatory elements in a nearby gene may activate genetic markers carried by the transposon in a process known as "enhancer trapping" (O'KANE and GEHRING 1987; BEIR *et al.* 1989; BELLEN *et al.* 1989; WILSON *et al.* 1989).

Identification of genes by *P* element insertional mutagenesis has been widely applied. In many instances, mutations are induced by mobilization of several *P* elements (KIDWELL 1986; ROBERTSON *et al.* 1988). In this way, mutations are generated in a background of multi-

ple *P* elements. Subsequent mapping, cloning and genetic analysis of a gene tagged in this manner is complicated by the additional *P* elements in the mutant strain. Mobilization of single *P* elements alleviates several of these problems. A large collection of mutant strains has been generated by this method (COOLEY *et al.* 1988a,b; BERG and SPRADLING 1991; KARPEN and SPRADLING 1992). The utility of this technique to induce mutations in a given gene is limited by the low insertion rate of *P* elements within any single locus. However, as the benefits of isolation of mutations caused by single *P* insertions are great, methods are being developed to enhance the rate of single-element insertion into a particular region of the chromosome (TOWER *et al.* 1993).

P elements act as insertional mutagens. Mutations can result from interruption of the open reading frame (RUBIN *et al.* 1982), interference with processing or stability of the mRNA (GEYER *et al.* 1988) or disruption of a regulatory region (KELLEY *et al.* 1987) of a gene. However, insertions of *P* elements within or near a gene are not always associated with a phenotype. For example, three *P* element insertions within the 5' flanking region of the *pourquoi-pas* (*wings-down*) gene did not produce a mutant phenotype (SEGALAT *et al.* 1992). These results suggest that *P* elements may not efficiently alter gene expression when integrated in the proximity of upstream regulatory sites. As *P* elements appear to insert preferentially into these control regions (KELLEY *et al.* 1987), it may be that a large proportion of transpositions near genes go unrecognized. Therefore, increasing the ability of *P* elements to disrupt the action of enhancers should dramatically improve the insertional mutation rate.

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A second well-characterized transposable element in *Drosophila* is the *gypsy* retrotransposon, which is 7.5 kb in length and contains long terminal repeats that flank a region encoding proteins homologous to retroviral proteins (MARLOR *et al.* 1986). These elements are effective mutagens since insertion of *gypsy* can disrupt gene expression even when it resides as much as 10–80 kb from a promoter (PEIFER and BENDER 1986; JACK *et al.* 1991). Several tissue-specific mutations caused by the insertion of *gypsy* have been identified (MODOLELL *et al.* 1983). In many cases, these mutations result from the binding of the *suppressor of Hairy-wing* [*su(Hw)*] protein to a region within the *gypsy* element near the 5' long terminal repeat (reviewed in CORCES and GEYER 1991). The 110-kD *su(Hw)* protein accumulates in most tissues throughout development (PARKHURST *et al.* 1988; HARRISON *et al.* 1993). Association of the *su(Hw)* protein with its *gypsy* binding region causes inactivation of enhancers when the *su(Hw)* binding region is placed between an enhancer and its promoter (GEYER *et al.* 1986; HOLDRIDGE and DORSETT 1991; JACK *et al.* 1991). Effects of the entire *gypsy* element can be duplicated by the *su(Hw)* binding region alone (HOLDRIDGE and DORSETT 1991; GEYER and CORCES 1992). This binding region can generally inactivate both enhancers and silencers present in the *Drosophila* genome (ROSEMAN *et al.* 1993).

The efficiency of *gypsy* in disrupting 5' enhancer function may make it a more effective mutagen than a *P* element. However, a major limitation for using *gypsy* as a mutagen is the inability to control its mobilization. In this paper, we describe the use of a composite *gypsy-P* transposon as a mutator element. This transposon was created by cloning sequences corresponding to the *su(Hw)* binding region between *P* element ends. We generated and characterized several hundred lines that carry a single composite element. We demonstrated that this modified transposon combines the mutagenic properties of *gypsy* and *P* elements, resulting in a higher mutagenic rate than found in standard *P* mutagenesis and production of mutations that are dependent on the activity of the *su(Hw)* gene. In addition, we found that the composite *gypsy-P* element generates a substantial number of detectable insertions into or near heterochromatic regions.

MATERIALS AND METHODS

***Drosophila* stocks:** Flies were raised at 25°C, 70% humidity on standard corn meal/agar medium. The mutations and chromosomes used in this study are described in LINDSLEY and ZIMM (1992).

Mobilization of *SUPor-P* in *M25* flies: The *M25* flies carry a deletion of both the *yellow* and *achaete* genes ($y^- ac^-$), the w^{1118} mutation and the composite *gypsy-P* element inserted on the *CyO* balancer chromosome at cytological location 60F. A diagram of this transposon, called *SUPor-P* for *suppressor-P* element, is shown in Figure 1A. This *P* element contains two marker genes. The first is a modification of the mini-*white*

gene. Mini-*white* contains 305 bp of 5' and 500 bp of 3' flanking DNA and a deletion of most of the first intron (PIRROTTA 1988). The mini-*white* gene was modified by insertion of *white* regulatory sequences (-1084 and -1465 relative to the transcription start site) upstream of -305 bp. This regulatory region contains both the eye and testes enhancers (QIAN *et al.* 1992) and directs a high level of *white* expression in the eye. Two 430 bp *su(Hw)* binding regions (*gypsy* sequences between nucleotides 647 and 1077, as numbered in MARLOR *et al.* 1986) flank the *white* gene to provide insulation of this gene from chromosomal position effects (ROSEMAN *et al.* 1993). The second marker gene is the intronless *yellow* gene. This *yellow* gene is 5.2 kb in length, contains 2.8 kb of 5' and 0.13 kb of 3' flanking DNA and lacks the tissue-specific bristle and tarsal claw enhancers (GEYER and CORCES 1987).

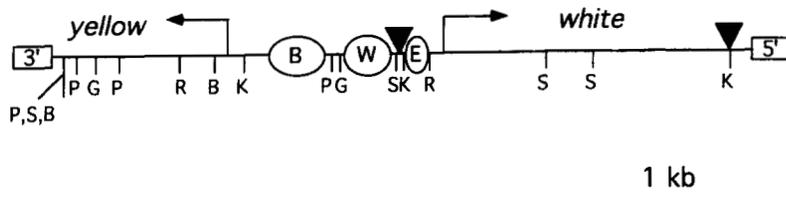
In experiment one, the *SUPor-P* was mobilized from the 60F location as shown in Figure 1B. Transpositions were obtained using a chromosomal source of transposase (*P[ry⁺Δ2-3](99B)* ROBERTSON *et al.* 1988). Females carrying *SUPor-P* were crossed to males that were $w^{1118}; CyO/Sb; Sb Δ2-3/TM6, Ubx$. Single males carrying both *SUPor-P* and $Δ2-3$ were mated in individual vials to females that were $y^- ac^- w^{1118}$ or $y^1 fs(1)Yb/FMO$. New mobilizations were identified as straight-winged (Cy^+) flies that were pigmented in the eye and body tissue (Figure 1B). Stocks were established by backcrossing to either $y^- ac^- w^{1118}$ or $y^1 fs(1)Yb/FMO$. In the F2 generation, males were preferentially chosen to establish a stock. However, if the only $y^+ w^+$, Cy^+ flies were females, then these individuals were chosen. This undoubtedly biased our recovery of insertions on the X chromosome. As *P* element mobilization often occurs premeiotically, each vial may have contained multiple F2 progeny with an identical mobilization event. For this reason, only one line was established from each vial, unless flies with different phenotypes were observed.

The chromosomal location of each *P* element was determined by a set of additional crosses in which males containing a new transposition were mated to $y^- ac^- w^{1118}/y^- ac^- w^{1118}; T(2,3)ap^{8a}/CyO, MKRS$ females. (*CyO* and *MKRS* are balancer chromosomes carrying the dominant *Cy* and *Sb* markers, respectively). X-linked insertions were identified because they did not produce $y^+ w^+$ sons in this cross, while Y-linked insertions were identified because the only $y^+ w^+$ progeny produced were male. In addition, insertions that mapped to the Y chromosome showed variegated pigmentation of the body and eye, *yellow^{var} white^{var}*. Possible autosomal insertions were mapped by mating $y^+ w^+; CyO; MKRS$ male progeny to $y^- ac^- w^{1118}$ females. The progeny from this cross were examined to determine whether the $y^+ w^+$ phenotype segregated away from either the second (*CyO*) or third (*MKRS*) chromosome.

Virgin females and males that were phenotypically y^+, w^+, Cy and *Sb* were mated together to determine whether a particular insertion had a recessive phenotype; between 150 to 200 progeny were examined. In all but two cases, the insertion was classified as lethal if the cross generated none of the nonbalancer class of flies that was being scored. In the two exceptional cases, adult escapers were present, at a frequency of less than 5% of the balancer class of flies. To determine whether a given insertion line was male or female sterile, between five and 10 homozygous *SUPor-P* females and males were mated together. If that cross generated no progeny, an additional cross of either five to 10 homozygous *SUPor-P* females or males to five to 10 $y^- ac^- w^{1118}$ males or females was carried out. Depending on the outcome of these crosses, the line was classified as male or female sterile.

Lines in which the $y^+ w^+$ markers did not segregate solely from the X, Y, second or third chromosomes most likely contained either fourth chromosome or multiple insertions. In

A.



B.

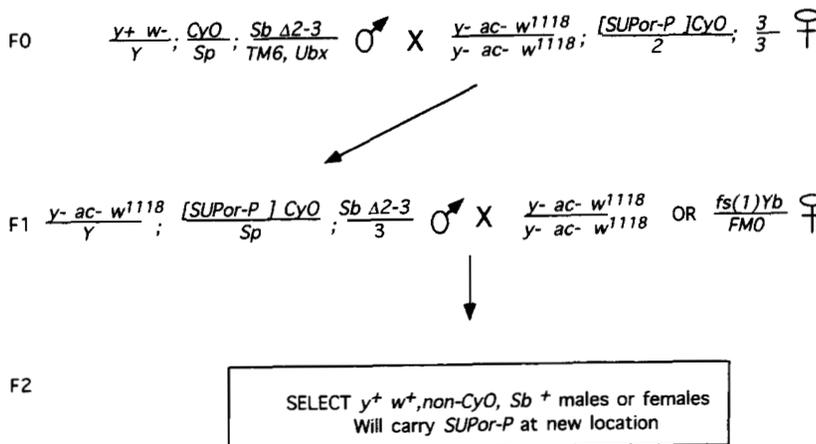


FIGURE 1.—Mobilization of a composite *gypsy-P* element for mutagenesis. (A) Structure of the mutator *P* element called *SUPor-P*. This transposon carries the *yellow* and *white* marker genes. The direction of transcription of each gene is indicated by the raised arrow. The *P* element ends are indicated by the small blocks at the end of the *yellow* and *white* genes. The 5' end of the *P* element is located at the 3' end of the *white* gene. Enhancers are shown by ovals; the *yellow* gene contains the wing (W) and body (B) enhancers; the *white* gene contains the eye (E) enhancer. Two *su(Hw)* binding regions (triangles) flank the *white* gene. The restriction enzyme symbols shown below the line are as follows: B, *Bam*HI; G, *Bgl*II; K, *Kpn*I; P, *Pst*I; R, *Eco*RI; S, *Sal*I. (B) Genetic scheme used to generate new insertion sites of *SUPor-P*. *SUPor-P* was mobilized from the *CyO* chromosome by *P[ry+Δ2-3](99B)*, abbreviated Δ2-3. Genetic symbols are described in LINDSLEY and ZIMM (1992).

these cases, the number of insertion sites was determined by Southern blot analysis that was designed to identify flanking restriction fragments. Multiple insertions gave a more complex restriction pattern than lines with one *P* element and were not considered further. Lines determined to contain a single insertion were categorized as fourth chromosome lines.

Mobilization of additional *P* elements containing *su(Hw)* binding regions: Four additional small-scale *P* element mobilizations (experiments two through five) were carried out. In experiment two, the starting *SUPor-P* element was located in the middle of 2L on a *CyO* balancer chromosome. This *P* element was mobilized as described above (Figure 1B). Twenty new mobilizations were obtained and mapped and their associated recessive phenotypes were determined. In experiment three, the starting *P* element was *SUPor-P light (lt)*, which is a derivative of *SUPor-P* that lacks the *white* eye enhancer but is otherwise identical to *SUPor-P* (see B.R. >white>B.R. in ROSEMAN *et al.* 1993). This *P* element was mobilized from position 92B on the *TM6* chromosome by crossing females carrying the *SUPor-P lt* to *w¹¹¹⁸*; *CyO/Sp*; Δ2-3 *Dr/TM6, Ubx* males. Single males carrying both *SUPor-P lt* and Δ2-3 were mated in individual vials to *y⁻ ac⁻ w¹¹¹⁸* females. New mobilizations were identified as *Dr* flies that contained pigmented eye and body tissues. Stocks were established by backcrossing to *y⁻ ac⁻ w¹¹¹⁸* and selecting *y⁺ w⁺*; non-*Dr* flies. We established 65 new lines, determined that 26 lines carried insertions on the *CyO* balancer chromosome and analyzed the remaining 39 for the associated recessive phenotype. Experiments four and five were mobilizations of *SUPor-P* elements located on either the X or second chromosome. These experiments generated 18 lines. The position of the starting

transposon in these lines is unknown, as they were lost prior to *in situ* localization. In summary, experiments two through five generated 77 lines that were analyzed for the associated recessive phenotype. Of these lines, 46 were also analyzed by *in situ* hybridization.

Suppression of lethal second chromosome insertions by mutations in *su(Hw)*: The *su(Hw)* mutant stock used in these studies was *y⁻ ac⁻ w⁶⁷ ct⁶ v¹ f¹*; *Ro/CyO*; *bx^{34r} su(Hw)^y/TM6, su(Hw)^f, Ubx*. This combination of *su(Hw)* alleles reverses the phenotypes associated with *gypsy* insertions and is female fertile. The *su(Hw)^y* allele is a deletion of the *su(Hw)* gene (HARRISON *et al.* 1992), whereas *su(Hw)^f* is a point mutation in one of the Zn fingers that retains some ability to bind DNA (HARRISON *et al.* 1993). The X chromosome in this stock carries two *gypsy*-induced alleles, *ct⁶ f¹* that are suppressed by *su(Hw)* mutations. This allowed identification of homozygous *su(Hw)* mutant flies. We studied only second chromosome lines due to the fact that *su(Hw)* is located on the third chromosome. Crosses were designed such that no recombination could occur between the *P*-element-containing chromosome and its homologue. Males carrying a lethal insertion of *SUPor-P* were crossed to virgin females of the above *su(Hw)* stock. Males in the second generation that were phenotypically *y⁺, w⁺, ct, f* and *Ro⁺* were selected. These males were genotypically *y⁻ ac⁻ w⁶⁷ ct⁶ v¹ f¹*; *SUPor-P/CyO*; *su(Hw)^y or ^f/+* and were backcrossed to the *su(Hw)* stock. Males and virgin females that were phenotypically *y⁺, w⁺, ct⁺, f⁺, Cy* were selected. These flies were homozygous for the *su(Hw)* mutations and balanced for the lethal second chromosome *SUPor-P* insertion. Males and females of this genotype were mated, and at least 100 *Cy* progeny were screened. Suppression of the *SUPor-*

TABLE 1
Summary of recessive phenotypes generated by mobilization of *SUPor-P* in *M25* flies

Chromosome	Percentage Insertion ^a	Percentage Lethals ^b	Percentage F. sterile ^b	Percentage M. sterile ^b	Percentage Visible ^b	Percentage Wt ^b
X	10 (62)	13 (8)	0 (0)	0 (0)	0 (0)	87 (54)
2	72 (432)	15 (65)	3 (13)	2 (9)	1 (3)	79 (342)
3	15 (88)	26 (23)	1 (1)	0 (0)	1 (1)	72 (63)
4	1.5 (9)	ND	ND	ND	ND	ND
Y	1 (6)	0 (0)	NA	0 (0)	0 (0)	100 (6)
Summary ^c	588	16 (96) ^d	2.5 (14) ^d	1.5 (9) ^d	1 (4) ^d	79 (465) ^d
Total insertions	601					

ND, not determined; NA, not applicable; F, female; M, male; Wt, wild type. Values in parentheses are number of lines.

^aPercentage of insertions on a given chromosome per total lines generated.

^bPercentage of insertions with a given phenotype per total number of insertions on a given chromosome.

^cSummary of the number of lines in which recessive phenotypes were analyzed (excludes fourth chromosome).

^dPercentage of insertions with a given phenotype per total number of chromosomes in the subset analyzed (no. phenotype/no. total insertions). This total excludes the insertions on the fourth chromosome.

P lethal phenotype resulted in the production of straight-winged (*Cy*⁺) progeny, which represented, in most cases, approximately one third of the homozygous *su(Hw)* flies.

In situ hybridizations: Determination of the cytological location of *SUPor-P* was done according to the procedure of LIM

(1993) using *white* DNA as a probe. This probe recognizes both the endogenous *white* gene at 3C and the transposon. *P*-element positions were determined with resolution to the lettered interval.

Effects of the Y chromosome on variegation of *SUPor-P*: A

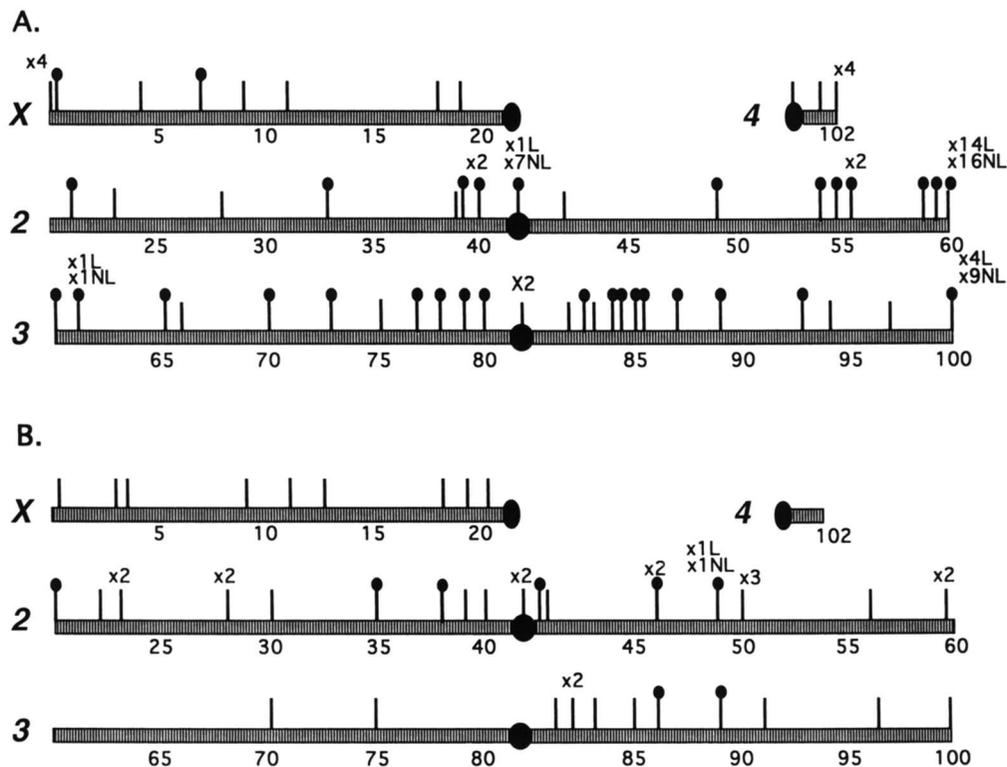


FIGURE 2.—Chromosomal locations of *SUPor-P*. (A) Sites of insertion of *SUPor-P* generated from the *M25* line, which carried *SUPor-P* at the 60F position. The location of *SUPor-P* elements associated with recessive lethal (lollipop, L) and nonlethal (bold line, NL) phenotypes are shown. In this study, *P* element localization was carried out primarily on lines which had an associated recessive lethal phenotype. The number after the X indicates how many independent lines had an insertion at that location. (B) Sites of insertion of *SUPor-P* generated by mobilizations from euchromatic positions. The locations of insertions associated with a recessive lethal (lollipop, L) and nonlethal phenotype (bold line, NL) are shown. Eleven of the localized elements were generated from a starting position in the middle of 2L (experiment 2) and 35 of the localized elements were generated from a 92B starting position (experiment 3). These 46 lines were chosen at random, independent of their associated recessive phenotype.

TABLE 2
Complementation analysis of *SUPor-P*-induced mutations

Line	Insertion site	Allele or deficiency tested	Failure to complement
546-1 ^a	7B	<i>ct</i> ⁶	Yes
203-1 ^a	33F	<i>Df(2L)a1: 21B8-C1; 21C8-D1, 22D1-2; 33F5-34A1</i>	No
274-2 ^a	55BC	<i>Df(2R)PC4: 55A-F</i>	No
411-1 ^a	55D	<i>Df(2R)PC4: 55A-F</i>	Yes
425-1 ^a	55D	<i>Df(2R)PC4: 55A-F</i>	Yes
286-4 ^a	61D	<i>Df(3L) emc², red¹: 61C3-C4; 62A8</i>	Yes
82c ^a	73CF	<i>Df(3L)std¹¹, Ki roe p[p]: 73B1; 73D1</i>	Yes
16-1 ^a	77A	<i>Df(3L)rdg C, th st in ri p[p]: 77A1; 77D1</i>	Yes
850-1 ^a	85A	<i>Df(3R)CA3: 84F2; 85A5-7</i>	Yes
59-2 ^a	87E	<i>Df(3R) ry n²⁷, Dfd cu dar: 87D1-2; 87F1-2</i>	Yes
126M19 ^b	100CD	<i>Df(3R)awd, KRB, ca: 100C-D</i>	Yes

^a*SUPor-P* lines generated from the 60F position.

^b*SUPor-P* lines generated from a non-60F position.

large percentage of mobilizations showed variegation of the marker genes, *yellow* and *white*. To determine whether this variegation was sensitive to modifiers of position effect variegation, we tested the effects of changing the Y chromosome dosage. Females carrying *SUPor-P* were crossed to males that carried an attached XY chromosome [*XY, y v*]. All male progeny from this cross were XO. The phenotype of these males was compared with that of males obtained from crossing *SUPor-P* containing females to *y⁻ ac⁻ w¹¹¹⁸*. Position effect variegation is very sensitive to culture conditions such as temperature and media. For this reason, these crosses were carried out under identical conditions at 25°. Similar crosses carried out with nonvariegating *SUPor-P* lines showed that these lines were not affected by Y chromosome dosage.

Effects of the *su(Hw)* protein on marker gene expression:

Several centric and telomeric insertion lines were obtained. To determine whether the presence of the *su(Hw)* protein provided protection from repressive position effects that are associated with these regions of the genome, lines containing these *SUPor-P* transposons were made homozygous mutant for *su(Hw)*. These crosses were carried out as described for the suppression studies. The resulting phenotypes were classified by considering both the level of marker gene expression and the amount of variegation observed.

RESULTS

Mobilization of a *P* element containing *su(Hw)* binding regions: The parental M25 line is a *y⁻ w⁻* strain that carries a *SUPor-P* element inserted on the *CyO* chromosome at cytological location 60F (Figure 1). The *SUPor-P* transposon carries modified *yellow* and *white* genes which confer a red/brown eye color and dark pigmentation of only body and wing cuticle. Transpositions of *SUPor-P* from the *CyO* balancer to another chromosome were identified as straight-winged flies that showed *w⁺* and/or *y⁺* expression. Altogether, 1759 fertile crosses were established and 655 independent lines were obtained. Our "jumping rate," defined as the percentage of vials containing at least one *y⁺ w⁺, Cy⁺* fly (BERG and SPRADLING 1991), was 37%. In most cases, the phenotype of flies carrying the mobilized *SUPor-P* element was indistinguishable from the parental line. However, a number of flies showed phenotypes that were variegated either for both the *white* and *yellow* genes, or only for the *yellow* gene.

TABLE 3
Chromosomal distribution of variegating lines obtained by mobilization of *SuPor P* in M25 flies

Chromosome	Percentage Insertions	Class I ^d	Class II ^e	Class III ^f
X ⁿ	8 (29)	100 (29)	0 (0)	0 (0)
2 ^a	73 (255)	82 (209)	7 (18)	11 (28)
3 ^a	15 (52)	77 (40)	4 (2)	19 (10)
4 ^a	3 (9)	0 (0)	100 (9)	0 (0)
Y ⁿ	1 (4)	0 (0)	100 (4)	0 (0)
Summary ^b		80 (278)	9.5 (33)	10.5 (38)
Total ^c	349			

Values in parentheses are number values.

^aPercentage of insertions with a given phenotype per total number of insertions on a given chromosome.

^bPercentage of insertions with a given phenotype per total inserts analyzed.

^cA subset of the total transposition lines were analyzed in this study.

^dWild-type eye and cuticle phenotype.

^eVariegated phenotype of both eye and cuticle.

^fVariegated phenotype of cuticle only.

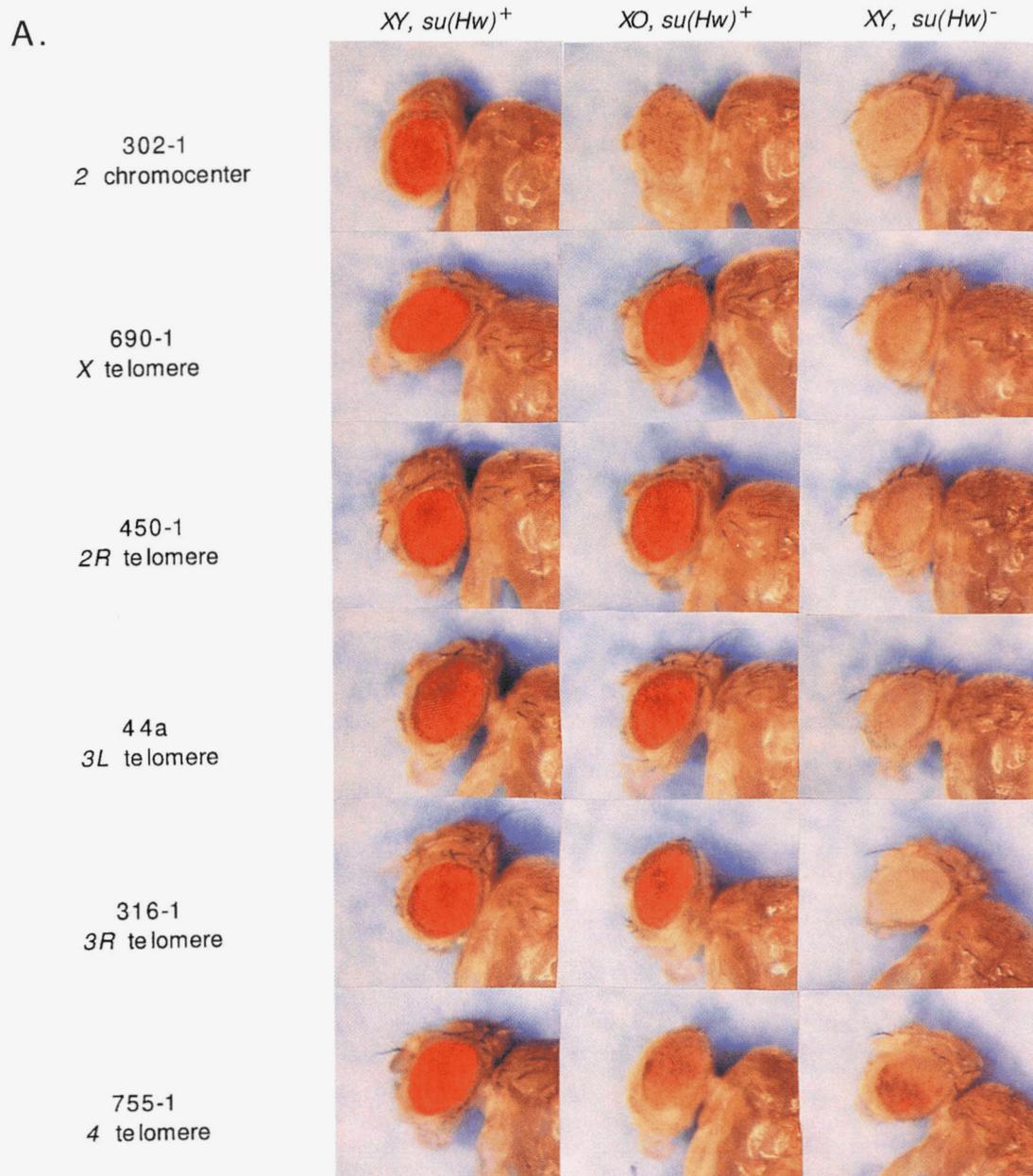


FIGURE 3.—Phenotypes of marker genes associated with *SUPor-P* insertions. (A) Eye phenotypes of male flies carrying a *SUPor-P* transposon integrated at either a centric or a telomeric location. Three genotypic backgrounds are shown for each insertion. (B) Body phenotypes of males carrying a *SUPor-P* transposon integrated at the same positions as in A.

Of the 655 lines, 601 were analyzed further (Table 1). In our mutagenesis protocol, the mobilization of *SUPor-P* was induced in males. New insertions could be recovered on either one X, the Y, one second (a transposition in *cis* on the *CyO* chromosome would be lost in our scheme), one third (the $\Delta 2-3$ chromosome was selected against), or two fourth chromosomes. Based on this analysis, we anticipated that there should be an equal number of insertions recovered on the second and third chromosomes. The frequency of X-linked insertions was expected to be reduced for two reasons. First, F2 males were usually used to establish stocks which biased against the isolation of X-linked

insertions. Second, *P* transposition events occur primarily in premeiotic germ cells. Thus, if the X-linked insertion caused inactivation of a cell autonomous gene essential for viability or fertility, this germ cell would fail to form a functional sperm. In contrast to these predictions, we found that most lines had insertions on the homologous second chromosome (72%), with only 15% of the lines containing an insertion on the third chromosome. A low percentage of insertions were recovered on the other chromosomes: X (10%), Y (1%) and fourth (1.5%) (Table 1).

Among these *SUPor-P* lines, a second bias was observed. Only 15% of the second chromosome insertions

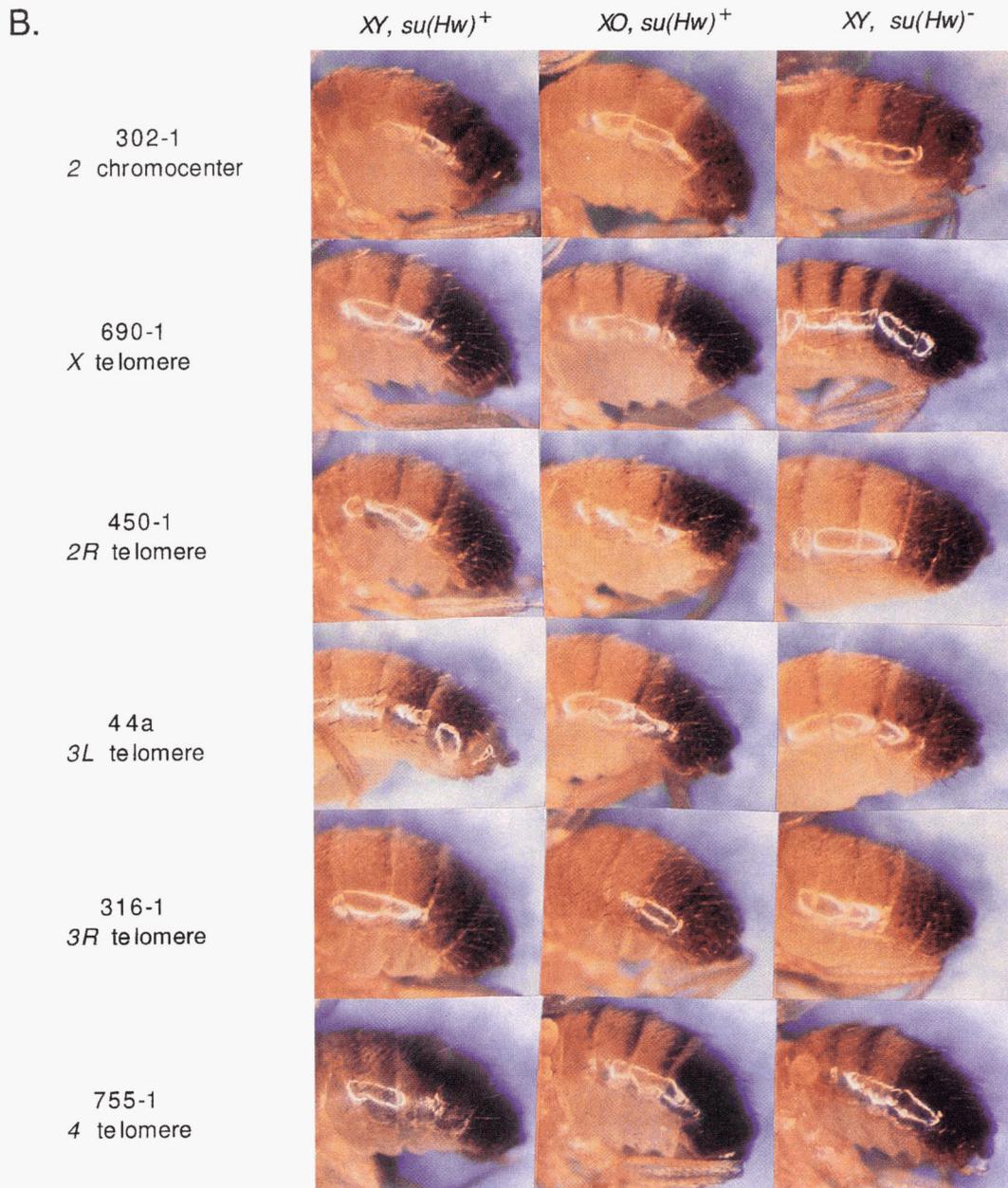


FIGURE 3.—Continued

were associated with a recessive lethal phenotype, in contrast to 26% for those on the third chromosome. In other mutagenic screens involving single *P* elements, the rate of recessive lethality of new insertions ranged from 10 to 17% for the second chromosome and 10 to 12% for the third chromosome (COOLEY *et al.* 1988a,b; BEIR *et al.* 1989; COOLEY *et al.* 1988a; KARPEN and SPRADLING 1992). Therefore, our lethality rate on the second was similar to previous studies, while that on the third chromosome was about 2.5-fold higher. These differences between the second and third chromosomes reflect the disproportionate recovery of lines that carry *SUPor-P* in a 60F location, the same cytological position as the parental *SUPor-P* insertion (see below). Subsequent analyses indicated that this anomaly is not

due to inherent properties of *SUPor-P*, but is caused by its starting location in the parental *M25* line (this will be examined in detail in a later section). For these reasons, we will not consider the 60F mutations in the following characterization of *SUPor-P*-induced lesions.

Complementation analysis of *gypsy-P*-element mutations: Insertion of a *P* transposon causes the majority of mutations isolated in single *P*-element mutageneses (COOLEY *et al.* 1988b; BEIR *et al.* 1989; BERG and SPRADLING 1989; ZHANG and SPRADLING, 1994). Based on the following data, we believe the same is true for the *SUPor-P*. The location of *SUPor-P* in several randomly chosen recessive lethal lines was determined by *in situ* hybridization (Figure 2). We then investigated whether any of our *SUPor-P*-induced mutations were uncovered

by known cytological deletions of these regions. In eight of 10 lines tested, the location of the mutations corresponded to the *SUPor-P* insertion site, as determined by failure to complement available deletions in the region of the transposon (Table 2). In addition, one allele of *cut* (*ct*) was recovered that carried a *SUPor-P* insertion at the appropriate cytological location, 7B. Thus, we find a strong correlation between the location of a lesion and the site of *SUPor-P* insertion for several mutagenic events.

A subset of *SUPor-P* mutations are suppressed in a *su(Hw)* mutant background: A potential advantage of the *SUPor-P* transposon as a mutagen is the generation of lesions that are dependent on the presence of the *su(Hw)* protein. We predicted that a subset of the *SUPor-P*-induced mutations should be suppressed in a *su(Hw)* mutant background. Forty-one randomly selected second chromosome lines carrying recessive lethal *P* element insertions were crossed into a *su(Hw)^v/su(Hw)^f* mutant background. In nine cases (22%), the lethality was reversed. The fact that the lethal phenotype of these mutations was sensitive to the allelic state of *su(Hw)* correlates the mutant phenotype with the presence of the *su(Hw)* binding regions. This indicates that these insertions would not produce a detectable phenotype if a standard *P* element was inserted at the same site.

A high percentage of transposons show chromosomal position effects: The *SUPor-P* transposon carries two visible, cell autonomous phenotypic markers, *yellow* and *white*. We noted that many lines generated from mobilization of the *M25 SUPor-P* did not express these markers in a wild type pattern. A random subset of 349 lines was characterized in more detail. Flies carrying this transposon could be classified into three phenotypic classes (Table 3, Figure 3). The predominant class I (80%, 278/349) had a marker phenotype identical to the parental *M25* strain, showing dark eye, body and wing color. Class II (9.5%, 33/349) showed a variegated phenotype in both the cuticle and eye: *yellow^{var}*, *white^{var}*. Class III (10.5%, 38/349) showed only a variegated cuticle coloration: *yellow^{var}*. Variegated phenotypes are often observed when euchromatic genes are placed next to or within heterochromatic portions of the chromosomes, such as the centromere or telomere (SPOFFORD 1976; HENIKOFF 1990). These position effects are proposed to result from a variable spreading of highly compacted chromatin, from the heterochromatic region into euchromatic DNA (LOCKE *et al.* 1988) or compartmentalization of heterochromatic genes into regions of the nucleus that are inaccessible to transcription factors (WAKIMOTO and HEARN 1990; DEVLIN *et al.* 1990). Gene expression is repressed if the euchromatic gene is in a heterochromatic region, whereas it remains active when in a euchromatic compartment.

Our chromosome mapping and *in situ* localization data provided insights into the origin of these phenotypes (Table 3, Figure 2). Several of the class II inser-

tions were located on the *Y* (4/33) and fourth (9/33) chromosomes, two chromosomes which are heterochromatic in nature. The *P* element was localized in six of the nine lines designated to the fourth chromosome by genetic mapping studies. In all cases, the fourth chromosome assignment was confirmed (Figure 2A). Although insertions into these heterochromatic chromosomes caused both *yellow* and *white* variegation, the level of expression from these reporter genes was dramatically different. Three of the four *Y* chromosome insertions had greatly reduced expression of both marker genes; eye color was essentially white with a few spots of pigment, and cuticle color was essentially yellow with a few spots of color. In contrast, lines carrying an insertion in the telomere of the fourth chromosome showed nearly normal levels of *white* and *yellow* gene expression and only light variegation (Figure 3). In the only fourth chromosome line localized to centric heterochromatin, pigmentation of the eye, body and wing cuticle was greatly reduced and showed extreme variegation.

The remaining *yellow^{var}*, *white^{var}* insertions (20/33) mapped to the second or third chromosome. In these cases, the level of marker gene expression varied greatly among lines, with most lines showing low to intermediate levels of pigmentation and moderate variegation (Figure 3). The *SUPor-P* element associated with ten of the *yellow^{var}*, *white^{var}* autosomal lines (50%, 10/20) was localized by *in situ* hybridization and found to reside in the centric region of the second or third chromosome (Figure 2A).

The *Y* chromosome is a strong modifier of position effect variegation, with loss of the *Y* chromosome resulting in increased variegation (SPOFFORD 1976). We tested whether the autosomal and fourth chromosome *yellow^{var}*, *white^{var}* lines were sensitive to the dosage of the *Y* chromosome. In these studies, females carrying the *SUPor-P* transposon were mated to attached *XY* males. All male progeny generated in this cross were *XO*. The level of pigmentation associated with the eye and body cuticle was compared with males generated in a parallel cross between *SUPor-P* females and *y⁻ac⁻w¹¹¹⁸* males. This allowed comparison of males with different doses of *Y* chromosomes. We tested sixteen *yellow^{var}*, *white^{var}* second chromosome lines, two *yellow^{var}*, *white^{var}* third chromosome lines and six *yellow^{var}*, *white^{var}* fourth chromosome lines for effects of the *Y* chromosome. In all cases, *XY* males showed substantially less variegation in the eye than *XO* males. One caveat of this experiment is that the genetic background of the *XO* and *XY* males differs, which may affect the observed phenotype. Position effect variegation is sensitive to a large number of modifiers that could differ in these backgrounds. If the enhanced eye phenotype in the *XO* background was due to the presence of dominant modifier mutations and was not related to the loss of the *Y* chromosome, then we would have expected to see substantial phenotypic variations in the eyes among the male progeny of

a given line. As this was not the case, these results suggest that the position effects on *white* expression can be modified by the dosage of the *Y* chromosome (Figure 3A).

The effects of the *Y* chromosome dosage on *yellow* expression depended upon the chromosome into which the *SUPor-P* element was inserted. Second and third chromosome *yellow^{var}*, *white^{var}* lines showed less *yellow* variegation in *XY* males than *XO*, while *yellow* expression in the fourth chromosome *SUPor-P* insertions did not change in these two backgrounds (Figure 3B). As with *white*, the *XO* males produced from a given line did not show substantial phenotypic variation, supporting the hypothesis that these effects are associated with changes in dosage of the *Y* chromosome.

Altogether, 23 of the 33 *yellow^{var}*, *white^{var}* *SUPor-P* lines were found to be inserted in heterochromatin. In 13 lines, *SUPor-P* resided in heterochromatic chromosomes (fourth and *Y*); in 10 lines, *SUPor-P* was localized by *in situ* hybridization to centric regions of the second or third chromosome. These results suggest that the *yellow^{var}*, *white^{var}* phenotype is an indicator of whether an insertion of *SUPor-P* is in or near heterochromatin.

The position of the *SUPor-P* element in approximately one third (13/38) of the class III lines was determined (Figure 2A). Twelve lines had *SUPor-P* transposons inserted at a telomere of one of the large chromosomes. These regions of the genome have been shown previously to repress expression of genes (HAZELRIGG *et al.* 1984; LEVIS *et al.* 1985; KARPEN and SPRADLING 1992; WALLRATH and ELGIN, 1995). Although, most of the class III insertions were located at a telomere, other lines shown to contain telomeric insertions either did not show any position effects on *yellow* expression or showed reduced but not variegated *yellow* expression and were not included in class III. This suggests that either the *su(Hw)* binding regions associated with *SUPor-P* protected both the *white* and *yellow* reporter genes from chromosomal position effects or that *SUPor-P* was not inserted into sequences that caused repression of gene expression. To distinguish between these possibilities, we crossed eleven telomeric insertion lines into a *su(Hw)* mutant background (Table 4, Figure 3). We found that the eye phenotype associated with all lines became much lighter and variegated in a *su(Hw)* mutant background. This demonstrates that the *su(Hw)* protein is effective at protecting *white* from repressive chromatin assembled at chromosome tips. The *yellow* phenotype associated with several lines also changed when placed in a *su(Hw)* mutant background, although this effect was less consistent (Table 4, Figure 3B).

We examined whether the phenotypes associated with telomeric insertions were sensitive to the dose of the *Y* chromosome, as was demonstrated for the heterochromatic insertions in the class II transposons. Females from several different telomeric insertion lines were mated to attached *XY* males as described above (Table

4, Figure 3). These experiments are subject to the same caveats as applied in the previous experiments. We found that loss of the *Y* chromosome failed to enhance the *white* phenotype. Furthermore, the *yellow* phenotype was enhanced in only four of the 11 lines (36%). Again, substantial phenotypic variations in the cuticle color were not observed among the male progeny of a given line, suggesting that in lines where *yellow* expression was affected, this was caused by the dosage of the *Y* chromosome. These studies indicate that the *Y* chromosome does not act as a general enhancer of telomeric position effects for insertions located on the *X*, second or third chromosomes.

Differential recovery of mutations on the autosomes: As noted previously, there was a bias in the recovery of *SUPor-P* insertions on the second chromosome. A series of experiments were done to examine the substantial contrasts in the rates of insertion and recessive lethality between the second and third chromosomes. The distribution of the *SUPor-P* elements in 21 of the 23 third chromosome recessive lethal lines was determined to test whether an insertional hot spot on this chromosome caused the increased mutational rate. In 18 of these 21 cases, the transposon associated with a recessive lethal phenotype was localized to a different chromosomal position (Figure 2A). Only one cluster of four insertions was detected, which occurred in the telomere of $\mathfrak{3R}$, cytological position 100F. This indicates that there is not a strong insertional hot spot of recessive lethal mutations on the third chromosome. However, there appears to be a general clustering of *SUPor-P* transposons at the $\mathfrak{3R}$ telomere, as noted by the localization of *SUPor-P* in nine additional nonlethal lines (Figure 2A).

We also tested whether a preexisting lethal was present that would increase the mutation rate on the third chromosome. Background lesions have affected original estimations of mutation rate in similar studies (COOLEY *et al.* 1988b; BELLEN *et al.* 1989). Complementation analysis was done with 10 of the lethal third chromosome lines (10/23). Each of these lines was crossed to the remaining lines in all pair-wise combinations. Complementation occurred in every case, indicating that the lethals represented independent, separable mutations. We conclude that for the third chromosome, the recessive lethals generated were distributed at a number of different sites.

The same conclusion could not be drawn when the second chromosome recessive lethal lines were analyzed in detail. Localization of the *SUPor-P* transposon in 27 lines associated with a second chromosome recessive lethal mutation showed that 14 contained an insertion at the telomere of $2R$, cytological position 60F, as did 80% (16/20) of a randomly selected group of nonlethal, nonvariegating second chromosome lines (Figure 2A). Complementation analysis was done between 11 of the 14 60F recessive lethal lines. Each of

TABLE 4
Phenotypes of marker genes in lines carrying telomeric insertions of *SuPor-P*

No. location	<i>yellow</i> phenotype			<i>white</i> phenotype		
	<i>su(Hw)</i> ⁺ , XY	<i>su(Hw)</i> ⁺ , XO	<i>su(Hw)</i> ⁻ , XY	<i>su(Hw)</i> ⁺ , XY	<i>su(Hw)</i> ⁺ , XO	<i>su(Hw)</i> ⁻ , XY
690-1, 1A	black	black	brn, slt var	red	red	or, hvy var
863-1, 1A	black	black	brn, slt var	red	red	or, md var
178-1, 60F	brn	brn	brn, slt var	red	red	or, ext var
315-1, 60F	dk brn	brn	dk brn	red	red	or, hvy var
450-1, 60F	brn, md var	brn, md var	brn, md var	red	red	or, ext var
50-2B, 60F	lt brn, md var	lt brn, md var	lt brn, md var	brn	brn	or, ext var
44a, 61A	lt brn, md var	lt brn, hvy var	lt brn, md var	red	red	w, ext var
108-1, 100F	black	black	tan, md var	red	red	w, ext var
316-1, 100F	lt brn, md var	tan, ext var	tan, md var	red	red	w, ext var
478-1, 100F	brn, md var	tan, hvy var	brn, md var	red	red	w, ext var
525-1, 100F	brn, md var	lt brn, md var	brn, hvy var	red	red	w, ext var

lt, light; dk, dark; var, variegation; slt, slight; md, medium; ext, extreme; hvy, heavy; brn, brown; or, orange; w, white. The level of *yellow* gene expression produced flies with pigmentation in the descending order: black > dark brown > brown > light brown > tan > yellow. The level of *white* gene expression produced flies with eye color in the descending order: red > brown > raspberry > orange > yellow > white. Variegation was classified in descending order: no variegation > slight variegation > medium variegation > heavy variegation > extreme variegation.

these 11 lines was crossed to the remaining lines in all pair-wise combinations. Complementation occurred in every case, indicating that a preexisting second chromosome lethal was not present and that the lethal mutations at this position resulted from multiple and separable lesions. This latter conclusion was further demonstrated by complementation tests using a deletion in the 60F region (kindly provided by N. PATEL). Thirteen lethal 60F alleles were tested, and four failed to complement this deficiency. We conclude that the second chromosome lines represent the biased recovery of lines with an insertion at cytological location 60F.

***SUPor-P* elements mobilized from other locations are not targeted to 60F:** Inclusion of some sequences within a *P*-element vector can alter the specificity of *P*-element insertion (KASSIS *et al.* 1992). To verify that the large number of 60F insertions obtained from mobilization of the *M25 SUPor-P* element was not influenced by a general targeting property of *P* elements carrying a *su(Hw)* binding region, small-scale mobilizations with four different *SUPor-P* or *SUPor-P lt* (a modified element lacking the *white* eye enhancer) transposons were carried out (experiments two through five). Forty-six randomly selected lines were mapped by *in situ* hybridization, and none contained an insertion at 60F (Figure 3B). We conclude that the clustering of *SUPor-P* elements in cytological position 60F that were recovered in the *M25* study was a consequence of the starting location of the *P* element. In the presence of a source of transposase, imprecise male recombination between the telomere of the parental *M25* chromosome and its homologue would give results consistent with this biased recovery.

***SUPor-P* elements cause a high rate of production of**

recessive lethal mutations: We determined the recessive phenotypes associated with 77 lines generated from these small-scale mobilizations (experiments two through five, Table 5). In this case, the recessive lethal phenotype associated with second chromosome insertions was 30% (14/46), while the third chromosome recessive lethality frequency was found to be 25% (5/20). These frequencies are very similar to that of the third chromosome lethal rate found in the original *M25* mutagenesis (26% or 23/88).

Taken together, these data suggest that the reduced frequency of mutations caused by presumed second chromosome insertions in the *M25* mutagenesis derives from the 60F insertion class. If we discount this second chromosome data due to this anomaly, we find that the frequency of recessive lethal mutations derived from the *M25* third chromosome events and both the second and third chromosomes from the four subsequent mutageneses is approximately 27% (42/154).

DISCUSSION

***SUPor-P* mutagenesis:** These studies examined whether addition of *su(Hw)* binding regions to a marked *P*-element increased the effectiveness of single *P*-element mutagenesis. This was predicted because the *su(Hw)* binding region is a potent mutagen of 5' regulatory regions (GEYER *et al.* 1986; HOLDRIDGE and DORSETT 1991; JACK *et al.* 1991; ROSEMAN *et al.* 1993), unlike other *P* transposons (SEGALAT *et al.* 1992). Several mobilization experiments were carried out using the *SUPor-P* transposon. As a measure of the mutagenic effectiveness of this transposon, we determined the frequency of production of recessive lethal mutations. We found that

TABLE 5
Summary of recessive phenotypes generated by mobilization of composite
***gypsy-P* elements not located at 60F**

Chromosome	Percentage insertions ^a	Percentage lethal ^b	Percentage F. sterile ^b	Percentage M. sterile ^b	Percentage visible ^b	Percentage Wt ^b	Percentage class II ^b
X	8 (6)	0 (0)	0 (0)	0 (0)	0 (0)	100 (6)	16 (1)
2	60 (46)	30 (14)	9 (4)	2 (1)	2 (1)	57 (26)	13 (6)
3	26 (20)	25 (5)	0 (0)	0 (0)	0 (0)	75 (15)	0 (0)
4	4 (3)	ND	ND	ND	ND	ND	100 (3)
Y	2 (2)	0 (0)	NA	0 (0)	0 (0)	100 (3)	100 (2)
Summary ^c	74	26 (19) ^d	5 (4) ^d	1.5 (1) ^d	1.5 (1) ^d	66 (49) ^d	16 (12)
Total	77						

F, female; M, male; Wt, wild type; N, number; ND, not determined; NA, not applicable. Values in parentheses are number values.

^a Percentage of insertions on a given chromosome per total lines generated.

^b Percentage of insertions with a given phenotype per total number of insertions on a given chromosome.

^c Refers to the number of lines in which recessive phenotypes were analyzed (excludes the fourth chromosome).

^d The percentage of insertions with a given phenotype per total number of chromosomes in the subset analyzed.

27% of the *SUPor-P* lines were associated with a recessive lethal phenotype (42/154; 95% confidence interval of 20 to 34%). This compares with a frequency of 12% (958/7825; 95% confidence interval of 11.5 to 13%) for recessive lethal mutations on the second and third chromosomes in the large mobilization screen reported by KARPEN and SPRADLING (1992), which is representative of the frequency obtained in other studies (COOLEY *et al.* 1988a,b; BIER *et al.* 1989). These data indicate that mobilization of *SUPor-P* caused a significant increase in the frequency of recessive lethal mutations.

A novel class of P-induced alleles are generated by insertion of *SUPor-P*: The increased frequency of mutagenesis is coincident with the production of alleles of *SUPor-P* that were suppressed by *su(Hw)* mutations. We found that 22% (9/41) of the second chromosome recessive lethal mutations were viable in a *su(Hw)* mutant background, suggesting that binding of the *su(Hw)* protein to *SUPor-P* caused the mutant phenotype. These suppressible alleles are unlikely to result from insertion of *gypsy* elements because new excisions or insertions of *gypsy* do not occur under hybrid dysgenic conditions (WOODRUFF *et al.* 1987; EGGLESTON *et al.* 1988). The 22% frequency of suppressible alleles is lower than was expected considering the 2.5-fold increase in mutagenicity. One possible explanation for the observed lower rate is that these suppression studies used a hypomorphic combination of *su(Hw)* alleles because *su(Hw)* null mutants are female sterile. This allelic combination completely suppresses somatic phenotypes caused by *gypsy* insertion but produces sufficient amounts of protein in the germline to allow fertility. Thus, it is possible that *su(Hw)* protein made in the germline is transported into the oocyte and remains at sufficient levels in the developing embryo to bind to *SUPor-P* and cause inacti-

vation of control elements required for some genes. Given this caveat, it is difficult to predict what percentage of alleles should be suppressible. However, the creation of *P*-element-induced alleles that are suppressed by mutations in the *su(Hw)* locus demonstrates that *SUPor-P* can expand the repertoire of mutations made by *P* mutagenesis.

The *SUPor-P* transposon may provide a means to recover insertions into regions of the genome not obtained by standard *P* mutagenesis: *P* elements appear to insert preferentially into euchromatic positions (ENGELS 1988; ZHANG and SPRADLING 1993; WALLRATH and ELGIN, 1995). The low recovery of marked *P* elements into heterochromatic regions has been proposed to result from compromised detection (ENGELS 1988; KARPEN and SPRADLING 1992; ZHANG and SPRADLING 1994). In fact, *P*-element mobilization under conditions that suppress position effect variegation allows recovery of insertions into heterochromatin (ZHANG and SPRADLING 1994). Mobilization of the M25 *SUPor-P* element generated many lines that carried insertions into heterochromatic regions of the genome. Although these lines show variegation of both *yellow* and *white*, the presence of the *su(Hw)* binding regions greatly reduced the negative effects of these regions. In a *su(Hw)* mutant background, variegation became more severe; and, in some cases, pigmentation was barely detectable (Table 4, Figure 3). The ability to isolate transposons inserted into heterochromatin appears to be a general feature of composite *gypsy-P* elements, as our smaller mobilizations (experiments two through five) produced 16% (12/77) of the lines showing *yellow* and *white* variegation (Table 5). These results support the notion that position effects limit the recovery of marked *P* elements into heterochromatin. Furthermore, they suggest that

a second method for obtaining a large number of insertions into this region of the genome is the mobilization of *P* elements carrying *su(Hw)* binding regions flanking a marker gene.

Chromatin assembled at telomeres and centromeres show different effects on expression of marker genes in *SUPor-P*: From the mobilization of the 60F *SUPor-P*, many transposons located at telomeric positions were obtained. In total, 18 lines had insertions on the X, 3L and 3R tips (Figure 2A). An additional 30 transposons were associated with the 2R telomere. Although these 60F insertions may have resulted from male recombination, they differ in the expression of the marker genes and therefore were useful to study telomeric position effects (Table 4).

The level of *white* expression was wild type or nearly wild type at all telomeric sites; whereas, there was range of *yellow* phenotypes, with some lines showing wild type pigmentation and others showing a low level of variegated coloration (Table 4, Figure 3). The phenotypes associated with these marker genes reflect the fact that *white* is completely flanked by *su(Hw)* binding regions, while *yellow* is not. This suggests that unlike heterochromatic position effects of the centromeres, fourth and Y chromosomes, telomeric position effects can be completely blocked by the *su(Hw)* protein. This observation that *su(Hw)* protein differentially insulates the *white* gene from repression in these genomic locations suggests that telomeric position effects are distinct. Additionally, the expression of marker genes present on *SUPor-P* elements inserted into centric regions and the fourth chromosome was sensitive to the Y chromosome dose, while expression of these genes was affected only in a minority of telomeric insertions on the second and third chromosomes. This second observation supports the idea that chromatin assembled at these telomeres has different properties from centric or fourth chromosome chromatin. These data are consistent with those of WALLRATH and ELGIN (1995), who found that expression of the *hsp 70-white* gene present on *P* elements inserted into telomeres of the second and third chromosomes was not altered by known modifiers of position effect variegation, including the Y chromosome.

The difference between repressive centric and telomeric chromatin may indicate that a unique set of proteins are involved in establishing these kinds of chromatin. Alternatively, this may reflect the fact that establishment of centric heterochromatin requires a greater amount of each protein than is needed for telomeric chromatin. Thus, if proteins which establish repressive chromatin interfere with the association of the *su(Hw)* protein, then the binding of this protein may be more difficult in centric positions than telomeric, producing a less effective block at these positions.

The mechanism by which the *su(Hw)* protein insulates the mini-*white* gene from position effects: Several

models have been proposed to explain how heterochromatin represses euchromatic gene expression. Most models evoke inactivation of transcription as the underlying basis for repression. The chromatin assembly model proposes that transcriptional inactivation results from chromatin compaction due to assembly of large protein complexes that associate into a higher order structures (SPOFFORD 1976; LOCKE *et al.* 1988). In this model, chromatin compaction initiates at particular sites and spreads along the chromosome; the closer a gene to the site of initiation, the more likely repression of gene expression will occur. Studies on chromosomal rearrangements that juxtapose euchromatin and heterochromatin provide some support for this hypothesis. In many rearrangements, inactivation of euchromatic genes occurs over long distances with the gradient of gene inactivation matching the change in morphology of the euchromatic region towards a more heterochromatic appearance (SPOFFORD 1976). In this context, the *su(Hw)* protein may block repression by heterochromatin by providing a road block to the subsequent spreading of repressive chromatin, either by its direct interaction with DNA or as a consequence of establishing a domain boundary.

A second model is the compartmentalization model (WAKIMOTO and HEARN 1990; KARPEN 1994). In this model, heterochromatin and euchromatin occupy different regions of the nucleus. It is envisioned that in these regions transcription factors required for expression of genes are distinct and that the two nuclear compartments are differentially enriched with the appropriate factors. Thus, genes requiring euchromatic transcription factors would be poorly expressed if localized into the heterochromatic nuclear compartment. This model is supported by the studies on the effects of chromosomal rearrangements on expression of heterochromatic genes (WAKIMOTO and HEARN 1990). In this context, it is possible that the *su(Hw)* protein blocks heterochromatic position effects by establishing an independent domain that encompasses the euchromatic gene and alters the compartment into which this gene is localized, thereby increasing its expression.

Both models described above are based on data obtained from studies of centric position effects. It is unclear whether these models adequately describe telomeric effects. Analysis of the *SUPor-P* insertion lines may provide insights into this question. In this regard, we found that several telomeric insertion lines showed disparate effects on the expression of the *SUPor-P* reporter genes. In these cases, *white* expression was wild type, while *yellow* expression was low and/or extremely variegated. This wild-type level of *white* expression required insulation by the *su(Hw)* protein (Table 4, Figure 3). Thus, if the telomeric position effects resulted from inappropriate compartmentalization in the nucleus and insulation by the *su(Hw)* protein occurred by altering the nuclear compartment of the telomeric transposon,

we would have expected that the *yellow* gene would also localize to the new compartment, and its gene expression would be largely unaffected. However, this is not observed. Alternatively, if telomeric repression resulted from the assembly of an altered, perhaps more compacted chromatin structure, and insulation by the *su(Hw)* protein occurred by blocking the assembly of the *white* gene into this repressive structure, then we would predict that *yellow* expression would be susceptible to repression, since this gene is not flanked by *su(Hw)* binding regions. This model is more consistent with our results and suggests that telomeric repression may not be caused from inappropriate compartmentalization. However, it is possible that the disparate *yellow* and *white* phenotypes are due to the fact that these genes are expressed in distinct cell types or that the threshold amount of each product required to elicit a phenotype differs. Thus, more experiments are required to sort out these possibilities.

In yeast, telomeric position effects are caused by the assembly of an altered chromatin structure which is initiated at the end of the chromosome and propagated a short distance in the proximal direction (RENAULD *et al.* 1993). Interestingly, in many *SUPor-P* telomeric insertion lines, the *yellow* gene was partially protected from repression by the *su(Hw)* protein (Table 4). These results may reflect differences in the orientation of the *SUPor-P* transposon relative to the end of the chromosome. For example, if the *SUPor-P* transposon was oriented such that *yellow* was more proximal than *white*, the *su(Hw)* protein could block the assembly of both genes into a repressive chromatin structure. However, if the *yellow* gene was more distal, the degree of repression would be unaffected by *su(Hw)* mutations. A second possible explanation for effects of the allelic state of *su(Hw)* on *yellow* expression is that association of the *su(Hw)* protein with *SUPor-P* could alter telomeric chromatin such that the *yellow* gene is subjected to fewer negative influences than in the absence of this protein. Determination of the orientation of the telomeric *SUPor-P* elements will provide insights into this question.

In summary, mobilization of a *P* element containing *su(Hw)* binding regions provides the opportunity to produce a new spectrum of *P*-induced alleles. Our findings demonstrate that mutations can be generated at a higher frequency using this composite transposable element and that a subset of these mutations are dependent solely on the presence of the *su(Hw)* protein. Furthermore, *SUPor-P* insertions can be recovered in regions of the chromosome normally repressive to marker gene expression due to insulation of position effects by binding of *su(Hw)* protein.

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