Type I Repressors of *P* Element Mobility

Gregory B. Gloor,* Christine R. Preston,† Dena M. Johnson-Schlitz,† Nadine A. Nassif,† Randall W. Phillis,‡ Wendy K. Benz,† Hugh M. Robertson§ and William R. Engels†

**Department of Biochemistry, University of Western Ontario, London, Ontario N6A 5C1, †Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706, ‡ Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01003, and §Department of Entomology, University of Illinois, Urbana, Illinois 61801*

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ABSTRACT

We describe here a family of *P* elements that we refer to as type I repressors. These elements are identified by their repressor functions and their lack of any deletion within the first two-thirds of the canonical *P* sequence. Elements belonging to this repressor class were isolated from *P* strains and were made *in vitro*. We found that type I repressor elements could strongly repress both a cytotype-dependent allele and *P* element mobility in somatic and germline tissues. These effects were very dependent on genomic position. Moreover, we observed that an element's ability to repress in one assay positively correlated with its ability to repress in either of the other two assays. The type I family of repressor elements includes both autonomous *P* elements and those lacking exon 3 of the *P* element. Fine structure deletion mapping showed that the minimal 3′ boundary of a functional type I element lies between nucleotide position 1950 and 1956. None of 12 elements examined with more extreme deletions extending into exon 2 made repressor. We conclude that the type I repressors form a structurally distinct group that does not include more extensively deleted repressor elements such as the KP element described previously.

P elements are Drosophila transposons best known for their usefulness in molecular genetic techniques and their interesting population and evolutionary biology (ENGELS 1989). Autonomous *P* elements are 2907 bp long, and are bounded by 31-bp inverted repeats (O'HARE and RUBIN 1983). Such elements have four exons, which together produce P transposase, a protein of 87 kDa required for *P* element mobility (KARESS and RUBIN 1984; RIO, LASKI and RUBIN 1986). Transposition is thought to occur by a DNA-only mechanism (ENGELS *et al.* 1990; GLOOR *et al.* 1991; KAUFMAN and RIO 1992). In addition to autonomous elements, smaller, nonautonomous *P* elements are also common. These elements can be derived from autonomous ones by deletion of some internal sequences (O'HARE and RUBIN 1983; O'HARE *et al.* 1992). The deletion end points are distributed throughout the *P* sequence but have a tendency to occur between short, directly repeated sequences (ENGELS 1989).

Most natural populations contain both autonomous and nonautonomous *P* elements. They are called P strains to distinguish them from old laboratory stocks (M strains) which lack *P* elements. The cross M ♀ × P ♂ yields "dysgenic" hybrids in which *P* elements are highly mobile, but the reciprocal cross results in relatively low levels of mobility (KIDWELL, KIDWELL and SVED 1977).

P element mobility is regulated on two levels. First, tissue-specific splicing of the transposase pre-mRNA ensures that the elements are mobile only in the germline (CHAIN *et al.* 1991; LASKI, RIO and RUBIN 1986; SIEBEL and RIO 1990). This specificity can be eliminated by the artificial removal of the 2-3 intron to create a "Δ2-3" element (LASKI, RIO and RUBIN 1986).

The second level of regulation, which is the subject of this report, applies to all tissues and prevents mobility within the P strains. In some P strains this regulation shows a limited form of maternal transmission (ENGELS 1979; SVED 1987) leading to the term "P cytotype." This maternal component is responsible for the reciprocal cross effect between P and M strains. One effect of the P cytotype is to reduce the expression of the transposase promoter (LEMAITRE and COEN 1991). Several lines of evidence suggest that an element-encoded repressor is responsible for this kind of regulation (ENGELS 1981; SVED 1987; MISRA and RIO 1990; O'HARE and RUBIN 1983; ROBERTSON and ENGELS 1989).

Several groups have tried to determine the structure of repressor-making *P* elements. In one study the authors used recombination to isolate naturally occurring repressor-making *P* elements from other *P* elements in the genome. They were able to segregate a *P* element lacking part of the 2-3 intron and part of exon 3 from some but not all of the other elements in a wild stock. The authors suggested that the internally

deleted element made repressor (NITASAKA, MUKAI and YAMAZAKI 1987). In other studies, a geographically widespread *P* element with a more extreme internal deletion that removed part of exons 1 and 3 and all of exon 2 correlated with the presence of *P* repressor (BLACK *et al.* 1987; JACKSON, BLACK and DOVER 1988; SIMMONS *et al.* 1990). BLACK *et al.* designated this element "*KP*." Finally, a recent report has shown that two closely linked insertions of apparently complete *P* elements can repress *P* mobility (RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1991).

Two studies that examined *in vitro* modified *P* elements for repressor have been reported. The first (ROBERTSON and ENGELS 1989) tested several mutated *P* elements for repressor. These mutations included frameshift mutations in each *P* element exon and mutations preventing splicing of the 2-3 intron. The authors observed that some insertions of two of the mutant elements could produce repressor, and concluded that the 66-kDa polypeptide predicted to be produced by exons 0 through 2 may be a P repressor molecule. Later work by another group has confirmed this result, and demonstrated that the 66-kDa protein can be detected by P-specific antiserum when P repressor is detected genetically (MISRA and RIO 1990).

Even though the genome of a typical wild-caught Drosophila strain contains many different element structures (O'HARE *et al.* 1983, 1992), previous work has described only three repressor-making element structures. Two of the elements, those capable of producing a 66-kDa protein and the autonomous elements, are large elements having at least the *P* exons 0 through 2. The other element, the *KP* element, is much smaller. The previous work has not attempted to determine systematically which *P* element structure(s) can make P repressor, or which sequences are required for its production. More importantly, it is unclear whether the three repressor-making elements represent different points of a continuous spectrum of *P* elements capable of making repressor. Alternatively, it is possible that P repressor-making elements can be divided into groups of similar elements with non-overlapping structures.

We report here that repressor-making *P* elements can be divided into at least two classes and that such an element can be placed into a class solely on the basis of its structure. One class is composed of large repressor-making elements. We have observed several elements of this type, all of which have the *P* sequences through exon 2 and at least the first nine nucleotides of the 2-3 intron. Further truncation at the 5′ end results in loss of repressor function. The second class is made up of the *KP* element and other small elements with similar deletions in exons 2 and 3 (in preparation). This report is concerned with the structural characterization of the larger class of elements.

We propose the term "type I" to refer to the larger elements and "type II" for the smaller ones. This naming system is neutral with respect to mechanism and leaves open the possibility of other classes of repressor elements being discovered. Furthermore, this system permits entire groups of similar elements to be easily referred to.

We have characterized type I P repressors in three ways. First, we isolated two naturally occurring repressor-making *P* elements as single insertions. We used these isolated elements to show that a single insertion of a naturally derived *P* element can make repressor in two assays. Secondly, we used *in vitro* mutagenesis to define systematically the sequence requirements for type I elements. We found that type I elements require some sequences for repressor activity that are absent from the type II elements. The results showed that type I and type II repressor elements have discrete structural characteristics. Finally, we demonstrated a profound effect of position on an element's ability to make P repressor. We observe that the proportion of sites that allow repressor production is a function of the element's structure.

MATERIALS AND METHODS

Genetic techniques: Flies were maintained on standard cornmeal-molasses-agar medium. Mating schemes are described in the text, and were done at room temperature (20–23°) unless noted. Genetic symbols not described here are in LINDSLEY and ZIMM (1992).

In vitro modified white$^+$-marked P element test constructs: Constructs were transformed by microinjection into embryos whose genotypes were a mixture of *w*; Δ2-3(99B)/*TM3 Sb* and *w*; Δ2-3(99B)/*TM6B* as described previously (ROBERTSON *et al.* 1988). The insertions were maintained in one of several ways: *X*-linked insertions were maintained in a *C(1)DX, y w f*; M cytotype genetic background; second-linked insertions were maintained across from the balancer chromosome, *CyO*, in a *white* genetic background; third-linked insertions were on one of the balancer chromosomes, and were maintained across from the opposite balancer (*i.e.*, *TM3*-linked insertions were across from the *TM6B* chromosome and vice versa) in a *white* genetic background.

Transposase-containing stocks: the stable transposase source *P*[*ry*$^+$Δ2-3](99B) (ROBERTSON *et al.* 1988), hereafter Δ2-3(99B), was used to mobilize the *in vitro* modified constructs. Our standard *P* strain was π_2 (ENGELS and PRESTON 1979).

Molecular techniques: Fly DNA preps-DNA for the polymerase chain reaction was prepared (GLOOR and ENGELS 1992) by mashing single flies in a 0.5-ml tube with a pipette tip using 50 μl of squishing buffer (10 mM Tris-Cl pH 8.2, 1 mM EDTA, 25 mM NaCl, and 200 μg/ml freshly diluted Proteinase K (Sigma)). The squished flies were incubated at 20–37° for 20–30 min, and the Proteinase K was inactivated by heating to 95° for 1–2 min. We used a method for estimating mRNA levels (BECKER-ANDRÉ and HAHLBROCK 1989) and determined that there is an average of 750 Drosophila haploid genomes/μl (range 57–1800, $n = 4$) in these preparations (this corresponds to a DNA concentration of approximately 100 pg/μl).

Polymerase chain reaction techniques: The polymerase chain reaction was used to amplify specific sequences essentially

as described (ERLICH 1989; SAIKI *et al.* 1988). Our reaction volumes were routinely 15 μl containing 1 μl of fly DNA and were cycled 30 times. We used the asymmetric PCR technique to sequence genomic DNA (GYLLENSTEN and ERLICH 1988). For this, our amplifications were in a 100-μl volume and were performed for 40 cycles. Following amplification, the reactions were extracted once with $CHCl_3$-isoamylalcohol to remove the mineral oil, ethanol precipitated and sequenced using the Sequenase kit (United States Biochemicals). The sequencing primer was annealed at 60° for 2 min, and the nucleotides were diluted 1:30 for this sequencing. Manganese was sometimes used to supplement the reaction mixtures as recommended by the manufacturer.

Inverse PCR: DNA for inverse PCR (OCHMAN, GERBER andHARTL 1988) was prepared from single flies as described above, except that the Proteinase K was inactivated by the addition of 1 μl of 0.1 M phenylmethylsulfonylfluoride (Sigma) to the fly prep following the incubation. The mixtures were heated to 65° for 10–15 min to denature any proteins not inactivated by the proteinase. Four microliters of fly supernatant (corresponding to approximately 400 pg of DNA) were added to 16 μl of 1.25× *Nde*II buffer (125 mM Tris-HCl pH 7.6, 12.5 mM $MgCl_2$, 188 mM NaCl, 1.25 mM DTT), and 0.5 μl (2.5 units) of *Nde*II was added (Life Technologies Inc.) and incubated at room temperature for 15 min. The enzyme was inactivated by heating to 65° for 15 min. Three microliters of digested DNA were added to 7 μl of ligation mix (5 mM $MgCl_2$, 20 mM DTT, 0.8 mM ATP), and 0.5 μl (50–100 NEB units) T4 DNA ligase (New England Biolabs) were added. The mixture was incubated at room temperature for 20–30 min, and the enzyme was inactivated by heating to 95° for 2–3 min. The ligated DNAs were amplified by adding 5 μl of ligation mix to 95 μl of 1× PCR reaction mix containing out-directed 20-mer oligonucleotide primers beginning at *P* element nt (nucleotide) 89 and 945. This scheme amplified genomic sequences flanking the *P* element 5′ end. We also used out-directed primers beginning at nt 2785 and 1918 to amplify sequences flanking the *P* element 3′ end. In both cases, the reactions were cycled 35 times and a single amplification product was observed, indicating the presence of a single *P* element end in the stock.

Molecular cloning: Molecular cloning techniques were performed according to standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982; SAMBROOK, FRITSCH and MANIATIS 1989). Enzymes were obtained from New England Biolabs, Life Technology Inc., and Promega Biotech. *P* element deletions were constructed by *Bal*31 exonuclease digestion starting at the unique *Bsm*I site, which cuts at nt 1972 of the *P* element. The deletions were blunted by treatment with the Klenow fragment of DNA Polymerase I and subcloned into pP3′*w*+, a vector designed for this purpose.

EXPERIMENTS AND RESULTS

Naturally occurring repressor elements

Isolation of repressor elements: We isolated several independent *P* elements that can make P repressor. Our strategy was to recover single *P* elements in a genetic background that allowed us to identify repressor-producing *P* elements. The indicator used for this isolation was a cytotype-dependent allele of the *vestigial* locus, $vg^{21\text{-}3}$ (WILLIAMS, PAPPU and BELL 1988). This allele has an extreme phenotype in the M cytotype that is partially or completely suppressed in the P cytotype (WILLIAMS, PAPPU and BELL 1988). Further work has shown that the P cytotype *per se* is not required, since a single repressor-making *P* element, not producing the P cytotype, can fully suppress the extreme vestigial phenotype (BENZ 1989). This effect serves as a convenient assay for P repressor function even though its mechanism remains unknown.

FIGURE 1.—The $vg^{21\text{-}3}$ assay for P repressor. This allele is a *P* element insertion in the *vestigial* gene that places it under the control of *P* repressor (WILLIAMS, PAPPU and BELL 1988). With increasing repressor strength, each succeeding class of wing is identified with the appearance of a specific vein or area of tissue. In the absence of repressor, the wing is fully vestigial and is scored as a 5.0. The first tissues to appear with repressor are the anterior crossvein and the second longitudinal vein. This phenotype is scored as a 4.0. In the next level, scored as 3.0, the wing becomes broader and the posterior crossvein appears. At level 2.0 the marginal vein and cell become complete. Finally, the second and third posterior cells and the tip are completed in a wild-type wing, which is scored as 1.0. Borderline phenotypes are scored as 1.5, 2.5, etc. The scale is similar to one described (BENZ 1989), except that the two phenotypes denoting the weakest levels of repressor have been combined. All wing phenotypes were scored in $vg^{21\text{-}3}/vg$ heterozygotes.

The $vg^{21\text{-}3}$ allele is a particularly good cytotype-dependent allele for isolating repressor-making *P* elements for three reasons. First, the allele has a range of readily visible phenotypes that are thought to reflect the strength of P repressor (Figure 1). Second, each insertion of a repressor-making *P* element shows a characteristic heritable suppression of the $vg^{21\text{-}3}$ allele. Finally, this assay does not require *P* element transposase, making it easier to recover repressor-making elements in isolation.

For this method to succeed we needed to ensure that the *P* elements in the P strains were mobilized at very low levels to increase the probability that only a single insertion of a repressor-making element would be isolated. Preliminary experiments with dysgenic crosses (where P strain males are mated to M strain females) suffered from two problems. Many of the test progeny were sterile or showed reduced fertility. Furthermore, we often recovered multiple new *P* insertions on each chromosome. This prevented further characterization of single repressor elements (unpublished data). We therefore took advantage of the observation that *P* elements transpose only rarely in "non-dysgenic" matings—when P strain females are

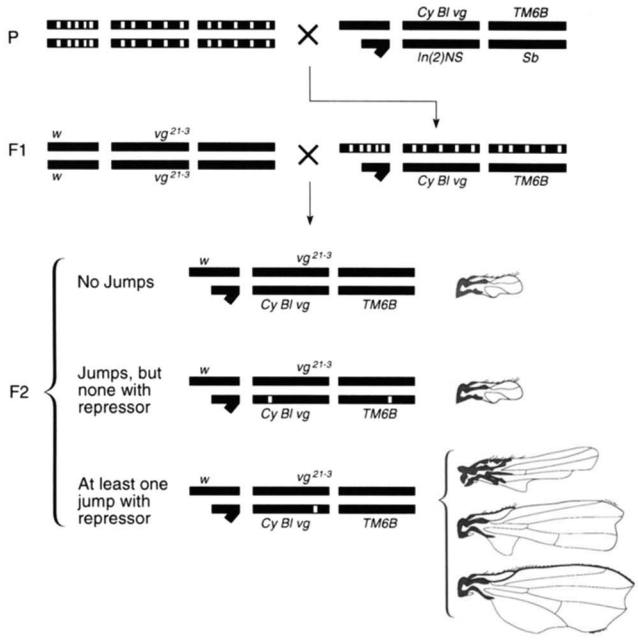

FIGURE 2.—Isolation of repressor-producing *P* elements. One of the mating schemes used to isolate single repressor-producing *P* elements is shown. In this example, transposase supplied by autonomous *P* elements on the P-derived chromosomes in the F_1 male mobilizes the elements (shown as open boxes) allowing them to jump to other sites. The F_2 sons with entirely M cytotype-derived chromosomes and with the $vg^{21\text{-}3}/vg$ genotype are scored for their wing phenotype. Most of these progeny are expected to have a nonsuppressed phenotype either because the progeny did not receive an element or because the newly transposed element did not make repressor. These possibilities are shown as the first two F_2 progeny genotypes. Those carrying a new repressor-producing insertion have a wing phenotype that is partially or completely restored to wild type, depending on the strength of the repressor. This class is represented by the third F_2 progeny genotype. Marked autosomes were used so that the appropriate genotypes could be selected. The M-derived second chromosome was *In(2L)Cy* + *In(2R)Cy* carrying dominant *Curly* and *Bristle* alleles and a recessive null *vg* allele. The M cytotype-derived third chromosome was either In*(3LR)TM6B* carrying the dominant *Tubby* and *Humeral* alleles, or a chromosome marked with the dominant allele of the *Stubble* locus.

mated to M strain males (EGGLESTON, JOHNSON-SCHLITZ and ENGELS 1988; ENGELS 1979). This reduced mobility allowed us to recover rare transpositions thus enhancing the likelihood of isolating single elements.

The mating scheme in Figure 2 was used to isolate elements from *P* cytotype stocks. Most of the F_2 sons had the extreme vestigial phenotype (class 5 in Figure 1), indicating that no repressor-making *P* elements were detected. The exceptional cases with a less extreme vestigial phenotype (classes 1–4 in Figure 1) were kept for further analysis. As summarized in Table 1, we recovered a total of 379 sons displaying some phenotypic suppression among 3483 total F_2 sons scored. The frequencies varied from just under half of the F_2 sons having suppression for line O86a to none in lines Arb78-61 and T-007. Since our screen selects only elements that transpose, we interpret this variability to reflect differences in the numbers of transposase- and repressor-making *P* elements in the genome as well as differences in their mobility.

Individual F_2 progeny that were presumed to have a repressor-making element present in their genomes were easily recognized since they had more wing tissue than was observed for $vg^{21\text{-}3}/vg$ flies in the absence of repressor. Individual male flies with wing phenotypes classified as being in the range of 1–4 (see Figure 1) were mated to $vg^{21\text{-}3}$ virgin females to map the location of the suppressive effect. Of the 379 repressor-positive progeny, 59 were mapped unambiguously to a chromosome. The rest were either sterile or appeared to have multiple inserts or had extreme transmissional instability. From the 59 mapped suppressers, we selected 32 independent lines for further analysis. Some slight instability was noted for 28 of them, indicating the presence of transposase activity. The remaining four lines initially appeared stable, but instability was observed when the lines were followed for several generations.

In addition to these 32 isolates, we also derived elements suppressing the vestigial phenotype from a recombinant-*X* chromosome originally from the Drosophila stock, π_2. *In situ* hybridization with a *P* element probe showed four *P* element sites on the tip of this chromosome, at least one of which made transposase (BENZ 1989). The chromosome's inherent transposase was used to mobilize the *P* elements. Independent transpositions that suppressed the $vg^{21\text{-}3}$ allele were recovered on each major chromosome. The resulting lines, designated A12(X), A12(2) and A12(3), were stable and used for further molecular analysis.

Molecular characterization of repressor elements: Thirty-two lines derived from the P cytotype genomes, and three lines from the recombinant-*X* chromosome were established from single males by mating them to M strain virgin females. The number of distinguishable *P* elements present in each line was then determined by polymerase chain reaction (PCR) amplification (SAIKI *et al.* 1988) of DNA extracted from single flies (MATERIALS AND METHODS). We used three strategies designed to amplify *P* elements. The first strategy used three primer pairs to amplify three segments of 964, 883 and 903 bp. The amplified segments are shown as B, C and D in Figure 3. Segment C would amplify using the *P* element in the $vg^{21\text{-}3}$ allele as a template, but segments B and D would not. The second strategy used two primer pairs that would amplify two overlapping segments of the *P* element genome. This strategy amplified segments A and D in Figure 3, and gave products of 1747 and 903 bp in size when a complete *P* element was used as the template. These primer pairs would not amplify the nonautonomous *P* element inserted in the $vg^{21\text{-}3}$ allele. Our strategies would also not amplify any *P*

TABLE 1

Natural repressor transpositions

Stock	Origin[a]	Crosses/progeny[b]	Progeny with vg^{21-3} suppression	Analyzed by PCR[f]	Transposase/no. tested
Agana[c]	np	40/290	5	1	—
Arb 78-61[d]	np	98/623	0	—	—
Cage 3[b]	np	47/432	1	—	—
Kerbinou[a]	np	43/349	2	2	—
Perra Wirra[a]	np	55/430	97	10	4/4
Texas 007[a]	np	56/334	0	—	—
8.31.15[b]	np	3/29	3	—	—
O86a[c]	Single P on Y	49/292	138	6	—
1039-3[c]	Single P injected	180/704	133	13	10/10

[a] "np" indicates stocks derived recently from natural populations.
[b] Number of males tested/total number of progeny.
[c] Origin described in BLACK *et al.* (1987).
[d] Origin described in ENGELS and PRESTON (1979).
[e] Origin described in PRESTON and ENGELS (1989).
[f] — = not tested.

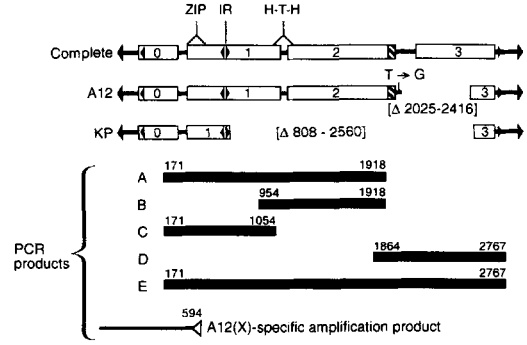

FIGURE 3.—Structures of *P* elements able to affect the expression of the vg^{21-3}allele. Exons of the transposase gene are shown as open boxes with transcription proceeding from left to right. Repeated DNA sequences are indicated by arrowheads. The hatched box shows a part of intron 3 that encodes an additional 15 amino acids when exons 2 and 3 are not spliced, resulting in a 66-kDa somatic product. Putative protein secondary structures of potential functional importance are also shown: ZIP = leucine zipper motif; H-T-H = helix-turn-helix motif. An inverted repeat DNA sequence of 17 bp is also shown (IR). The top two elements are those recovered in our initial screen. The bottom element is a *KP* element. The DNA sequence of A12, between positions 1–2855, was determined. There is a deletion between positions 2025 and 2416, and a T-G transversion at position 2022. The five fragments amplified by PCR during the screening of these elements are indicated below as dark lines. The oligonucleotides to amplify these segments were 20 nt long, and their 5′ ends were at the nucleotide indicated. The location of the *P*-specific 20 base primer on the *P* element map that was used to amplify the 5′ end of the A12(X) insertion by inverse PCR is shown as the open arrow. A 720-bp band was observed in this amplification indicating that the genomic primer began about 125 bp away from the *P* element insertion. The sequence of this genomic primer was: 5′(GTTGCCTTCCGCTCGCTTCG)3′. This amplified product is shown as thin black line.

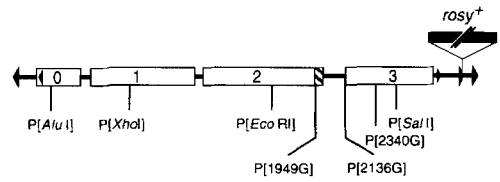

FIGURE 4.—*In vitro*-modified *P*[*ry*$^+$] elements. The *in vitro* modified, *rosy*-marked elements have been described (KARESS and RUBIN 1984; LASKI, RIO and RUBIN 1986; RIO, LASKI and RUBIN 1986). The *Alu*I mutation is an 8-bp insertion, the *Xho*I, *Eco*RI and *Sal*I mutations are four base insertions. The 1949G and 2136G mutants are single base changes that destroy the consensus splice sites. The 2340G mutation is a single base change that introduces an in-frame amber stop codon. Each element can be followed genetically by its associated *rosy*$^+$ phenotype. A full description of Drosophila lines transformed with these constructs and the repression properties of lines transformed with them has been reported (ROBERTSON and ENGELS 1989).

elements with an internal deletion that removed sequences close to the *P* element ends since the 5′ ends of our PCR primers began 171 and 140 bp from the *P* 5′ and 3′ ends. However, we assumed that we would be able to amplify almost every mobile *P* element with this strategy since about 150 bp of the *P* 5′ and 3′ sequences are required for transposition (MULLINS, RIO and RUBIN 1989). As controls, we used genomic insertions of either *P*[*Eco*RI] or *P*[*Sal*I] elements. These elements differ from the canonical sequence by the insertion of 4 bp at either the *Eco*RI or *Sal*I sites (Figure 4) (KARESS and RUBIN 1984). This difference was undetectable with our electrophoresis conditions. Only lines that contained a single amplification product following agarose gel electrophoresis were analyzed further. Our third strategy amplified segment E in Figure 3. This strategy was used to search for any elements with an internal deletion that would not amplify with the first two methods. No such elements were observed. The first and second strategies were used for all the analyses. The third method was used for all 13 elements examined from 1039-3, for four elements from Perra Wirra, and for six elements from O86a.

The results showed that all of the 32 lines derived from the P-cytotype strains had apparent complete *P* elements. The three derived from the recombinant *X* chromosome had an incomplete *P* element, designated A12. Direct sequencing of the element in the A12(3) line showed that it had a deletion of 391 bp, spanning nucleotide positions 2025–2416 (Figure 3). There was also a T→G transversion at position 2022. The other two lines and the original recombinant-*X* chromosome carried an element with the same deletion, as indicated by PCR with a primer specific for this deletion (16 nt on the 5′ side of the deletion and 4 on the 3′ side). No amplification was observed with lines containing the control templates *P*[*Eco*RI], *P*[*Sal*I], or a single *KP* element. Furthermore, no amplification was observed when π_2 DNA, or DNA from eight other independent wild-caught P stocks from a wide geographic range, was used as a template. This indicates that the A12 deletion was likely produced in one of the dysgenic crosses during the isolation of this recombinant chromosome.

Note that only type I elements were recovered using this screen. There are three potential reasons that no type II elements were recovered: First, only some flies from the Perra Wirra and Kerbinou stocks showed any evidence of *KP* elements in their genomes, whereas the other stocks did not (unpublished data). Whereas *KP* elements are not the only type II elements, they form a large fraction of them (unpublished data). Moreover, one of the lines we used to isolate repressors, 1039-3, was derived from a single injected autonomous element (PRESTON and ENGELS 1989) and therefore started with no type II elements. Second, our molecular screen discriminated against type II elements that would not produce PCR products with primers for segments A–D but would produce a shorter fragment with the primers for segment E (Figure 3). Only 4 of the 23 amplifications with the E primers were done on $vg^{21\text{-}3}$ suppressers derived from P stocks that were retrospectively found to carry *KP* elements (unpublished data). Finally, type II elements would be harder to detect in our screen due to their less pronounced effect on the $vg^{21\text{-}3}$ allele (in preparation).

Testing type I elements for transposase: We selected 14 of the lines classified as having complete *P* elements plus the three sublines containing the A12 element to test for the presence of transposase. Males from each line were mated to M-cytotype females homozygous for the sn^w allele, and the sons were progeny-tested for stability of sn^w in their germline. Previous work (ENGELS 1984; ROIHA, RUBIN and O'HARE 1988) has shown that sn^w, a *P* insertion mutation at the *singed* locus, is highly mutable to the sn^e and $sn^{(+)}$ alleles in the presence of P transposase and usually displays at least some instability even when repressor elements are present (MISRA and RIO 1990; ROBERTSON and ENGELS 1989). The results showed that each of the 14 apparently complete *P* elements, including the four that were initially transmitted stably in our previous mapping crosses, did produce transposase. However, none of the three A12 sublines produced transposase, as expected since part of exon 3 is deleted (Figure 3).

In summary, we analyzed 35 naturally occurring *P* elements selected for suppression of the $vg^{21\text{-}3}$ allele and found two structures. One was the complete *P* element as defined by both its structure and ability to make transposase; the other was an internally deleted element that retained exons 0, 1, and 2 and part of the 2-3 intron.

Loss of type I elements results in loss of repressor: The repressor-making elements were originally detected by the ability of new insertions of the elements to suppress the $vg^{21\text{-}3}$ allele. Direct confirmation that the putative repressor-making *P* elements were responsible for the suppression of the $vg^{21\text{-}3}$ allele was provided by an experiment to determine whether loss of suppression was correlated with loss of the element. Males in which the putative repressor-making element was mobilized in the germline were crossed to $vg^{21\text{-}3}$ females, and the progeny were selected for transposase-induced loss of the suppressive effect. This was observed as the restoration of the nonsuppressed phenotype of the $vg^{21\text{-}3}$ allele. Siblings that retained the suppression of the $vg^{21\text{-}3}$ allele served as controls. Flies with the extreme vestigial phenotype were expected to have a partial or complete deletion of the repressor-producing element, but the element should be intact in the control siblings.

Transposase was supplied by the autonomous elements themselves or, in the case of A12, by the Δ2-3(99B) element. PCR amplification was used to determine the state of the putative repressor-making element in the selected and control offspring. In the case of A12, it was important to avoid amplifying other copies that might have appeared by transposition during the generation when transposase was present. Therefore, we first used inverse PCR (MATERIALS AND METHODS) to determine the DNA sequence flanking the A12(X) element in order to construct a primer that would be specific for this particular insertion (Figure 3). We found that this step was not necessary for the autonomous elements, which only rarely transposed.

We tested three independent restorations of the extreme vestigial phenotype from one of the complete *P* element lines. One of the three showed a complete loss of the putative repressor-making element. The second had an extensive internal deletion that removed all of exons 0 and 1, and part of exon 2. The third element was deleted for all of exon 0 and part

of exon 1. In addition, this element also had a rearrangement that was not characterized further. All the complete elements appeared to be intact in each of the 13 independent control siblings. The loss-of-suppression and the control groups were significantly different by Fisher's exact test ($P < 0.002$). Eight independent restorations of the extreme vestigial phenotype were tested from A12(X). All eight had lost the A12-specific amplification product, thus indicating that at least part of the repressor-making element had been deleted. Seven independent control siblings were also analyzed in the same manner, and all seven retained the A12-specific amplification product. The loss-of-suppression and the control groups were significantly different by Fisher's exact test ($P < 0.0002$).

These results confirm that the *P* elements shown in Figure 3 are responsible for the suppressive effect on the $vg^{21\text{-}3}$ allele.

Type I elements can repress *P* element mobility: The $vg^{21\text{-}3}$ assay is an indirect measure of P repressor. As a more direct test of P repressor, we selected five of our repressor elements isolated as described above to test for the ability to prevent pupal lethality. This assay measures an element's ability to reduce the transposase-induced lethality which occurs when many *P* elements are mobilized in the soma (ENGELS *et al.* 1987; ROBERTSON and ENGELS 1989). Pupal lethality was measured for the second-linked insertions by performing the cross:

$$\frac{CyBlvg^{*}}{+}\ \varphi \times \frac{CyO}{Birm2}\ ;\ \frac{Dr\Delta 2\text{-}3(99B)}{P[SalI](89D)}\ \delta.$$

In this mating, a female with the repressor (shown as an *) on a dominantly marked autosome is mated to a male with the genotype indicated and the progeny are allowed to develop at 28°. The *Birm2* chromosome is the second chromosome from the *Muller-5 Birmingham* stock (ENGELS *et al.* 1987). This chromosome carries many copies of nonautonomous *P* elements that are mobilized by the transposase source Δ2-3(99B). This mobilization normally results in lethality at the pupal stage, but it is repressed in the males by the $P[ry^{+}\ SalI](89D)$ element. The progeny of this mating that receive both the *Birm2* chromosome and Δ2-3(99B) fail to eclose unless transposase is repressed. The proportion of progeny that eclose is indicative of repressor strength (ROBERTSON and ENGELS 1989).

The results of this assay are shown in Table 2. Line A of the complete element and the A12(X) and A12(3) lines significantly repressed pupal lethality. The results demonstrate that some insertions of the complete and A12 elements can repress *P* element mobility in the soma, and are true repressors of *P* element mobility. Furthermore, this shows that the $vg^{21\text{-}3}$ assay used to isolate these elements selects for repressor-making *P* elements.

TABLE 2

Pupal lethality repression by natural repressor elements

Element (line)[a]	$vg^{21\text{-}3}$ phenotype	Experimental class[b]	Survival controls[c]	Lethality controls[d]
Complete (A)	1.0	32	105	10[e]
Complete (B)	4.0	2	149	1
A12(X)	1.5	58	439	3
A12(2)	2.5	4	92	1
A12(3)	1.5	171	128	0

[a] Two of the complete *P* repressor lines derived from 1039-3 (Table 1) were selected for their relative stability and designated Complete (A) and Complete (B).

[b] The number of survivors of the pupal lethality genotype carrying the tested repressor. Their genotype is: *Birm2*/Repressor; Δ2-3(99b).

[c] The survival control column shows the number of flies without the pupal lethality genotype that lack either the *Birm2* chromosome or the transposase source. This serves as a measure of the number of siblings in the adjacent columns that potentially could have eclosed. The genotypes were usually *CyO*; Δ2-3(99B) except in the case of the A12(3) insertion where the genotype was *Birm2*; A12(3). The former survives because it lacks the *Birm2* transposase and the latter because it lacks Δ2-3(99B).

[d] The lethality control column shows the number of flies with the pupal lethality genotype that lack repressor and is a test of the efficiency of the pupal lethality assay.

[e] Eight of the ten survivors from complete (A) are made up of two clusters, one of three and one of five survivors. These may represent transpositions of the autonomous element in the generation prior to testing. All the other survivors in this column were recovered as single events.

Sequence requirements for type I repressors: The results presented above indicate that type I repressors are found in P strains, and probably play an important role in *P* regulation. Therefore, we wanted to find the sequence limits required to encode such a repressor.

We used *in vitro*-modified *P* elements to investigate the sequence requirements for P repressor encoded by type I elements. A set of frameshift and splicing mutations was employed to obtain the gross map of the sequence required for repressor. This was followed by deletion mapping to yield an accurate placement of the 3′ end. Each construct was tested by the $vg^{21\text{-}3}$ and pupal lethality assays described above. Both these assays measure P repressor in somatic tissues.

Previous work indicates that only a portion of the genomic insertion sites of a given repressor element will yield a detectable level of repressor expression (ROBERTSON and ENGELS 1989). Therefore, it was necessary to test each construct in a large number of genomic positions. A positive repressor test for any insertion site is sufficient, but a negative conclusion can only come after many insertion sites have been tested with none showing repressor function. Our strategy was to mobilize each element to be tested and screen the progeny with one or both of the repressor

TABLE 3

Gross mapping of repressor requirements using the $vg^{21\text{-}3}$ assay

Element	Total independent transpositions	
	rosy$^+$ transpositions	$vg^{21\text{-}3}$ suppression
P[*Sal*I]	27	17
P[1949G]	6	3
P[*Eco*RI]	12	0
P[*Xho*I]	23	0

assays. Thus, many random insertion sites can be screened without constructing stocks to test each individually.

Gross structure mapping: We first tested a group of elements that contained either a small insertion causing a frameshift mutation in one of the transposase exons or a point mutation in one of the 2-3 intron's splice junctions. Each is marked with the wild-type allele of the *rosy* gene. These constructs were produced by KARESS and RUBIN (1984) and by RIO, LASKI and RUBIN (1986). The structures of these elements are summarized in Figure 4. Repressor tests for some of these elements have been reported previously (ROBERTSON and ENGELS 1989), but only a small number of insertion sites were tested.

The elements to be tested (*P*[*Sal*I], *P*[1949G], *P*[*Eco*RI] and *P*[*Xho*I]) were located on either the *X* or second chromosome. The second-linked elements were tested for their ability to suppress the $vg^{21\text{-}3}$ allele by the cross:

$$C(1)DX,\ y\ f;vg^{21\text{-}3};\ ry\ \female \times \frac{CyBlvg}{P[ry^+]};\ \frac{ryDr\Delta 2{-}3}{ry\ +}\ \male.$$

The *P*[ry^+] element is mobilized by the Δ2-3(99B) transposase source in the germline of the fathers. New insertions of the element are detected as non-Dr progeny with a Cy Bl; ry^+ phenotype. Those with some suppression of $vg^{21\text{-}3}$ allele indicate that the new insertion expresses repressor. The elements located on the *X* chromosome were tested in an analogous mating scheme.

The data for this experiment are summarized in Table 3. Two of the *rosy*-marked elements, *P*[1949G] and *P*[*Sal*I], were able to suppress the $vg^{21\text{-}3}$ allele; two other elements, *P*[*Eco*RI] and *P*[*Xho*I] could not. The difference between *P*[*Sal*I] and the two nonsuppressing elements was significant using Fisher's exact test ($P < 0.006$ for *P*[*Eco*RI], and $P < 0.0002$ for *P*[*Xho*I]). The two suppressing elements were not significantly different from each other by the same test ($P = 0.86$).

The same elements were next tested for their ability to suppress pupal lethality. For each element we performed the cross:

TABLE 4

Pupal lethality assay for repressor[a]

Element	Transpositions/ total progeny[b]	Non-Δ2-3 control siblings[c]	Estimated transpositions[d]	Survivors[e]
P[*Alu*I](86E)	10/279	4340	155	0
P[*Eco*RI](89A)	51/470	3620	392	0
P[1949G](55F]	76/697	887	96	26
P[2136G](45D)	115/565	575	117	15
P[2340G](53F)	12/182	640	42	5
P[2340G](42B)	49/330	400	59	3
P[*Sal*I](49D)	17/104	1415	231	17
P[*Sal*I](52C)	63/629	975	97	27

[a] All the assays were performed at 21°, except those testing *P*[1949G](55F] and *P*[2136G](45D), which were done at 25°.

[b] This column shows the results of a parallel experiment testing the mobility of each element. The data shows the ratio between the number of transpositions recovered and the number of flies scored.

[c] This column shows the number of siblings without the pupal lethality genotype. For both the second- and third-linked elements it represents the number of flies lacking the transposase source.

[d] This column is the product of the ratio in the transposition rate column and the number of control siblings. It represents an approximation of the number of transpositions tested for repressor. In this case the third linked elements' transposition rates represent transpositions to only the *X* and second chromosome whereas pulpal lethality rescue could occur by a transposition to the *X*, second and third chromosome. Consequently, the number of transpositions tested is underestimated.

[e] The survivors column is the number of survivors of pupal lethality observed.

$$C(1)DX,Birm3\ \female \times \frac{CyO}{P[ry^+]};\ \frac{rySB\Delta 2{-}3}{ry\ +}\ \male.$$

As before, the *P*[ry^+] elements were mobilized in the male parents. Offspring that are phenotypically Curly and Stubble are not normally recovered because of the lethal interaction between Δ2-3(99B) and nonautonomous *P* elements on the *Birm3* chromosome (ENGELS *et al.* 1987). However, they will survive if they are rescued by repressor from a new jump of the *P*[ry^+] element (ROBERTSON and ENGELS 1989). Each such survivor was then confirmed by crosses to sn^w to check the presence of Δ2-3(99B), as indicated by bristle mosaicism, and to ry^{506} to confirm the presence of the *P*[ry^+] element. The number of transpositions was estimated for each element by mating males with the same genotype to *C(1)DX, y f; ry* virgin females and comparing the number of rosy and rosy$^+$ progeny among the offspring that were Cy and Sb$^+$. The transposition rate of the third-linked elements was determined using a mating scheme where the rosy phenotype was scored for the F_2 progeny that did not receive either the Sb Δ2-3 chromosome or the chromosome that the test element was originally inserted on. The results (Table 4) indicate that only the four mutants 3′ to the *Eco*RI site produced repressor.

Fine structure mapping of type I repressor requirements: It is possible that the type I repressor elements and the type II elements represent two ends

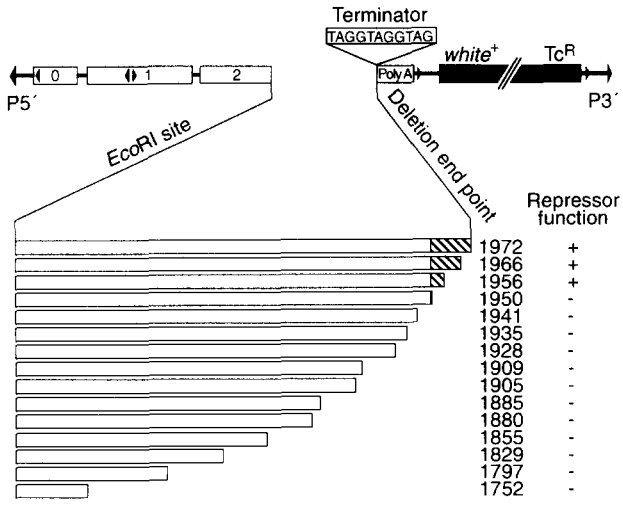

FIGURE 5.—The *white*-linked deletion end points. A vector, pP3′*w*+, was designed so that any *P* element 5′ end cloned into a unique *Pst*I site would have a common stop site for transcription and translation and would be linked to a functioning *white* gene. The vector was built from several component parts: The tetracycline resistance gene is the *Eco*RI-*Ava*I fragment derived from pAT153 (MANIATIS, FRITSCH and SAMBROOK 1982). The P3′ end is the 3′ terminal *Dra*I fragment from pπ25.1 (O'HARE and RUBIN 1983) cloned into the *Ava*I site of pAT153. A three-frame termination sequence was inserted by oligonucleotide mutagenesis into the *Pst*I site of pAT153. This regenerated the *Pst*I site. The polyA site is the *P* element *Sal*I-*Nla*IV fragment (O'HARE and RUBIN 1983). The *white* gene is an *Eco*RI-*Bss*HII fragment derived from pCaSpeR (PIRROTTA 1988). The *white* gene and the tetracycline resistance genes are not shown to scale. The linkage of the deletion end points to the vector is shown below in an expanded scale to show the deletion end points in more detail. *P* element deletions were derived from pπ25.1, a plasmid that contains an autonomous *P* element. The deletions were cloned into a unique *Pst*I site introduced before the terminator and sequenced to confirm their orientation and end-point. The extra 15 amino acids that are translated at the end of exon 2 when the 2-3 splice is not made are shown as a hatched line. The junction between exon 2 and the 2-3 intron is between nucleotides 1947 and 1948 (LASKI, RIO and RUBIN 1986).

of a continuous series of P repressors. Therefore, we decided to define more precisely the 3′ end limit sequence of the type I repressor that could have an effect in our assays. A series of deletion mutations in this region was produced, and each was cloned into the vector, pP3′w^+ (Figure 5) constructed for this purpose. This vector was designed so that transcription and translation of each mutant would initiate and terminate at the same sites. This ensures that each deletion was tested for P repressor in the same molecular background.

The mutants tested for repressor are also shown in Figure 5. Each of the 15 elements was named according to the first *P* element nucleotide deleted. Mating schemes similar to those presented previously were used to test each element for its ability to suppress the cytotype-dependent $vg^{21\text{-}3}$ allele and to repress pupal lethality. These matings were carried out in a *white* mutant background to allow transpositions of the *white*-marked test elements to be detected phenotypically.

We tested between 34 and 124 insertions of each element in the $vg^{21\text{-}3}$ assay, and at least 70 transpositions of each in the pupal lethality assay. Table 5 summarizes the data for these tests. Only three deletion end points consistently gave suppression of the $vg^{21\text{-}3}$ allele and repression of pupal lethality. These elements had deletion end points at nt 1956, nt 1966 and nt 1972. Elements with more extreme deletions, *i.e.*, those with end points 5′ of nt 1956, were not observed to have a significant effect in either assay.

This position-dependent expression of *P* elements can be used to measure the relative strength of each repressor-making element. The proportion of insertions of an element that make repressor will be taken as an indicator of its repressor potential.

We observed such an effect with the deletion mutants (Table 5). The repressor-making elements Δ1972, Δ1966 and Δ1956 inserted to make repressor with characteristic frequencies in both the $vg^{21\text{-}3}$ assay and the pupal lethality assay. In each case, the element with the smallest deletion made repressor in the most insertion sites and the element with the largest deletion made repressor in the fewest.

These results indicate that there is a boundary between a repressor-making and a nonrepressor-making element between nt 1956 and 1950. There were 12 constructs that did not make repressor with deletion end points between the smallest type I element and the *KP* element. This result indicates that the type I and type II element structures do not overlap, and can be classified into two structural groups.

Position effects

The above data along with previous results (MISRA and RIO 1990; ROBERTSON and ENGELS 1989) indicate that the ability of a given *P* element to function as a repressor is sensitive to its genomic position. However, these data have been primarily qualitative, classifying each element as either positive or negative for repressor production. In the following experiments we take a quantitative approach in order to determine the distribution of these position effects and to ask whether various insertion sites are correlated in their repressor function in different assays.

Repressor distribution: We selected GD (gonadal dysgenic) sterility as an easily quantifiable phenotype for use in describing the frequency distribution. GD sterility occurs when a large number of *P* elements are mobilized in the germline. It is especially pronounced in females and is enhanced by elevated temperature during late embryonic and early larval development (ENGELS and PRESTON 1979; SCHAEFER, KIDWELL and FAUSTO-STERLING 1979). The result is early death of germ cells (NIKI and CHIGUSA 1986) leading to agametic adults. GD sterility can be pre-

TABLE 5

Repressor tests of *white*-marked deletion constructs

Element	vg^{21-3} suppression assay		Pupal lethality repression assay		
	white$^+$ transpositions[a]	*white*$^+$ vg^{21-3} suppressors[b]	Total *white*$^+$ transpositions[c]	Independent transpositions[d]	*white*$^+$ P.L. repressors[e,f]
Δ1972	87	56	70	23	23
Δ1966	53	24	100	40	19
Δ1956	124	8	254	51	2
Δ1950	89	0	85	27	0
Δ1941	99	0	107	26	0
Δ1935	41	1	73	20	0
Δ1928	54	0	84	24	0
Δ1909	34	0	151	33	0
Δ1905	38	0	81	22	0
Δ1885	86	0	72	27	0
Δ1880	74	2	230	25	0
Δ1855	—[g]	—	107	27	0
Δ1829	48	0	88	27	0
Δ1797	42	0	95	31	0
Δ1752	—	—	76	25	0

[a] This column shows the total number of independent *white*$^+$ transpositions in the vg^{21-3} assay.
[b] This column shows the number of independent *white*$^+$ suppressors of the vg^{21-3} allele recovered.
[c] The total number of transpositions inferred from siblings with a nonpupal lethal genotype in the pupal lethality assay is shown in this column.
[d] This column shows the minimum number of independent transpositions in the pupal lethality assay. This actually represents the number of vials with one or more transpositions and so is an underestimate.
[e] This column shows the total number of flies surviving the pupal lethality genotype.
[f] Only a proportion of flies that carry a repressor-making element survive the pupal lethality phenotype (ROBERTSON and ENGELS 1989). The proportion that survive is dependent on the strength of the repressor-making insertion. It is appropriate, then, to look at the bulk population of flies to determine the element's repressor activity. Therefore, one measure of the repression ability of an element is the ratio of new insertions to survivors of pupal lethality.
[g] — = not tested.

vented by repressor elements (ROBERTSON and ENGELS 1989; RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1991).

We selected 35 insertions of the type I repressor elements described above and tested them for the ability to rescue females from GD sterility. The insertions were obtained by mobilizing the elements *P*[Δ1956], *P*[Δ1966], *P*[Δ1972] and several of the *P*[*ry*$^+$] elements classified previously (Tables 3 and 4) as being positive for repressor. New insertions were selected by expression of the *white* or *rosy* gene carried by the element. Individual females heterozygous for each insert were then crossed to π_2 males. The daughters were raised at 28°, aged as adults for several days, and then pressed between two glass plates to determine the presence or absence of oocytes. A female was classified as fertile if one or more oocytes was observed. The daughters that did not receive the repressor, as indicated by segregation of dominantly marked balancer chromosomes, were used as controls. Figure 6 shows the results of testing 4375 repressor-bearing females and 1515 controls. One insert provided near complete rescue of GD sterility and another gave partial rescue. The rest had little or no ability to prevent GD sterility in this cross.

Correlation between repressor phenotypes: In the

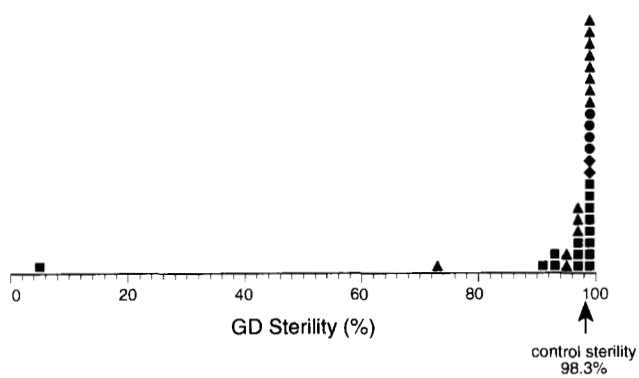

FIGURE 6.—Frequency distribution of position effect for repression of GD sterility. Each point represents the proportion of sterile female offspring for one insertion site of the element noted. Results for each insertion is indicated by a symbol ●: *P*[*ry*$^+$]; ▲: *P*[Δ1956]; ■: *P*[Δ1966]; ◆: *P*[Δ1972]. Lower sterility percentage indicates stronger repressor. We scored an average of 135 progeny/insertion.

experiments described above we utilized several distinct assays for repressor function. Each assay measures repressor in a particular set of tissues and developmental times. To study the nature of position effects, it was of interest to determine whether these effects operate in the same way for different assays. We chose seven inserts of the *P*[Δ1966] element, four of them on chromosome 2 and three on the *X*, to compare their ability to repress by three of our assays:

TABLE 6

Position effects of the *P*[w^+ Δ1966] element on three measures of repressor

Insert[a]	$vg^{21\text{-}3}$	Pupal lethality[b] ± SE		GD sterility ± SE[c]
		Control	Repressor	
1966x	5	0.99 ± 0.01	0.96 ± 0.02	0.99 ± 0.01
1966w	5	1.00	0.86 ± 0.05	0.94 ± 0.03
1966r	3	1.00	0.18 ± 0.15	1.00
1966k	3	1.00	0.97 ± 0.03	0.97 ± 0.01
1966v	3	1.00	0.69 ± 0.06	0.91 ± 0.05
1966j	5	0.95 ± 0.01	0.58 ± 0.07	0.98 ± 0.01
1966d	1.5	0.98	0.06 ± 0.14	0.05 ± 0.03

[a] Inserts 1966d, 1966j and 1966v were on the *X* chromosome, and the others were on chromosome 2.

[b] Pupal lethality was estimated as 1 − survivors/expected *Birm2*; Δ2-3(99B). The class of *Birm2*; Δ2-3(99B) progeny lacking repressor was used for the control column. The expected numbers and standard errors were computed by maximum likelihood as described (ENGELS *et al.* 1987). For the *X*-linked elements an additional interaction factor was included for genotypes in which both the *FM7* chromosome and Δ2-3(99B) were present in order to obtain an acceptable fit.

[c] A binomial distribution was assumed for computing the standard errors of GD sterility. This assumption is justified since sterility either occurs or does not occur in each female independently.

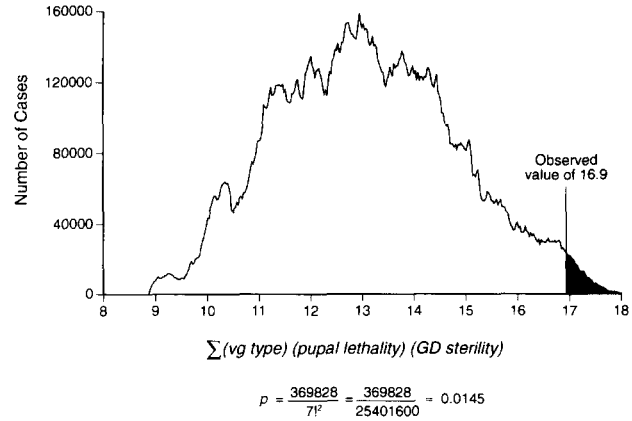

FIGURE 7.—Frequency distribution of the triple correlation coefficient describing the relationship between three assays for repressor activity over seven inserts of the *P*[Δ1966] element. The data in Table 6 were permuted in all possible ways ($7!^2$) to obtain the distribution of the test statistic under the null hypothesis of no correlation. The values ranged from 8.9–18.0. This range was divided into 500 sections to construct the plot. See text for further explanation.

$vg^{21\text{-}3}$ suppression, rescue of pupal lethality and rescue of GD sterility.

The effect on the $vg^{21\text{-}3}$ allele was determined for each element by inspection of $vg^{21\text{-}3}/vg$ heterozygotes with one copy of the repressor element. The GD sterility assays were described in the previous section. We measured pupal lethality for the second chromosomal repressor elements by the same cross used previously for this purpose, and for *X*-linked elements we used the cross:

$$\frac{*}{FM7}\,♀ \times \frac{CyO}{Birm2}\,;\,\frac{Sb\Delta 2\text{-}3}{P[SalI](89D)}\,♂.$$

In this mating * indicates the repressor-bearing chromosome. The *P*[*SalI*] element was included to prevent lethality of the parent.

The crosses, which were set up at 25°, each yield eight phenotypic classes, two of which include *Birm2* and Δ2-3(99B) and are therefore susceptible to pupal lethality. One of the two susceptible classes in each cross had the putative repressor element and the other did not. Counts of these and the other six classes from each cross were used in a multiplicative viability model described previously (ENGELS *et al.* 1987) to estimate how many progeny of the susceptible classes would have survived to adulthood had there been no pupal lethality.

The results in Table 6 provide some indication that position effects act similarly for the three assays. In particular, insert 1966d had the strongest repressor among the seven inserts for all three assays. However, the correlation was not perfect. For example, insert 1966r had moderate repressor for both $vg^{21\text{-}3}$ and pupal lethality but none for GD sterility. One possibility is that this insert produces repressor somatically but not in the germline. We employed a permutation test (KENDALL and STUART 1973) to determine the statistical significance of the three-way correlation. Since all three measures are necessarily non-negative with smaller values indicating stronger repressor, the sum of the products of the three values can be used as a test statistic for such a correlation. We computed this statistic for each of the 25,401,600 permutations, ($7!^2$), to determine the distribution shown in Figure 7. Only 369,828 of them had a value equal to or greater than the observed case. We therefore conclude with significance level $P = 0.015$ that genomic position effects are positively correlated for these three assays.

Position effects *vs.* element-internal changes: One potential weakness of the above conclusion is that some of what we interpret as position effects might actually be due to changes within the repressor elements. Specifically, inserts such as 1966x and 1966w have no repressor function by any assay and therefore could have suffered internal deletions resulting in an irreversible loss of repressor potential. To test this possibility, we mobilized the 1966x and 1966w inserts by crossing in the Δ2-3(99B) element and screening the $vg^{21\text{-}3}/vg$ progeny for their vestigial phenotypes. Each set of crosses yielded at least one offspring whose wing phenotype indicated repressor. Phenotype 3 as defined in Figure 1 was observed for 1966x and type 1.5 for 1966w. We conclude that the lack of repression from the original insertion sites was due to position effects rather than element-internal differences.

DISCUSSION

The structure of type I repressors: The results described here define the structure of one class of

repressor-making *P* elements. Type I repressor elements have at least the entire sequence of exons 0, 1 and 2 of the transposase gene, and the first 9 base pairs of the 2-3 intron (Table 5 and Figure 5). Elements with more extreme deletions did not make detectable repressor. In contrast, type II repressors are deleted for most of exon 1 and all of exon 2. Therefore, type I elements require sequences for their function that type II elements lack. These results can thus be used to define the distinction between these two types of P repressor-making *P* elements. Furthermore, a study of type II elements has demonstrated functional differences between these two repressor classes (in preparation).

The *P* element sequence has two known landmarks near the 3′ boundary of type I elements. One is the splice donor site of the 2-3 intron (RIO, LASKI and RUBIN 1986) and the other is the sequence involved in tissue-specific splicing (CHAIN *et al.* 1991). However, neither of these is sufficient for type I repressor function, since one of our constructs (Δ1950) has both sites, yet it lacks repressor function.

Mechanism of type I repressors: These experiments did not directly test how type I repressors inhibit *P* element mobility. Nevertheless, some conclusions can be drawn about their mechanism of action.

In one model, *P* element ends are postulated to titrate out the transposase or other factors required for *P* transposition (SIMMONS and BUCHOLZ 1985). The repression we describe for type I elements cannot be explained in this way because each deletion construct was tested in an identical genotype in the presence of the same number of *P* element ends.

Another suggestion is that antisense RNA produced by some *P* elements causes *P* repression (RASMUSSON, RAYMOND and SIMMONS 1993). This model is unable to explain the repression abilities of the deletion construct we tested. The strongest evidence that this model does not apply to our results is that the *white* gene would not be expressed if an outside promoter were transcribing the noncoding strand of the gene. All of our repressors had pigmented eyes, indicating *white* gene expression. Furthermore, these constructs are very similar at the RNA level, beginning and ending at the same control signals (Figure 5). Finally, the 3′ ends of the *P* elements in the constructs were flanked by a relatively large amount of DNA. This DNA, composed of the Drosophila *white* gene and the tetracycline resistance gene from pBR322, would be expected to serve as a buffer against an outside promoter transcribing antisense RNA. Note that this conclusion does not imply that titration and antisense repression do not occur for *P* elements in general, but rather that the particular effects of type I repressor elements must employ a different mechanism.

Our data are more consistent with the idea of a polypeptide repressor being produced by the type I elements (MISRA and RIO 1990). We observed that the proportion of insertions of the deletion elements that made repressor followed the order, Δ1972 > Δ1966 > Δ1956, with a large number of insertion sites tested for each element. These results suggest that progressively fewer insertion sites of the more extremely deleted elements can make sufficient repressor to be detected by our assays. The simplest explanation is that sequences near the end of exon 2 and the beginning of the 2-3 intron are required for the stability of a polypeptide repressor made by the type I element.

There are three proposed mechanisms of *P* element regulation by a polypeptide: transcriptional regulation, binding site competition and multimer poisoning (ENGELS 1989; RIO 1990, 1991). They are not mutually exclusive and are probably interdependent.

The main observation supporting the first two models is that P transposase represses transcription *in vitro* by binding to a site at the *P* element 5′ end (KAUFMAN, DOLL and RIO 1989; KAUFMAN and RIO 1991). This binding prevents the transcription factor TFIID from binding an overlapping site at nucleotides 44–70. In addition, type I repressors reduce expression of the *P* promoter *in vivo* (LEMAITRE and COEN 1991; LEMAITRE, RONSSERAY and COEN 1992), perhaps also by preventing TFIID binding. It is reasonable to assume that repressor bound to this site in addition to reducing transcription from the *P* promoter and preventing transposition *in trans* would also prevent transposase binding and repress transposition *in cis*. The transcriptional regulation and binding site competition models described above can thus be viewed as different consequences of repressor binding to the *P* element ends. The multimer poisoning model depends on the number of subunits of transposase required for accurate DNA binding. If more than one subunit is required, then truncated transposase molecules (repressors) could inhibit transposase by preventing either DNA binding or transposase's enzymatic activity (ENGELS 1989). Unfortunately, the number of transposase subunits required for function is unknown, but it is reasonable to assume from studies on bacterial transposons that P transposase is acting as part of a multimeric protein-DNA complex (BAINTON, GAMAS and CRAIG 1991; HANIFORD, CHELOUCHE and KLECKNER 1989; LAVOIE *et al.* 1991).

Position effects: The *P* element promoter is very sensitive to its context (O'KANE and GEHRING 1987; WILSON, Bellen and GEHRING 1990). Genes linked to this promoter display a wide variation in the level and specificity of their expression. This expression depends on the influence of one or more nearby tissue-specific enhancer elements (WILSON *et al.* 1989). In

this analysis of *P* regulation, we have observed that *P* elements capable of making repressor do so in only a proportion of their insertion sites. This position effect might explain the lack of repressor previously reported for a complete *P* element (ROBERTSON and ENGELS 1989). The proportion of sites with detectable repressor is characteristic of both the assay used to measure repressor and the structure of the repressor element.

Each assay measures repressor expression in a different tissue and time of development. The specificity of these assays may be useful in the designing of enhancer trap screens for specific tissues. Such a screen would use an enhancer trap construct that expressed a type I repressor element with its carboxyl-terminal end fused to an indicator gene. New transpositions of the repressor would be recovered by their ability to repress in the tissue of interest and further characterized by a tissue-specific staining pattern. The advantage of this approach would be that only those insertions expressed in the tissue of interest would need to be examined further. We have described two assays that may be useful for this approach. The GD sterility assay measures repressor produced early in the developing germline, whereas the $vg^{21\text{-}3}$ assay measures repressor during the time that expression of the *vestigial* gene is important for wing development. It is noteworthy that approximately 25% of *P*-induced mutations on the *X* chromosome are cytotype dependent (H. ROBERTSON, unpublished data). Thus, it is likely that many already described *P* insertions are cytotype dependent alleles and could be used for this purpose.

Type I repressors in nature: Despite the fact that wild strains of Drosophila have many different *P* element structures (BLACK *et al.* 1987; O'HARE *et al.* 1992; O'HARE and RUBIN 1983), our screen for naturally occurring type I repressors yielded one internally deleted element and several independent cases of complete *P* elements. Previous studies have also implicated deleted *P* elements (NITASAKA, Mukai and YAMAZAKI 1987) and complete *P* elements (LEMAITRE, RONSSERAY and COEN 1992; RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1991) from nature as potential repressors. O'HARE *et al.* (1992) have argued from the distribution of *P* element structures in the π_2 strain that complete *P* elements might be the predominant type I repressors in natural populations. Our data tend to support this suggestion. We recovered only one independent case of an internally deleted type I repressor, the A12 element, as opposed to many complete elements. Furthermore, the A12 element was not found in the parental π_2 strain nor in several other *P* strains examined suggesting that it arose *de novo* in the crosses prior to its isolation.

In this report we primarily used somatic rather than germline assays for repressor. Somatic assays are more accurate since they are simpler and allow larger sample sizes. Note, however, that our results (Figure 6 and Table 6) indicate a good correlation between somatic and germline repression. This result agrees with work reported previously (ROBERTSON and ENGELS 1989).

Maternal inheritance: Early studies of *P* element regulation revealed a maternally inherited component (ENGELS 1979). One model for this effect (O'HARE *et al.* 1992) requires the presence of the 2-3 intron for maternal inheritance of cytotype. More recently, B. LEMAITRE, S. RONSSERAY and D. COEN (unpublished data) have elaborated on this model and provided evidence for it. According to this model, only the type I repressor elements would be involved in the maternal component because the type II elements lack the 2-3 intron. However, both types can contribute to the chromosomal component.

Repressor or transposase? If complete elements are responsible for both transposase and repressor, what determines which function is active? One possibility is that position effects are involved. It is possible that an element's position determines whether a complete element produces predominantly repressor or transposase. The autonomous element(s) studied by RONSSERAY, LEHMANN and ANXOLABÉHÈRE (1991) appear to be an example of *P* elements that make repressor by virtue of their position. Another possibility advanced recently by O'HARE *et al.* (1992) is that the 2-3 intron may be spliced from the *P* element pre-mRNA with different efficiencies in M and P cytotype females. Some support for this hypothesis is given by the results of LEMAITRE, RONSSERAY and COEN (1992), who observed that the levels of *P* element transcription were dramatically lower in the P cytotype *vs.* the M cytotype germline. It should be noted, however, that no such differences have been observed with constructs designed to test for such splicing differences (LASKI and RUBIN 1989).

There are also other possible control points in the splicing pattern of the *P* element transcript. Two other minor splicing patterns have been observed in the 1-2 intron (LASKI and RUBIN 1989) and in the 2-3 intron (CHAIN *et al.* 1991). The alternate splice of the 2-3 intron is especially attractive as a potential regulatory splice since it introduces a small 32 base exon between exons 2 and 3. This alternately spliced mRNA might be expected to produce a type I repressor if the resulting polypeptide were stable.

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