A Role for the *KP* Leucine Zipper in Regulating *P* Element Transposition in *Drosophila melanogaster*

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ABSTRACT

The *KP* element can repress *P* element mobility in *Drosophila melanogaster*. Three mutant *KP* elements were made that had either two amino acid substitutions or a single amino acid deletion in the putative leucine zipper domain found in the *KP* polypeptide. Each *KP* element was expressed from the actin 5C proximal promoter. The wild-type control construct strongly repressed *P* element mobility, measured by the GD sterility and sn^w mutability assays, in a position-independent manner. The single amino acid deletion mutant failed to repress *P* mobility regardless of its insertion site, while repression of *P* element mobility by the double amino acid substitution mutants was position dependent. The results show that the leucine zipper of the *KP* polypeptide is important for *P* element regulation. This supports the multimer-poisoning model of *P* element repression, because leucine zipper motifs are involved in protein-protein interactions.

P ELEMENTS in *Drosophila melanogaster* are among the best-characterized eukaryotic transposons. Despite their utility in a wide variety of genetic techniques, the molecular mechanisms that regulate *P* element mobility have remained enigmatic.

Autonomous *P* elements are 2907 bp long and are bounded by 31-bp inverted repeats (O'HARE and RUBIN 1983). The *cis*-acting sites required for transposition are found within the terminal 150 bp of both transposon ends (MULLINS *et al.* 1989). Together, the four exons found in autonomous *P* elements produce the 87-kD *P* transposase required for *P* element mobility (KARESS and RUBIN 1984; RIO *et al.* 1986). Tissue-specific splicing of the transposase pre-mRNA ensures that the elements are mobile only in the germline (LASKI *et al.* 1986). This specificity can be eliminated by the artificial removal of the 2-3 intron to create a Δ2-3 element (LASKI *et al.* 1986). Regulation of this splice is dependent on host-encoded proteins acting at *cis* sites on the *P* element (LASKI and RUBIN 1989; SIEBEL and RIO 1990; CHAIN *et al.* 1991; TSENG *et al.* 1991). *P* transposition occurs by a DNA-only mechanism (ENGELS *et al.* 1990; GLOOR *et al.* 1991; KAUFMAN and RIO 1992).

Smaller nonautonomous *P* elements are also commonly found in the Drosophila genome. These elements can be derived from autonomous ones by deletion of internal sequences (O'HARE and RUBIN 1983; O'HARE *et al.* 1992). Natural populations of Drosophila contain both classes of *P* elements (ENGELS 1989). Strains derived from some recently sampled populations (P strains) contain many autonomous and nonautonomous *P* elements whose mobility can be deregulated under certain conditions. Other Drosophila stocks that were collected earlier in this century and maintained as laboratory stocks lack *P* elements and are called M strains. The cross M ♀ × P ♂ yields "dysgenic" hybrids in which *P* elements are highly mobile, while the reciprocal cross maintains the *P* elements in a relatively immobile state (KIDWELL *et al.* 1977).

P element mobility is regulated on at least two levels by a *P*-encoded polypeptide. First, *P* elements encode proteins that repress their own transcription both *in vitro* and *in vivo* (KAUFMAN and RIO 1991; LEMAITRE and COEN 1991; LEMAITRE *et al.* 1993; RONSSERAY *et al.* 1993). This transcriptional regulation can be inherited maternally, accounting for at least some of the reciprocal cross difference seen in the matings between M and P strain flies (LEMAITRE *et al.* 1993; RONSSERAY *et al.* 1993).

The second type of *P*-encoded regulation acts to repress transposase activity, even when transposase transcription is directed by a heterologous promoter (STELLER and PIRROTTA 1986). This type of regulation is likely the result of a repressor protein that forms an inactive multimer with either transposase or with a host protein required for transposition (ENGELS 1989; RIO 1990, 1991).

There are two nonoverlapping structural classes of repressor-making *P* elements (GLOOR *et al.* 1993). The type I elements produce a repressor protein of ~66 kD that is colinear with the amino terminal end of the 87-kD transposase protein. Their coding sequence contains all the first three *P* element exons and the 2-3 intron sequence up to at least nucleotide 1956. Type I repressor-making elements have been isolated from wild populations and constructed *in vitro* (NITASAKA *et*

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al. 1987; ROBERTSON and ENGELS 1989; MISRA and RIO 1990; GLOOR *et al.* 1993).

The other class of repressor elements is much smaller and is sometimes referred to as type II repressor elements (GLOOR *et al.* 1993). Type II repressor-making elements encode a smaller protein that is colinear with the amino terminal end of transposase for about the first 22 kD. The coding region for these elements is always truncated near the middle of exon 1 (BLACK *et al.* 1987; JACKSON *et al.* 1988; RASMUSSON *et al.* 1993). Type II elements are geographically widespread and are not typically associated with the P cytotype, although they can transmit repressor maternally (BLACK *et al.* 1987; RASMUSSON *et al.* 1993). One particularly well-characterized element is called the *KP* element, which has a deletion between nucleotides 808-2560 that fuses exons 1 and 3 out of frame (BLACK *et al.* 1987).

The aim of this paper is to investigate the molecular requirements for repressor production of the *KP* element. Inspection of the amino acid sequence of the translated *KP* protein reveals only one recognizable protein motif; a putative leucine zipper (RIO 1991). The leucine zipper domain is the site of specific contact between many proteins (LANDSCHULTZ *et al.* 1988; DANG *et al.* 1989; BUSCH and SASSONE-CORSI 1990). This domain is especially well represented in transcription factors, although the coiled coil structure that leucine zippers adopt is a very widespread protein-protein contact domain (COHEN and PARRY 1994). In this report, a single amino acid deletion mutant in the *KP* leucine zipper completely abolished *KP* repressor function. Point mutants that exchanged leucine residues in the contact region for other amino acids had only partial function. These results indicate that the *KP* leucine zipper is an important structural domain involved in regulating *P* element mobility.

MATERIALS AND METHODS

Genetic techniques: Flies were maintained on standard cornmeal-molasses-agar medium. Mating schemes are described in the text, and all matings were incubated at room temperature (23-24°C) unless noted. Genetic symbols not described here are in LINDSLEY and ZIMM (1992).

Transposase-containing stocks: The stable transposase source *P*[ry_+ $\Delta 2$-3](*99B*) (ROBERTSON *et al.* 1988), hereafter referred to as Δ2-3(99B), was used as our standard transposase stock. Our standard P strain was *w*Harwich (KIDWELL *et al.* 1977).

In vitro modified white$^+$-marked P element test constructs: Constructs were transformed by microinjection into embryos whose genotypes were *y w;Ki* Δ2-3(99B)/+ as described previously (ROBERTSON *et al.* 1988). Only injected male flies were mated to maintain linkage between *Ki* and Δ2-3(99B). The insertions were maintained in one of two ways: X-linked insertions were maintained in a *C(1)DX, y w f*M cytotype genetic background. Autosomal insertions were made homozygous by selectively mating sibling flies with a more intense eye color over several generations.

Meiotic recombination: Ten of the *P*[*w*act*KP*] elements were on *X* chromosomes marked with alleles of *white* and *forked*. Meiotic recombination in female flies with the genotype: *w P*[*w*act*KP*] *f/w snw* was used to make a chromosome that had the *P*[*w*act*KP*] and the sn^w allele. Only recombinant progeny with a forked$^+$ phenotype were retained. Recombinant chromosomes were not recovered for seven insertion lines because they were too close to either the *singed* or *forked* genes for meiotic recombinants to be recovered easily.

Molecular techniques: Molecular cloning techniques were performed according to standard procedures (MANIATIS *et al.* 1982; SAMBROOK *et al.* 1989). Enzymes were obtained from New England Biolabs, Life Technology Inc., and Promega Biotech.

Polymerase chain reaction: The polymerase chain reaction was used to amplify specific sequences essentially as described (SAIKI *et al.* 1988; ERLICH 1989; GLOOR *et al.* 1993). Our reaction volumes were routinely 15 μl containing 1 μl of fly DNA and were cycled 30 times. Drosophila DNA for the polymerase chain reaction was prepared as described previously (GLOOR *et al.* 1993). Recombinant PCR was done according to standard techniques (HIGUCHI 1990). We noted that recombinant PCR was more efficient if the first five amplification cycles had an annealing step that lasted for 5 min (GLOOR *et al.* 1991). The annealing steps in later amplification cycles were reduced to 1 min each. Care was taken to amplify for as few cycles as possible to minimize any errors introduced by PCR. All cloned sequences that were amplified with the PCR were examined by DNA sequencing. All errors were corrected by subcloning and the constructs were confirmed by DNA sequencing before transforming the constructs into Drosophila.

actKP fusion constructs: The act*KP* fusions were constructed by recombinant PCR (HIGUCHI 1990). The actin 5C-specific oligonucleotides were act1: 5′ (TTCTTGAATTCAGTGACGTAGG) 3′, and act2: 5′ (CACACACAACCTTTGAAAGGAATGACTGG) 3′. The *P* element-specific oligonucleotides were P1: 5′ (GAAAGGTTGTGTGTCACGA) 3′ and P2: 5′ (ATCAACATCGACGTTTCCAC) 3′. Recombination was effected by mixing the act1/act2 amplimer with the P1/P2 amplimer and amplifying with the act1 and P2 oligonucleotides. These products fused the actin 5C proximal promoter fragment from nucleotides −381 to 30 to the *KP* element sequence starting at 90 and were cloned into the vector *P*[*w*alL*KP*] (NASSIF *et al.* 1994) by replacing the *Eco*RI/*Xho*I fragment in the vector with the amplified fragment. The leucine zipper region was mutated by making oligonucleotides with the required base changes and introducing the mutations by recombinant PCR.

EXPERIMENTS AND RESULTS

These experiments were designed to test the role of a putative leucine zipper in the *KP* element, located between amino acids 101 and 122 (RIO 1991), in *KP*-mediated repression of *P* mobility. Figure 1 shows a diagram of the *white$^+$* marked transformation vector, *P*[*w*alL] (NASSIF *et al.* 1994), with the *KP* element placed under control of the actin 5C proximal promoter (act*KP*). This construct is named *P*[*w*act*KP*wt] in the results that follow. Three different constructs were made that had mutations introduced in the leucine zipper (sequences shown in Figure 1). Two constructs altered both the first and fourth leucine residues; one changed leucine residues at amino acid positions 101 and 122 to valine and histidine (*P*[*w*act*KP*VH]), the other changed these residues to isoleucine and arginine (*P*[*w*act*KP*IR]). A third construct deleted a codon for the isoleucine residue at position 112. This construct

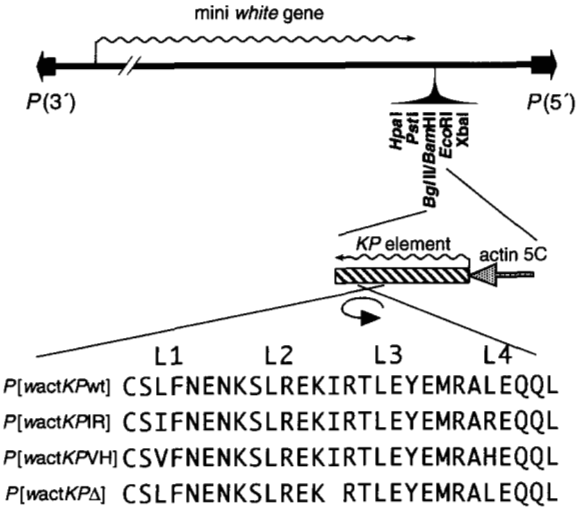

FIGURE 1.—The structure of *P*[*w*act*KP*]. This construct contains a *KP* element under the control of the actin 5C proximal promoter (CHUNG and KELLER 1990). The act*KP* fusion is cloned into the *Xho*I and *Eco*RI sites of the plasmid p*P*[*w*alL] (NASSIF *et al.* 1994). The transcripts of both act*KP* and the *white* gene transformation marker are shown as the wavy lines. The sequence of the *KP* leucine zipper is shown below. Note that this sequence is in the opposite orientation to the vector, with the peptide's amino terminal end on the left.

will be called *P*[*w*act*KP*Δ]. The generic constructs will be referred to as *P*[*w*act*KP*].

The constructs were transformed into the Drosophila germline using standard techniques (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1982; ROBERTSON *et al.* 1988). We established six independent *P*[*w*act*KP*wt] lines, four independent *P*[*w*act*KP*Δ] lines, nine independent *P*[*w*act*KP*IR] lines and seven independent *P*[*w*act*KP*VH] lines. Analysis of the genomic DNA of the transformant lines by Southern blotting confirmed that each line used in the subsequent analysis contained a single *P*[*w*act*KP*] element insertion (data not shown). These fly lines were tested for the ability of the *P*[*w*act*KP*] element to repress *P* element mobility as measured by both the gonadal dysgenic (GD) sterility assay and the sn^w mutability assay.

GD assay: This assay measures repression in the germline of the dysgenic progeny that result from a mating between a strong P strain male (such as *w*;Harwich) and an M strain female (KIDWELL *et al.* 1977). Such progeny are normally sterile because *P* elements are mobilized at high levels in their germline causing massive cell death (NIKI and CHIGUSA 1986). Repression of *P* element mobility is indicated by an increase in the proportion of fertile female progeny (ROBERTSON and ENGELS 1989; RONSSERAY *et al.* 1991; GLOOR et al. 1993).

X-linked insertions of *P*[*w*act*KP*] were tested in the cross: */*FM7, y* sn^{X2} *B* ♀ × *w*; Harwich ♂, where the * denotes the *P*[*w*act*KP*] element.

Autosomal insertions of the *P*[*w*act*KP*] elements were tested for their ability to repress GD sterility in the mating: *w*;*;* ♀ × *w*; Harwich ♂, where the * again denotes the *P*[*w*act*KP*] element.

In these matings, a *P*[*w*act*KP*] element was located either on the *X* chromosome or was homozygous on either the second or third chromosome. Three or four males from the *w*; Harwich strain were mated with a single virgin female. Each cross was incubated at 29°C until progeny started to eclose. The F_1 progeny were placed into separate vials and incubated at room temperature for 2 days to allow their germlines to fully mature. Progeny carrying a *P*[*w*act*KP*] element could be unambiguously identified by their orange eye color. The sibling female *FM7/w;* Harwich progeny lacking the *P*[*w*act*KP*] element were used as the controls in these experiments. The females were tested for fertility by squashing them between glass plates and examining them for the presence of eggs. A fly was considered fertile if at least one egg was present. Between 4 and 17 individual matings were set up for each transformant line, and ≥50 female offspring were tested for each line.

The results for the GD sterility assay are shown in Table 1. Almost all (98%) of the control flies that lacked a *P*[*w*act*KP*] element were sterile. Every insertion of the wild type *P*[*w*act*KP*wt] element strongly repressed gonadal sterility (range 1–12% sterility). In contrast dysgenic flies carrying *P*[*w*act*KP*Δ] were generally sterile (range 85–100% sterility). It should be noted that fertile progeny were not evenly distributed between the vials of *P*[*w*act*KP*Δ], but were found in only two of the five vials. In contrast to *P*[*w*act*KP*wt], the levels of repression provided by different insertions of *P*[*w*act*KP*IR] and *P*[*w*act*KP*VH] was strongly dependent on their insertion site. Different insertions of both mutant elements were represented at both extremes of repressor activity.

sn^w hypermutability assay: The second assay measured each repressor insertion for its ability to prevent Δ2-3(99B)-induced hypermutability of the sn^w (*singed-weak*) allele (ENGELS 1979b). The sn^w allele is a double *P* element insertion allele of the *sn* locus. Excision of one *P* element from the sn^w allele changes the phenotype to an extreme singed phenotype (sn^r), while excision of the other results in a wild-type phenotype ($sn^{(+)}$) (ROIHA *et al.* 1988). Repressor activity in this assay is determined by measuring the proportion of progeny that are sn^r, with increasing repressor activity indicated by a decrease in the proportion of sn^r progeny (ROBERTSON and ENGELS 1989).

Insertions of the *P*[*w*act*KP*] constructs were tested in the cross *C(1)DX, y w f* ♀ × *w* sn^w *; *; (*Sb* Δ2–3(99B)/ *) ♂.

In this mating the *P*[*w*act*KP*] element (shown as a *) was located on either the *X*, second or third chromosome. Single males with the *P*[*w*act*KP*] element were mated with three to five attached-*X* females at 24°C. The *X* chromosome was recovered in the male progeny and Sb^+ flies were examined for their singed pheno-

TABLE 1

Repression of *P* element mobility by the *P*[*w*act*KP*] lines

Construct	Line	Site[c]	GD sterility[a] Average[d]	GD sterility[a] n[e]/m[f]	sn^w mutability[b] Average	sn^w mutability[b] n/m
P[*w*act*KP*wt]	1	*III*	0.124 ± 0.029	7/129	0.033 ± 0.013	13/454
	2	*II*	0.086 ± 0.033	11/70	0.047 ± 0.018	17/386
	3	*III*	0.039 ± 0.022	7/77	0.013 ± 0.004	14/850
	4	*X*	0.098 ± 0.017	11/317	ND[g]	
	5	*II*	0.030 ± 0.021	10/67	0.024 ± 0.008	33/572
	6	*III*	0.011 ± 0.008	8/187	0	14/344
P[*w*act*KP*Δ]	1	*X*	0.994 ± 0.006	6/163	ND	
	4A	*II*	0.997 ± 0.003	10/307	0.24 ± 0.008	17/588
	4B	*III*	0.990 ± 0.010	12/99	0.291 ± 0.006	10/357
	5	*II*	0.852 ± 0.038	5/88	0.314 ± 0.026	9/175
P[*w*act*KP*IR]	1	*X*	0.949 ± 0.011	13/428	ND	
	2	*III*	0.939 ± 0.013	17/313	0.216 ± 0.032	14/784
	3	*X*	0.084 ± 0.018	9/238	0	10/228
	4	*III*	0.035 ± 0.020	7/85	0.006 ± 0.006	5/323
	5	*X*	0.211 ± 0.025	5/261	ND	
	6	*III*	0.029 ± 0.016	11/104	0.024 ± 0.012	15/720
	7	*III*	0.520 ± 0.058	4/75	0.033 ± 0.007	15/934
	8	*III*	0.218 ± 0.036	5/133	0.042 ± 0.013	19/781
	10	*X*	0.997 ± 0.003	16/379	0.393 ± 0.029	17/801
P[*w*act*KP*VH]	1	*X*	0.333 ± 0.029	12/273	ND	
	2	*II*	0.500 ± 0.038	17/172	0.041 ± 0.016	8/318
	2A	*X*	0.370 ± 0.020	15/581	ND	
	3	*X*	0.262 ± 0.034	8/172	0.088 ± 0.023	7/363
	4	*X*	0.237 ± 0.039	7/118	ND	
	5	*II*	0.104 ± 0.029	12/115	0.062 ± 0.017	10/209
	6	*III*	0.980 ± 0.014	6/99	0.461 ± 0.043	13/778
Construct			0.984 ± 0.005[h]	43/630	0.290 ± 0.037	20/926
Transposase			ND		0	20/2504

[a] The proportion of sterile dysgenic flies.
[b] The proportion of progeny that were sn^e.
[c] Chromosome of insertion.
[d] Weighted averages and standard errors were calculated by the method of ENGELS (1979a).
[e] The number of independent vials scored for each experiment.
[f] The total number of flies with the appropriate genotype scored.
[g] ND, not determined.
[h] Derived from the *P*[*w*act*KP*wt]/2FM7 × *w*;Harwich cross.

type. We did not score $sn^{(+)}$ bristles because of their similarity with sn^w in hemizygous males.

The repression ability of the lines examined are given in Table 1. Control experiments using flies lacking a construct showed a sn^e mutability rate of 29%. This result is similar to those obtained previously (ENGELS 1979b; ROBERTSON and ENGELS 1989) and was similar to the result seen for insertions of *P*[*w*act*KP*Δ] (range: 24–31%). The control mating without transposase did not produce any sn^e progeny (0%). Insertions of *P*[*w*act*KP*wt] strongly repressed sn^w hypermutability. Between 0 and 5% of progeny had the sn^e phenotype. The *P*[*w*act*KP*IR] and *P*[*w*act*KP*VH] lines varied in their ability to repress sn^w hypermutability. The proportion of sn^e flies for *P*[*w*act*KP*IR] ranged from 0% line to 39%, while *P*[*w*act*KP*VH] ranged from 4 to 46%. Two of the lines had sn^w mutation rates that were significantly greater than the negative control (39 and 46% *vs.* 29%). The elevated rates are not explained by the occurrence of a few large clusters. While this variability cannot be explained it is similar to the variation observed by other investigators (ROBERTSON and ENGELS 1989).

Comparison of repressive abilities: Each *P*[*w*act*KP*] element was tested for its ability to repress GD sterility and sn^w mutability in a variety of insertion sites. The main interest of this study, however, was to determine the position independent effect of the mutations on the repressive ability of the *KP* element. To do this, the mean repression ability of each pair of constructs was compared by a permutation test that assessed the difference between the means without relying on assumptions about the underlying distribution (FISHER 1935; KENDALL and STUART 1973). The test results for all the pairwise comparisons are shown in Table 2. The repression ability of both *P*[*w*act*KP*wt] and *P*[*w*act*KP*Δ] were significantly different from each other and from both

TABLE 2

Comparison of mean repressor ability

Element	Element				Assay
	P[*w*act*KP*wt]	*P*[*w*act*KP*Δ]	*P*[*w*act*KP*IR]	*P*[*w*act*KP*VH]	
P[*w*act*KP*wt]	—	**0.005**[a]	**0.01**	**0.002**	GD[b]
	—	**0.02**	0.19	**0.02**	sn^{w}[c]
		—	**0.03**	**0.006**	GD
P[*w*act*KP*Δ]		—	0.06	0.17	sn^{w}
			—	0.4	GD
P[*w*act*KP*IR]			—	0.26	sn^{w}

[a] Boldface values represent significant differences between the means.
[b] *P* value for the GD sterility assay.
[c] *P* value for the sn^{w} mutability assay.

double-point mutants in the GD sterility assay. However, only the *P*[*w*act*KP*wt] and the *P*[*w*act*KP*Δ] showed a significant difference in their repressive ability in the sn^{w} mutability assay. The sn^{w} assay had less power to discriminate between the mutants because of the smaller number of insertions tested.

Correlation between repressor assays: We also wanted to compare the ability of the two assays to measure *P* repressor activity. Both the above experiments measured *P* repressor function in the Drosophila germline, and Figure 2 shows a scatter plot of the repressive ability of each element in the GD sterility and the sn^{w} mutability assays. The correlation between the two data sets was 0.904, indicating that the ability of an element to repress *P* mobility in one assay was a strong predictor of its ability to repress in the other. We employed a random permutation test (KENDALL and STUART 1973) to measure the significance of this correlation, both with the complete data set ($P = 0.0000012 \pm 0.00000035$; mean ± SE), and with a data set that excluded the *P*[*w*act*KP*Δ] elements, which did not repress in any assay, (correlation = 0.891, $P = 0.000023 \pm 0.000005$). We conclude that the ability of the two assays to measure *P* repressor are positively correlated.

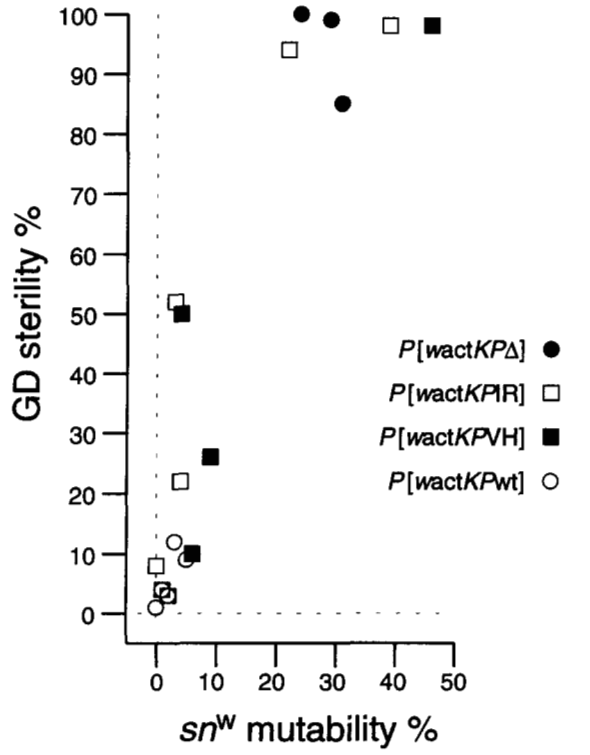

FIGURE 2.—Assay dependence of *P* repressor. The values in Table 1 for lines tested in both the GD sterility assay and the sn^{w} mutability assay are plotted. Stronger repressors are found near the origin. The correlation between the two data sets was 0.904.

DISCUSSION

Repression of *P* mobility by act*KP* elements: These results show that single insertions of an actin5C-driven *KP* element can strongly repress *P* element mobility in both the GD sterility and sn^{w} mutability assays. Previous workers have shown position-dependent expression of *P* repressor activity when the repressing *P* element was directed by the native *P* promoter (ROBERTSON and ENGELS 1989; MISRA and RIO 1990; GLOOR *et al.* 1993; RASMUSSON *et al.* 1993). In our experiments, repression by the *P*[*w*act*KP*wt] element was virtually position independent, but repression by both double-point mutants was position dependent. Insertions of the *P*[*w*act*KP*Δ] element did not repress *P* element mobility in either the GD sterility or the sn^{w} mutability assays in any of the insertion sites.

One possibility for the difference in repressive abilities between the wild-type and the mutant *P*[*w*act*KP*] elements was that the mutations may have affected the stability of the *KP*-encoded protein. We attempted to address this point by raising antibodies directed against the *KP* peptide. However, three independent affinity purified antibody preparations proved unsuitable for Western blotting experiments (data not shown).

In a second attempt to determine the structural integrity of the mutant *KP* proteins, we used two programs that predicted the secondary structure of the mutated region. Each program is expected to be >70% accurate

(KNELLER *et al.* 1990; ROST and SANDER 1993). Prediction of the putative secondary structure of the mutated region showed that none of the mutations disrupted the predicted secondary structure. Further analysis showed that the predicted secondary structure for the leucine zipper region was very similar in *P* elements isolated from *D. melanogaster, Scaptomyza pallida* and *D. bifasciata* (SIMONELIG and ANXOLABEHERE 1991; HAGEMANN *et al.* 1992).

We suggest that the variation in the repressive abilities of the mutated *KP* elements reflects an intrinsic difference in their ability to repress *P* mobility rather than gross differences in their structure or stability. Because each construct is expressed from an identical promoter, it is likely that the four *P*[*w*act*KP*] elements could produce equivalent amounts of repressor protein if inserted in equivalent genomic positions. The nearly position independent repression by the *P*[*w*act*KP*wt] element is explained if we assume the strong actin5C promoter produces enough repressor protein in all genomic insertion sites to pass the threshold level of repression in these assays. However, equivalent amounts of protein produced by the double-point mutants in their insertion sites would not always be sufficient to repress *P* element mobility.

Mechanism of repression by *KP* elements: It is likely that more than one type of regulation contributes to the repression of *P* element mobility in any given Drosophila strain. There are two models for polypeptide-independent repression: titration of *P* transposase in the presence of many *P* element ends (SIMMONS and BUCHOLZ 1985) and antisense RNA production (RASMUSSON et al. 1993). Neither of these models applies to the above experiments because single insertions of the test elements were used and all were transcribed from a common promoter. Furthermore, if antisense repression was involved, it would be difficult to explain the consistently strong repression by the *P*[*w*act*KP*wt] elements and the consistently weaker repression by the mutant elements.

Several other models for *P* repression require a *P* encoded polypeptide: transcriptional regulation, binding site competition, and multimer poisoning (ENGELS 1989; RIO 1991). The transcriptional regulation and binding site competition models described above can be viewed as different consequences of repressor binding to the *P* element ends. Such binding could repress transposition in *cis* by interfering with transposase binding at the same site and in *trans* by reducing the amount of transposase produced. These models are supported by observations that *P* transposase represses its own transcription *in vitro* by binding to a site at the *P* element 5′ end (KAUFMAN *et al.* 1989; KAUFMAN and RIO 1991). In addition, repressor-making *P* elements reduce expression of the *P* promoter *in vivo*, (LEMAITRE and COEN 1991; LEMAITRE *et al.* 1993; RONSSERAY *et al.* 1993). Transcriptional regulation cannot be the only mode of regulation because *P* elements whose expression is directed by a heterologous promoter can still be partially repressed (STELLER and PIRROTTA 1986). It should be noted that the *KP* element lacks the putative helix-turn-helix DNA binding motif found in *P* transposase (RIO *et al.* 1986), so it may not directly bind to the *P* element ends.

The third model, multimer poisoning, requires that *P* transposase acts as part of a multimeric DNA-protein complex, similar to those found in bacterial transposons (HANIFORD *et al.* 1989; BAINTON *et al.* 1991; LAVOIE *et al.* 1991). In this case *P* repressor proteins, which are truncated forms of transposase, could exclude transposase from the complex and prevent transposition.

Leucine zipper domains are common sites of contact between interacting proteins, especially transcription factors (BUSCH and SASSONE-CORSI 1990; HURST 1994). It is now recognized that the leucine zipper adopts the coiled coil structure that is a common protein-protein interaction motif (COHEN and PARRY 1994). Our results indicate that the *KP* leucine zipper, found between amino acids 101-122 in the *KP* amino acid sequence, is one of the protein domains involved in repressing *P* element transposition. The results support the multimer poisoning model of *P* repression as one type of *P* regulation, although it does not exclude the other two polypeptide-dependent regulatory mechanisms.

What then is the binding partner for the *KP* leucine zipper? One likely candidate is *P* transposase itself. In this case the *KP* peptide would interfere with transposase oligomerization and prevent an active transposase complex from assembling on the *P* element end. Another possibility is that the *KP* leucine zipper binds to a host protein that is required for transposition. One candidate for this would be the inverted repeat binding protein that binds to the terminal 16 nucleotides of the *P* element 31 bp inverted repeat (RIO and RUBIN 1988). The IRBP protein bound at the *P* element end could also serve to tether the *KP* protein to the *P* element end. Such a tethered *KP* protein could repress transposition in any of the following ways: by interfering with *P* element transcription, by preventing transposase binding to the *P* element ends, and by preventing *P* transposase from interacting with host proteins required for transposition.

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