A *hobo* Transgene That Encodes the *P*-Element Transposase in *Drosophila melanogaster*: Autoregulation and Cytotype Control of Transposase Activity

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ABSTRACT

Drosophila were genetically transformed with a *hobo* transgene that contains a terminally truncated but otherwise complete *P* element fused to the promoter from the Drosophila *hsp70* gene. Insertions of this *H(hsp/CP)* transgene on either of the major autosomes produced the P transposase in both the male and female germlines, but not in the soma. Heat-shock treatments significantly increased transposase activity in the female germline; in the male germline, these treatments had little effect. The transposase activity of two insertions of the *H(hsp/CP)* transgene was not significantly greater than their separate activities, and one insertion of this transgene reduced the transposase activity of *P(ry*$^{+}$*, Δ2-3)99B*, a stable *P* transgene, in the germline as well as in the soma. These observations suggest that, through alternate splicing, the *H(hsp/CP)* transgene produces a repressor that feeds back negatively to regulate transposase expression or function in both the somatic and germline tissues. The *H(hsp/CP)* transgenes are able to induce gonadal dysgenesis when the transposase they encode has *P*-element targets to attack. However, this ability and the ability to induce *P*-element excisions are repressed by the P cytotype, a chromosomal/cytoplasmic state that regulates *P* elements in the germline.

P-TRANSPOSABLE elements have proven to be valuable tools in the genetic analysis of Drosophila (Engels 1989). Natural and modified elements have been used as agents for mutagenesis and gene tagging, and appropriately marked elements have been used as vectors for genetic transformation. These applications have succeeded so richly because *P*-element activity can be controlled in experimental crosses. The key factors in this control are a *P*-encoded transposase, which catalyzes *P*-element excision and transposition, and a cellular state that regulates transposase activity.

P elements were discovered because of their involvement in a syndrome of germline abnormalities called hybrid dysgenesis (Kidwell *et al.* 1977). This syndrome includes high frequencies of mutation and chromosome breakage, transmission ratio distortion, male recombination, and gonadal dysgenesis. The traits of hybrid dysgenesis are seen in the offspring of crosses between certain types of Drosophila strains. M strains have a cellular state, the M cytotype, that permits *P*-element activity, whereas P strains have a complementary state, the P cytotype, that represses *P*-element activity (Engels 1979a). *P* elements are found in the genomes of P strains, where, for the most part, they are quiescent; they are also found in a subset of M strains that have been given the designation M′ or pseudo-M (Bingham *et al.* 1982). True M strains do not have *P* elements in their genomes. Dysgenic hybrids are produced when P males are crossed to M (or M′) females. The hybrids produced by the reciprocal cross typically do not show dysgenic traits or they show them with decreased frequency, because the P cytotype, which is maternally transmitted to the offspring, represses *P*-element activity.

P strains possess structurally complete *P* elements, 2.9 kilobases (kb) long, that encode a *trans*-acting transposase (O'Hare and Rubin 1983; Engels 1984; Karess and Rubin 1984). The coding sequence in these elements is partitioned among four exons designated 0, 1, 2, and 3. In the germline, the intervening sequences of transcripts from complete *P* elements are removed by splicing to produce an mRNA that encodes an 87-kD polypeptide, the transposase. In the somatic tissues, the intervening sequence between exons 2 and 3 is not spliced out (Laski *et al.* 1986). Because this sequence contains a stop codon, the incompletely spliced RNA encodes a smaller polypetide of 66 kD. P strains also have numerous incomplete *P* elements in their genomes (O'Hare *et al.* 1992). Most of these appear to have been derived from complete elements by deletions of internal sequences. These elements do not encode a catalytically active transposase, but they usually possess the terminal and subterminal sequences, which the transposase recognizes and attacks. Consequently, they can be excised and transposed. Several lines of evidence indicate that some incomplete *P* elements encode polypeptides that repress transposase activity (Black *et al.* 1987; Jackson

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et al. 1988; Rasmusson *et al.* 1993; Andrews and Gloor 1995). The 66-kD polypeptide produced by alternate splicing of complete *P*-element transcripts also appears to function as a repressor of transposase activity (Robertson and Engels 1989; Misra and Rio 1990; Gloor *et al.* 1993).

P elements are mobilized when the P transposase is produced in the M cytotype. This situation occurs in the offspring of crosses between P males and M females. It also occurs when transgenes that have been engineered to produce the transposase are introduced into M cytotype strains. However, because the standard procedure for introducing transgenes into the Drosophila genome involves transformation with a *P*-element vector, such transgenes are inherently unstable (Steller and Pirrotta 1986; Cooley *et al.* 1988; Simmons *et al.* 1996). The transposase they encode catalyzes their own excision and transposition. This article describes the properties of transgenes that are stable in the presence of the P transposase. A terminally truncated but otherwise complete *P* element has been introduced into the Drosophila genome by means of a transformation vector that was derived from a *hobo* transposable element. Like *P* elements, *hobo* elements are found in some Drosophila strains, and the largest of them, ~3 kb long, encodes a *trans*-acting transposase that is specific for *hobo* elements (Blackman *et al.* 1989; Calvi *et al.* 1991). Because the *hobo* transgenes are stable in the presence of the P transposase, they should be valuable tools for genetic analysis in Drosophila. These transgenes should also help to elucidate the complex mechanisms that control *P*-element activity in nature.

MATERIALS AND METHODS

Drosophila stocks and husbandry: Chromosomes and genetic markers are described in Lindsley and Zimm (1992). Fly cultures were maintained on a standard cornmeal-molasses-dried yeast medium at 25°, unless otherwise noted. Genetic manipulations of *hobo* transgenes were performed in stocks that lacked natural sources of the hobo transposase. In addition, these stocks lacked naturally occurring *P* elements, except where noted. When needed, heat shocks were administered to flies throughout their development and reproductive lives by placing cultures in a 37° cabinet incubator for 40 min once a day.

Mutability assays for P-transposase activity in male and female germlines: Measurements of transposase activity employed a genetic assay that detects excisions of either of two incomplete *P* elements that are responsible for a mutant allele of the *X*-linked *singed* (*sn*) gene (Engels 1979b; Roiha *et al.* 1988). This allele, called *weak singed* (sn^w), causes a mild malformation of the bristles in the adult cuticle. When one or the other of the *P* elements in sn^w is excised by transposase activity in the germline, the sn^w allele changes to either an *extreme singed* (sn^e) or a pseudo-wild (sn^+) allele, with corresponding visible changes in the bristle phenotype the next generation.

To measure transposase activity in the male germline, sn^w males carrying one or more autosomal sources of the P transposase were crossed individually to three or four *C(1)DX, y f* females. Because these females carry attached-*X* chromosomes, the *singed* allele from the father is transmitted patroclinously to his sons. These sons were classified for bristle phenotype and counted, and the proportion showing either the extreme or the pseudo-wild singed phenotypes was used as the index of transposase activity in the father's germline. When the modified *P* transgene *P(ry*$^+$*, Δ2-3)99B* (Robertson *et al.* 1988; Robertson 1996) was used as the source of the P transposase, the *C(1)DX, y f* females used in the test crosses had the genetic background of the π_2 P strain. This genetic background represses the somatic instability of sn^w induced in the offspring by *P(ry*$^+$*, Δ2-3)99B* and thereby permits these offspring to be classified unambiguously for bristle phenotype (Robertson and Engels 1989).

To measure transposase activity in the female germline, sn^w/sn^+ females carrying one or more autosomal sources of the P transposase were crossed individually to three or four *y* sn^3 *v car* males. In heterozygous combination with a sn^e derivative of sn^w, sn^3 causes an extreme singed phenotype; however, in heterozygous combination with sn^w, the phenotype is weak singed. The weak singed and extreme singed progeny from the crosses were counted and the proportion that were extreme singed was used as the index of transposase activity in the mother's germline. In these tests the phenotypically wild-type class was ignored because of the preexisting sn^+ allele in the mother's genotype.

The progeny emerging in the test crosses to measure P-transposase activity in the germlines of either males or females were counted until the 17th day after the crosses were established, unless otherwise noted. Statistical differences were evaluated by *z*-tests.

Gonadal dysgenesis assay for P-transposase activity in the female germline: To test for the induction or repression of gonadal dysgenesis, flies from the strains to be tested were mass mated at 21° for 2 days; the mated females were then separated into individual culture vials, which were reared at 29° for 12 days. The progeny of these cultures were transferred to fresh culture vials, and after 2–3 days of maturation at 21°, the females among them were examined for egg production by squashing them between two glass slides. A solution of blue dye was placed between the slides to help visualize the eggs. Females lacking eggs were considered to have gonadal dysgenesis. Statistical differences were evaluated by the Mann-Whitney rank sum test.

Molecular techniques: Standard procedures were used to extract and manipulate DNA. A *hobo* transformation vector that contained a terminally truncated but otherwise complete *P* element fused to the *hsp70* promoter was constructed by cloning the *Eco*RV/*Xba*I fragment from pCaSpeR/*hsp70/CP* (Simmons *et al.* 1996) into the polycloning region of pMartini, a plasmid derived from pBS-SK (B. Calvi, personal communication). The cloned fragment contained the *hsp70* promoter fused to the complete *P* element (abbreviated *CP*) sequence from base pair 39 to base pair 2871 in the canonical *P* sequence (O'Hare and Rubin 1983). The resulting plasmid was digested with *Not*I, which cleaves on either side of the polycloning region of pMartini, and the liberated fragment, which contained the *hsp/CP* fusion, was cloned into the unique *Not*I site of pHawN, a *hobo* transformation vector marked with the mini-*white* gene (B. Calvi, personal communication). The structure of the *P* element in the resulting plasmid, denoted p*H(hsp/CP)*, was verified by DNA sequencing. Two differences between the *P* sequence in p*H(hsp/CP)* and the canonical *P* sequence were noted: a deletion of 2864G, which lies outside the transposase coding region, and a synonymous substitution of C for 1104T in a valine codon.

Southern analysis of genomic DNA was accomplished by capillary transfer of the DNA from an agarose gel to Hybond

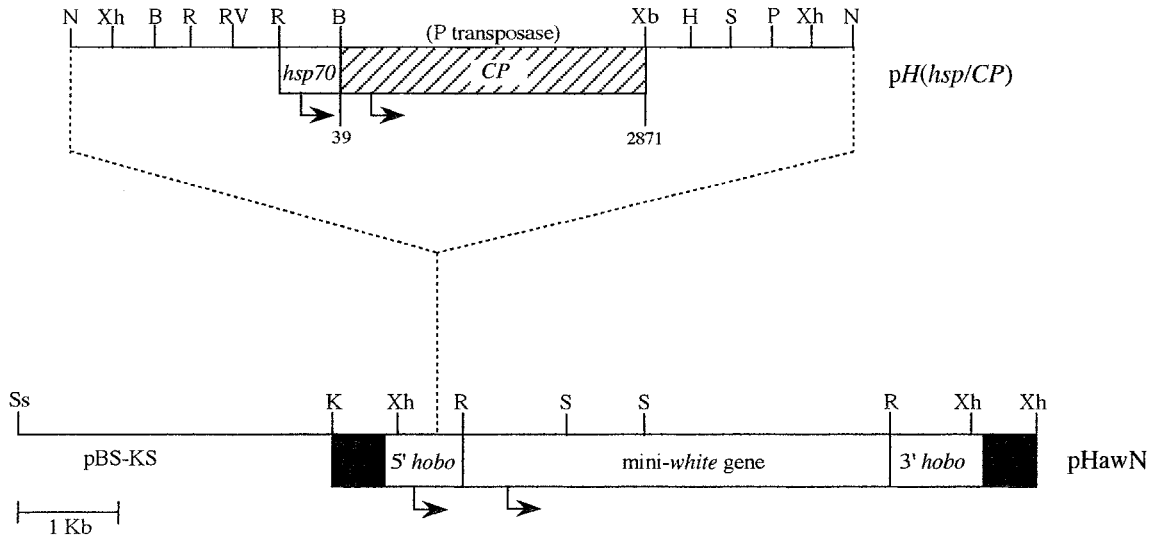

FIGURE 1.—Structure of p*H(hsp/CP)*. The plasmid was constructed by inserting the *hsp70/CP* fusion gene into the unique *Not*I site of the marked *hobo* element in the pHawN transformation vector; pBS-KS is the Bluescript backbone of this transformation vector. Bent arrows indicate the direction of transcription. Enzyme symbols: B, *Bam*HI; H, *Hin*dIII; K, *Kpn*I; N, *Not*I; P, *Pst*I; R, *Eco*RI; RV, *Eco*RV; S, *Sal*I; Ss, *Sst*I; Xb, *Xba*I; Xh, *Xho*I. The regions showing the restriction sites flanking the *hsp70/CP* fusion gene are not drawn to scale.

membranes using an alkaline transfer solution. After air drying, the membranes were hybridized with a ^{32}P-labeled DNA probe generated by randomly priming DNA synthesis from a purified *P*-element PCR product. This product was made by using primers complementary to sequences in the inverted terminal repeats to amplify genomic DNA from a strain containing a single complete *P* element. The blots were washed and exposed to X-ray film to produce autoradiograms.

Genetic transformation with p*H(hsp/CP)*: A mixture of the plasmids p*H(hsp/CP)* at 300 ng/μl and pHBL1 (CALVI and GELBART 1993) at 100 ng/μl was injected into preblastoderm embryos from a *hobo*- and *P*-element-free *y* w^{67c23} stock using standard procedures for the genetic transformation of Drosophila; pHBL1 is a "helper" plasmid that encodes the hobo transposase. Surviving embryos were reared to adulthood and mated individually to *hobo*- and *P*-element-free *w; TM3, Sb/TM6, Tb* flies; any offspring showing pigmented eyes were mated individually to *w; TM3, Sb/TM6, Tb* flies to establish stocks transformed with the *H(hsp/CP)* transposon. The chromosomal location of the transposon in these stocks was determined by analyzing segregation patterns in crosses with balancer chromosomes carrying dominant markers.

RESULTS

***H(hsp/CP)*, a *hobo* transgene that produces the *P*-element transposase in the germlines of male and female Drosophila:** The structure of the *hobo* transformation vector containing the gene for the *P*-element transposase fused to the promoter of the Drosophila *hsp70* gene is shown in Figure 1. This vector, denoted p*H(hsp/CP)*, is ~13 kb long; in addition to the *hsp70/P* transposase gene fusion, it contains the mini-*white* gene as a phenotypic marker. Both the *hsp70/P* transposase gene fusion and the mini-*white* gene in the vector are situated between the ends of an incomplete *hobo* element. In the presence of the hobo transposase, the modified 10-kb-long *hobo* transposon in p*H(hsp/CP)* can be inserted into the Drosophila genome.

One insertion of this transposon was obtained by germline transformation. This insertion was mapped to chromosome *2* and a stock homozygous for it was obtained by inbreeding. Southern blotting experiments with *Bam*HI- or *Sal*I-digested genomic DNA indicated that the stock carried a single insertion of the *H(hsp/CP)* transgene, which, because it is situated on chromosome *2*, is denoted *H(hsp/CP)2*.

The sn^w mutability assay was used to determine if the *H(hsp/CP)2* transgene could produce the P transposase in the male and female germlines. Homozygous *w* sn^w females were crossed to homozygous *y w; H(hsp/CP)2* males and their sons and daughters were crossed appropriately to detect P-transposase-catalyzed mutations of sn^w occurring in the germline. The test crosses were reared at 25° without the administration of heat shocks. The results (Table 1) show that the *H(hsp/CP)2* insertion could induce sn^w mutability in both the male and female germlines. The mutation rate, which indicates the level of transposase activity, is >50% in the male germline and >10% in the female germline. However, these rates are not directly comparable because the data for the males include the pseudo-wild derivatives of sn^w whereas those for the females do not. If the sn^+ flies are excluded from the male data, the mutation rate is 0.382, which is still more than three times the observed mutation rate in females. This difference could be due to greater expression of *H(hsp/CP)2* in the male germline or to some aspect of the mutation process itself; for example, a greater number of cell divisions during the development of the male germline could provide more opportu-

TABLE 1

Destabilization of *sn*^{w} by *H(hsp/CP)2* in male and female germlines

Sex	No. vials	sn^{w}	sn^{+}	sn^{e}	Total	Mutation rate[a]
Male	30	186	92	115	393	0.526[b]
Female	26	447	—	65	512	0.127[c]

[a] Weighted mean.
[b] Proportion sn^{+} or sn^{e} among total.
[c] Proportion sn^{e} among total.

TABLE 2

Destabilization of *sn*^{w} by *H(hsp/CP)2* and various P strains in the male germline

Strain	No. vials	sn^{w}	sn^{+}	sn^{e}	Total	Mutation rate[a]
H(hsp/CP)2	49	684	179	156	1019	0.326 ± 0.018
Harwich-w	50	633	130	147	910	0.305 ± 0.022
Nem12	50	842	49	61	952	0.108 ± 0.011
ν_6	50	1425	47	64	1536	0.076 ± 0.010
π_2	50	962	98	80	1140	0.157 ± 0.019
Still2	49	868	13	34	915	0.054 ± 0.007

[a] Unweighted mean ± SE.

nities for mutations to occur. The males that were tested in these experiments were also examined for evidence of *sn*^{w} mutability in the soma, *i.e.*, for sn^{e} or sn^{+} bristles on the body. No such evidence was found.

Additional experiments were performed to compare the *sn*^{w} mutability induced by *H(hsp/CP)2* in the male germline to that induced by different P strains. These strains included Harwich-w (Kidwell *et al.* 1977) and π_2 (Engels 1979a,b), both known to be strong inducers of *sn*^{w} mutability and gonadal dysgenesis; Nem12 and Still2 (listed as N12 and S2 in Kocur *et al.* 1986), both known to be moderate inducers of *sn*^{w} mutability and gonadal dysgenesis; and ν_6 (Engels and Preston 1981), a strain known to induce some *sn*^{w} mutability but little gonadal dysgenesis. Males from each strain were crossed to *w sn*^{w} females at 21° (to prevent gonadal dysgenesis in the offspring) and their sons were tested at 25° for *sn*^{w} mutability. The results in Table 2 show that in the male germline *H(hsp/CP)2* is a more powerful inducer of *sn*^{w} mutability than any of the P strains tested. The observed mutation rate for the *w sn*^{w}*; H(hsp/CP)2/+* flies, 0.326, is, however, less than the value of 0.526 obtained in the preliminary experiments described above. Thus, the culturing temperature of the initial cross affects the level of P-transposase activity encoded by the *H(hsp/CP)2* transgene.

Transposase activity of two insertions of *H(hsp/CP)*: A second insertion of the *H(hsp/CP)* transgene was obtained on chromosome *3*. Preliminary experiments demonstrated that this insertion, denoted *H(hsp/CP)3*, was homozygous viable and fertile and that it was capable of destabilizing the *sn*^{w} mutation in both the male and female germlines, but not in the male soma. Crosses between the homozygous *H(hsp/CP)2* and *H(hsp/CP)3* strains, followed by inbreeding, produced a strain that was homozygous for both of these insertions. Several sets of experiments were then conducted to compare the combined transposase activities of these two insertions with their separate transposase activities. In each experiment, homozygous *w sn*^{w} females were crossed with males homozygous for one or two of the insertions, and the offspring were tested for germline *sn*^{w} mutability.

Transposase activity in the male germline: Four sets of experiments were carried out to investigate the transposase activity of the *H(hsp/CP)* transgenes in the male germline (Table 3). In sets I and II, transposase activity was measured both with and without a heat shock administered to the tested flies. In sets III and IV, no heat shock was given; in addition, in set III the tested flies were reared at 21° instead of 25°.

When *H(hsp/CP)2* was tested by itself in the absence of any heat shocks, the estimated mutation rate for flies reared at 21° was 0.318, which is consistent with the previous estimate (0.326). The three estimates for flies reared at 25° were 0.498, 0.563, and 0.593, which are reasonably consistent with each other and with the preliminary estimate (0.526); the lowest of these values came from an experiment in which the flies were scored only on day 14. When *H(hsp/CP)3* was tested by itself in the absence of any heat shocks, the estimated mutation rate for the flies reared at 21° was 0.328. For the flies reared at 25°, the estimated mutation rates were 0.434, 0.497, and 0.484. These results indicate that at 25°, *H(hsp/CP)3* is slightly less effective than *H(hsp/CP)2* at inducing *sn*^{w} mutability. [By *z*-tests, two of the three comparisons between *H(hsp/CP)2* and *H(hsp/CP)3* were statistically significant at the 5% level.] At 21°, however, the two insertions appeared to be equally effective at causing *sn*^{w} mutability.

When heat shocks were administered to the tested flies, all the mutation rates were increased by small amounts. For *H(hsp/CP)2*, these rates were 0.575 (by a *z*-test significantly greater than the corresponding non-heat-shock value) and 0.605 (not significantly greater), and for *H(hsp/CP)3*, they were 0.528 (significantly greater) and 0.536 (not significantly greater). Heat-shock treatment therefore appears to enhance *H(hsp/CP)*-encoded transposase activity in the male germline, albeit slightly.

When the *H(hsp/CP)2* and *H(hsp/CP)3* insertions were combined in the same genotype, *sn*^{w} mutability was greater than when the insertions were tested by themselves in four of six comparisons, but it was significantly greater in only one of these comparisons. Furthermore,

TABLE 3

Destabilization of *sn*^{w} by *H(hsp/CP)2* and *P(ry*$^+$*, Δ2-3)* transgenes under different conditions in the male germline

Transgenes	Heat shock	No. vials	sn^w	sn$^+$	sn^e	Total	Mutation rate[a]
			Experiment I[b]				
CP2[c]	−	91	540	239	257	1036	0.498 ± 0.021
CP3[d]	−	95	731	292	243	1266	0.434 ± 0.020
CP2; CP3	−	93	476	292	295	1063	0.539 ± 0.021
CP2	+	82	424	264	270	958	0.575 ± 0.025
CP3	+	82	451	262	284	997	0.528 ± 0.023
CP2; CP3	+	87	494	285	323	1102	0.531 ± 0.027
			Experiment II				
CP2	−	48	697	460	433	1590	0.563 ± 0.015
CP3	−	49	813	425	368	1606	0.497 ± 0.015
CP2; CP3	−	49	574	521	492	1587	0.637 ± 0.017
Δ2-3[e]	−	21	165	359	208	732	0.765 ± 0.035
CP2	+	49	482	388	343	1213	0.605 ± 0.018
CP3	+	50	622	360	345	1327	0.536 ± 0.020
CP2; CP3	+	50	421	422	352	1195	0.663 ± 0.016
Δ2-3	+	25	195	372	218	785	0.748 ± 0.028
			Experiment III[f]				
CP2	−	49	1041	236	249	1526	0.318 ± 0.015
CP3	−	49	1162	256	291	1709	0.328 ± 0.015
CP2; CP3	−	50	1062	304	280	1610	0.363 ± 0.024
Δ2-3	−	50	725	747	421	1893	0.616 ± 0.021
			Experiment IV				
CP2	−	50	491	386	380	1257	0.593 ± 0.024
CP3	−	50	894	453	412	1759	0.484 ± 0.017
CP2; CP3	−	48	547	447	436	1430	0.587 ± 0.025

[a] Unweighted mean ± SE.
[b] Males were scored only on day 14; in all other experiments, males were scored on days 13 or 14 and again on day 17.
[c] *CP2 = H(hsp/CP)2.*
[d] *CP3 = H(hsp/CP)3.*
[e] *Δ2-3 = P(ry*$^+$*, Δ2-3)99B.*
[f] The males tested in this experiment were reared at 21°.

TABLE 4

Destabilization of *sn*^{w} by *H(hsp/CP)* transgenes under different conditions in the female germline

Transgenes	Heat shock	No. vials	sn^w	sn^e	Total	Mutation rate[a]
			Experiment I[b]			
CP2[c]	−	75	1336	124	1460	0.085 ± 0.010
CP3[d]	−	85	1587	124	1711	0.078 ± 0.009
CP2; CP3	−	90	1708	186	1894	0.100 ± 0.009
CP2	+	68	1233	286	1519	0.195 ± 0.013
CP3	+	72	1223	324	1547	0.219 ± 0.015
CP2; CP3	+	51	924	317	1241	0.255 ± 0.016
			Experiment II			
CP2	−	48	1529	252	1781	0.137 ± 0.013
CP3	−	49	1618	208	1826	0.110 ± 0.010
CP2; CP3	−	47	1445	295	1740	0.170 ± 0.014
CP2	+	46	1493	446	1939	0.232 ± 0.012
CP3	+	47	1540	382	1922	0.197 ± 0.011
CP2; CP3	+	35	944	410	1354	0.305 ± 0.025
			Experiment III			
CP2	−	47	1731	370	2101	0.164 ± 0.014
CP3	−	49	1879	459	2338	0.196 ± 0.014
CP2; CP3	−	47	1368	496	1864	0.249 ± 0.023

[a] Unweighted mean ± SE.
[b] Flies were scored only on day 14; in all other experiments, flies were scored on days 13 or 14 and again on day 17.
[c] *CP2 = H(hsp/CP)2.*
[d] *CP3 = H(hsp/CP)3.*

heat shocks did not increase *sn*^{w} mutability in the double-insertion flies. These results suggested that the transposase activity of the *H(hsp/CP)* transgenes is limited in the male germline.

In experimental sets II and III, the stable *P(ry*$^+$*, Δ2-3)99B* transgene was also tested for induction of *sn*^{w} mutability in the male germline. This transgene contains a modified *P* element that expresses the transposase in the soma as well as in the germline (Robertson *et al.* 1988). Homozygous *w sn*^{w} females were crossed to homozygous *P(ry*$^+$*, Δ2-3)* males and their *w sn*^{w}*; P(ry*$^+$*, Δ2-3)/+* sons, all mosaic for bristle phenotype because of the somatic activity of the *Δ2-3*-encoded transposase, were tested for germline *sn*^{w} mutability. For the flies that had been reared at 25°, the observed mutation rate was 0.765 without heat shock and 0.748 with heat shock; for the flies that had been reared at 21°, the observed mutation rate was 0.616. By *z*-tests, these rates are significantly greater than any of those seen with the *H(hsp/CP)* transgenes, even when the *H(hsp/CP)* transgenes were combined, under comparable experimental conditions. Thus, the transposase activity encoded by the *P(ry*$^+$*, Δ2-3)99B* insertion is not subject to the same limitation as that encoded by the *H(hsp/CP)* insertions.

Transposase activity in the female germline: The *H(hsp/CP)* insertions were also tested for their abilities to destabilize *sn*^{w} in the female germline (Table 4). In two sets of experiments, both heat-shocked and non-heat-shocked flies were tested; in a third set of experiments, no heat shocks were given. The results of the first two sets of experiments indicate that the heat-shock treatment increased the mutation rate by a factor of 2–3. These increases were seen in each of the tested groups within each set of experiments. By the nonparametric sign test, this pattern of results is statistically significant ($P = 0.0156$). Thus in the female germline, the transposase activity of the *H(hsp/CP)* transgenes is enhanced by heat shocks.

With or without heat shocks, the observed mutation rates of females carrying two *H(hsp/CP)* transgenes were not significantly greater than those of females carrying only one. Thus, the transposase activity of the *H(hsp/CP)* transgenes appears to be limited in the female germline as well as in the male germline.

Repression of the somatic transposase activity of *P(ry*$^+$*, Δ2-3)99B* by *H(hsp/CP)* insertions: The *P(ry*$^+$*, Δ2-*

TABLE 5

Repression of somatic transposase activity by *H(hsp/CP)* transgenes

Transgene	Transmission	Heat shock	Pigmented sons: sn^w	Pigmented sons: Mosaic sn	White sons: sn^w	White sons: Mosaic sn
CP2[a]	Maternal	−	27	19	0	36
CP3[b]	Maternal	−	1	41	0	33
CP2	Paternal	−	13	27	0	41
CP3	Paternal	−	1	37	0	60
CP2	Maternal	+	31	31	0	36
CP3	Maternal	+	0	34	0	27
CP2	Paternal	+	15	24	0	51
CP3	Paternal	+	1	49	0	80

[a] *CP2 = H(hsp/CP)2.*
[b] *CP3 = H(hsp/CP)3.*

3)99B insertion produces the P transposase in somatic tissues because the last intron in the *P*-transposase gene, which is not removed in these tissues by splicing, has been deleted by construction. A male carrying this insertion and the sn^w allele has a mosaic of weak singed, extreme singed, and wild-type bristles on its cuticle because the transposase produced by *P(ry*$^+$*, Δ2-3)* destabilizes sn^w in the bristle precursor cells during development (Laski *et al.* 1986). The *H(hsp/CP)* insertions, by contrast, cannot produce the P transposase in the somatic tissues because they possess the last intron of the *P*-transposase gene. Instead, these insertions would be expected to produce a truncated polypeptide that acts as a repressor of transposase activity (Robertson and Engels 1989; Misra and Rio 1990). To determine if the *H(hsp/CP)* insertions could produce this repressor, *w* sn^w females were crossed to *w; H(hsp/CP)* males, and then their *w* sn^w*; H(hsp/CP)/+* sons were crossed to *C(1)DX, y f; P(ry*$^+$*, Δ2-3)99B* females and their *w* sn^w*/w +*; *H(hsp/CP)/+* daughters were crossed to *w; P(ry*$^+$*, Δ2-3)99B* males. The male offspring from these "reciprocal" crosses were classifed by eye color [pigmented, *i.e.*, carrying *H(hsp/CP)*, or not pigmented, *i.e.*, not carrying *H(hsp/CP)*] and by bristle phenotype, and those with either a weak singed or a mosaic bristle phenotype were counted. In one set of experiments daily heat shocks were administered to all the crosses and in another set, they were not.

The results of these experiments (Table 5) show that *H(hsp/CP)2* repressed somatic transposase activity, although only partially, whereas *H(hsp/CP)3* did not. Even with heat shocks, *H(hsp/CP)3* had little ability to repress somatic transposase activity. Repression by *H(hsp/CP)2* was stronger when the transgene was transmitted maternally; however, administration of heat shocks to the *H(hsp/CP)2* test cultures did not seem to affect its repression ability.

TABLE 6

Regulation of *P(ry*$^+$*, Δ2-3)99B*-induced germline sn^w mutability by *H(hsp/CP)*

Transgenes	No. vials	sn^w	sn^+	sn^e	Total	Mutation rate[a]
CP2[b]	50	606	382	346	1334	0.547 ± 0.019
Δ2-3[c]	46	328	640	508	1476	0.780 ± 0.015
CP2; Δ2-3(C)[d]	48	523	460	453	1436	0.633 ± 0.019
CP2; Δ2-3(G)[d]	48	482	608	452	1542	0.671 ± 0.025

[a] Unweighted mean ± SE.
[b] *CP2 = H(hsp/CP)2.*
[c] *Δ2-3 = P(ry*$^+$*, Δ2-3)99B.*
[d] These tests were performed with two replicate stocks, C and G.

Repression of the germline transposase activity of *P(ry*$^+$*, Δ2-3)99B* by *H(hsp/CP)2*: Tests with two insertions of the *H(hsp/CP)* transgene indicated that the germline transposase activity of these transgenes is significantly less than that of the *P(ry*$^+$*, Δ2-3)99B* transgene. To determine if a *H(hsp/CP)* transgene could affect the germline transposase activity of the *P(ry*$^+$*, Δ2-3)99B* transgene, two replicate stocks homozygous for both *H(hsp/CP)2* and *P(ry*$^+$*, Δ2-3)99B* were constructed. Males from these stocks were then crossed to *w* sn^w females and their *w* sn^w*; H(hsp/CP)2/+*; *P(ry*$^+$*, Δ2-3)99B/+* sons were tested for sn^w mutability; *w* sn^w*; H(hsp/CP)2/+* and *w* sn^w*; P(ry*$^+$*, Δ2-3)99B/+* males were also tested for comparison. All the test cultures were incubated at 25° and no heat shocks were applied. From the results (Table 6), it is clear that the *H(hsp/CP)* transgene adversely affects the transposase activity of the *P(ry*$^+$*, Δ2-3)99B* transgene. With the *P(ry*$^+$*, Δ2-3)99B* transgene alone, the sn^w mutation rate was 0.780, which is consistent with previous results obtained under similar conditions (*cf.* Table 3); when the *H(hsp/CP)2* transgene was combined with *P(ry*$^+$*, Δ2-3)99B*, the sn^w mutation rate decreased significantly. Thus, the *H(hsp/CP)2* transgene actually represses the transposase activity of the *P(ry*$^+$*, Δ2-3)99B* transgene in the male germline.

Repression of *H(hsp/CP)*-encoded transposase activity by the P cytotype: The P cytotype is jointly determined by the *P* elements on the chromosomes and by maternally inherited cytoplasmic factors (Engels 1979a). Two types of analyses were performed to determine if the transposase activity encoded by the *H(hsp/CP)* transgenes could be regulated by the P cytotype. First, *H(hsp/CP)* transgenes were tested for their ability to induce gonadal dysgenesis (GD) in the daughters of P-cytotype females and, second, *H(hsp/CP)* transgenes were tested for their ability to destabilize sn^w in the grandsons of P-cytotype females.

Effect of the P cytotype on H(hsp/CP)-induced GD: Five P-cytotype strains were studied in the gonadal dysgenesis experiments. Southern analysis indicated that all five

TABLE 7

Induction and repression of gonadal dysgenesis by various strains

Strain used as ♂ or ♀	Cytotype	Induction of GD ♂ × *y w* ♀♀			Repression of GD: ♀ × Harwich-w ♂♂ (NHS)			Repression of GD: ♀ × Harwich-w ♂♂ (HS)		
		No. vials	No. flies	%GD[a]	No. vials	No. flies	%GD	No. vials	No. flies	%GD
Harwich-w	P	30	461	100 ± 0.0	30	387	7.7 ± 1.5	30	443	2.8 ± 1.3
Nem12	P	25	421	57.2 ± 3.7	20	302	0.3 ± 0.3	16	265	0.0 ± 0.0
π_2	P	30	492	100.0 ± 0.0	26	217	1.9 ± 0.9	29	498	0.3 ± 0.3
Still2	P	30	401	84.1 ± 2.6	25	277	1.2 ± 0.8	26	319	0.2 ± 0.2
ν_6	P(Q)	30	477	8.2 ± 2.0	25	404	0.7 ± 0.5	27	534	5.7 ± 2.0
Sexi.4	M′	19	181	1.0 ± 0.7	33	542	100.0 ± 0.0		Not tested	Not tested
y w	M	28	224	11.3 ± 5.0	28	443	100.0 ± 0.0	28	558	99.8 ± 0.2

NHS, no heat shock; HS, heat shock.
[a] Unweighted mean ± SE throughout table.

possessed numerous *P* elements in their genomes. Two M-cytotype strains were also included in these studies: *y w*, a true M strain that is devoid of *P* elements and Sexi.4, a pseudo-M (or M′) strain that has numerous incomplete *P* elements in its genome (Rasmusson *et al.* 1990). Table 7 presents data showing the basic characteristics of these seven strains. First, four of the five P strains were able to induce significant frequencies of gonadal dysgenesis when males from them were crossed to females from the true M strain. The only exception was ν_6, which, because of its weak ability to induce gonadal dysgenesis, has previously been classified as a special type of P strain called Q (Engels and Preston 1981). Neither the M or M′ strains induced significant gonadal dysgenesis in these crosses. Second, all five P strains (including ν_6) were effective repressors of the gonadal dysgenesis induced when Harwich-w males were crossed to M-cytotype females, either with or without heat shocks. Thus, all five strains clearly possess the P cytotype.

Table 8 shows the results of reciprocal crosses between the seven test strains and a strain homozygous for two insertions of the *H(hsp/CP)* transgene. When the test strains provided the male parent for the cross, the frequency of gonadal dysgenesis among the daughters was about the same as that seen in comparable crosses with the control M strain (*cf.* Table 7). However, there were two exceptions. When males from either the ν_6 or Sexi.4 strains were used in the crosses, the frequency of gonadal dysgenesis among the daughters was increased (30.2% for ν_6 and 14.1% for Sexi.4) over that seen in the control crosses (8.2% for ν_6 and 1.0% for Sexi.4); by the Mann-Whitney rank sum test, both increases are significant. Thus, the doubly homozygous transgene strain cannot repress the induction of gonadal dysgenesis by different P strains—*i.e.*, it has the M cytotype—and this strain actually enhances the dysgenesis-inducing potential of Q and M′ males, presumably by activating the *P* elements they transmit to the offspring.

When males from the doubly homozygous transgene stock were crossed to P, Q, M, and M′ females, either with or without heat shock, little gonadal dysgenesis was observed among the daughters except when the cross involved M′ females, in which case the GD frequency was 35.9% without heat shock and 63.9% with heat shock. These highly significant results indicate that paternally derived *H(hsp/CP)* transgenes induce gonadal dysgenesis in the offspring of M′ females and that this induction is enhanced by heat shocks. However, these transgenes do not induce gonadal dysgenesis in the offspring of true M females, which do not have *P* elements to attack, nor do they induce it in the offspring of P females, which inherit the repressive P cytotype.

Effect of the P cytotype on H(hsp/CP)-induced sn^w mutability: P-cytotype control of *H(hsp/CP)*-encoded transposase activity was also investigated by using the *sn^w* mutability assay. Harwich-w P-cytotype females were crossed to *w sn^w* males and their *w sn^w*/Harwich-w daughters were crossed to *y w* males or to males homozygous for one or two *H(hsp/CP)* transgenes. The *w sn^w* sons of these crosses, which carried zero, one, or two *H(hsp/CP)* insertions in their chromosomes, were then tested for germline *sn^w* mutability. In one set of experiments daily heat shocks were given to the flies in the last two generations; in another set they were not.

In the absence of *H(hsp/CP)* transgenes, germline *sn^w* mutability was 0.208 with heat shock and 0.222 without heat shock. Transposase-producing *P* elements derived from the Harwich-w genome evidently were able to destabilize *sn^w* in these test groups. In the presence of *H(hsp/CP)* transgenes, higher mutation rates were observed, but the increases were not statistically significant (Table 9). Moreover, these rates were not nearly so high as those seen in flies derived from crosses lacking the

TABLE 8

Effect of *H(hsp/CP)* transgenes on induction and repression of gonadal dysgenesis by various strains

Strain used as ♂ or ♀	Cytotype	♂ × *CP2; CP3*[a] ♀♀			♀ × *CP2; CP3* ♂♂ (NHS)			♀ × *CP2; CP3* ♂♂ (HS)		
		No. vials	No. flies	%GD[b]	No. vials	No. flies	%GD	No. vials	No. flies	%GD
Harwich-w	P	26	297	100 ± 0.0	30	334	0.0 ± 0.0	30	463	2.0 ± 0.8
Nem12	P	30	260	53.0 ± 5.8	18	170	0.0 ± 0.0	18	193	0.0 ± 0.0
π_2	P	30	491	100 ± 0.0	23	144	0.5 ± 0.5	30	287	0.5 ± 0.5
Still2	P	30	391	94.0 ± 1.6	15	147	0.0 ± 0.0	14	95	1.0 ± 1.0
ν_6	P(Q)	30	396	30.2 ± 4.0	30	372	0.0 ± 0.0	30	504	1.8 ± 0.9
Sexi.4	M′	20	82	14.1 ± 5.3	36	381	35.9 ± 4.6	23	206	63.9 ± 6.5
y w	M	49	555	0.6 ± 0.3	29	189	2.0 ± 1.0	30	268	1.8 ± 1.8

NHS, no heat shock; HS, heat shock.
[a] *CP2; CP3 = H(hsp/CP)2; H(hsp/CP)3.*
[b] Unweighted mean ± SE throughout table.

Harwich-w grandmaternal contribution, *i.e.*, in the absence of the P cytotype (*cf.* Table 3). Thus, the induction of sn^w mutability by the *H(hsp/CP)* transgenes is repressed by the P cytotype.

DISCUSSION

The *H(hsp/CP)* transgenes are effective producers of the P transposase in the germlines of male and female Drosophila. The transposase is produced constitutively from these transgenes, either because transcription is initiated at the *hsp70* promoter without heat-shock induction or because it is initiated at the *P*-element promoter, which is structurally intact and located downstream of the *hsp70* promoter. Constitutive expression of *P(hsp70/CP)* transgenes has been reported previously (Steller and Pirrotta 1986; Simmons *et al.* 1996). Steller and Pirrotta (1986) argued that the transcription of these transgenes is initiated at the *hsp70* promoter because a transgene in which the gene for neomycin resistance (*neo*) had been inserted between the *hsp70* promoter and the transposase gene generated much lower levels of transposase activity than a transgene without this insertion. Steller and Pirrotta (1986) hypothesized that the *neo* insertion interfered with the proper translation of RNAs that had been initiated at the *hsp70* promoter. These investigators also found that heat-shock treatments significantly increased the level of transposase activity encoded by the *P(hsp70/neo/CP)* transgene, but not that encoded by the *P(hsp70/CP)* transgene. In their experiments, transposase activity was assessed by measuring sn^w mutability in the male germline; mutability in the female germline was not studied. In our experiments, heat-shock treatments significantly increased the transposase activity encoded by the *H(hsp/CP)* transgenes in the female germline; in the male germline the heat-shock treatments had little effect.

The *H(hsp/CP)* transgenes are genetically stable as long as *hobo* elements encoding the hobo transposase are not present. In contrast, *P(hsp/CP)* transgenes are inherently unstable because they produce the transposase that catalyzes their own movement. For most genetic applications, *H(hsp/CP)* transgenes should therefore be superior to *P(hsp/CP)* transgenes. Only one genetically stable transposase-encoding *P* transgene, $P(ry^+, \Delta 2\text{-}3)99B$, is currently available. When this transgene inserted into the genome, it was altered so that it cannot be acted on effectively by the transposase (Robertson 1996). Thus, it does not excise or transpose at noticeable frequencies. The *Jumpstarter* element, another *P* transgene that encodes the P transposase (Cooley *et al.* 1988), is genetically unstable.

When two *H(hsp/CP)* transgenes are present in the genome, their combined ability to destabilize the sn^w allele is not significantly greater than their separate abilities, but it is much less than the destabilizing ability of the $P(ry^+, \Delta 2\text{-}3)99B$ transgene. These observations imply that the transposase activity of the two *H(hsp/CP)* trans-

TABLE 9

Induction of sn^w mutability by *H(hsp/CP)* transgenes in males with P-cytotype grandmothers

Transgene	Heat shock	No. vials	sn^w	sn^+	sn^e	Total	Mutation rate[a]
None	−	49	816	87	105	1008	0.222 ± 0.030
CP2[b]	−	47	699	132	102	933	0.293 ± 0.035
CP3[c]	−	47	658	161	134	953	0.343 ± 0.033
CP2; CP3	−	44	493	134	131	758	0.355 ± 0.041
None	+	50	988	107	99	1194	0.208 ± 0.026
CP2	+	43	690	101	94	885	0.239 ± 0.038
CP3	+	43	764	139	110	1013	0.266 ± 0.036
CP2; CP3	+	46	755	108	127	990	0.285 ± 0.042

[a] Unweighted mean ± SE.
[b] *CP2 = H(hsp/CP).*
[c] *CP3 = H(hsp/CP).*

genes is limited. This limitation can be explained in three ways. First, the two transgenes might compete for transcription factors to initiate RNA synthesis, from either the *hsp70* or the *P*-element promoters controlling the transposase gene. Second, the transcripts of the transgenes might compete for splicing factors that are required for correct processing of the transposase pre-mRNA, in particular, for removal of the last intron. Third, a transgene product might feed back negatively to limit transposase expression or function.

The key observation that discriminates among these hypotheses is that a *H(hsp/CP)* transgene reduces the transposase activity encoded by the *P(ry*$^+$*, Δ2-3)99B* transgene, in both the soma and the germline. This effect is difficult to explain by competition for transcription or splicing factors because (1) the *P(ry*$^+$*, Δ2-3)99B* transgene naturally produces a high level of transposase activity, (2) this activity does not depend on an *hsp70* promoter for expression, and (3) the RNA transcribed from the *P(ry*$^+$*, Δ2-3)99B* transgene is partially preprocessed. Rather, the repression of *P(ry*$^+$*, Δ2-3)99B* is better explained by a product of *H(hsp/CP)* that acts as a negative regulator of transposase expression or function. This product is unlikely to be the transposase itself. A better candidate is the 66-kD polypeptide encoded by *P*-element RNAs that retain the last intron. Such RNAs are produced naturally in somatic tissues because of a factor that inhibits this intron's splicing, and they also appear to be produced in the germline (Misra and Rio 1990; Roche *et al.* 1995).

Transgenes and plasmids designed to produce the 66-kD polypeptide repress *P*-element mobility *in vivo* (Misra and Rio 1990; Gloor *et al.* 1993; Handler *et al.* 1993); furthermore, the 66-kD polypeptide has been found in the oocytes of a P strain (Misra and Rio 1990). It has therefore been proposed that this polypeptide naturally plays an important role in *P*-element regulation in the germline and that it might be the basis for the P cytotype. According to one model (O'Hare *et al.* 1992; Roche *et al.* 1995), *P* elements are weakly transcribed in the P cytotype because the 66-kD polypeptide acts as a transcriptional repressor. When little *P*-element RNA is produced, the splicing machinery tends to leave the last intron in *P*-element transcripts, and this leads to the synthesis of more 66-kD polypeptide instead of the 87-kD transposase. An autoregulatory loop in which the 66-kD polypeptide feeds back to minimize transcription from the *P*-element promoter is thereby established.

The 66-kD polypeptide can repress transcription from the *P*-element promoter in the soma (Lemaitre and Coen 1991; Lemaitre *et al.* 1993). However, it is not clear if it does so in the germline. The fact that the transposase activity encoded by the *H(hsp/CP)* transgenes is limited suggests that the 66-kD polypeptide has a genuine autoregulatory role in the germline. It is not clear, however, if the 66-kD polypeptide is the basis of the P cytotype.

This chromosomal/cytoplasmic state represses the transposase activity encoded by the *H(hsp/CP)* transgenes. When males carrying these transgenes are crossed to M′ females, their daughters are dysgenic, especially when the cultures are treated with heat shocks. The M′ females used in these crosses came from an inbred strain that has 43 incomplete *P* elements in its genome (Rasmusson *et al.* 1990); however, none of these *P* elements has regulatory ability. The gonadal dysgenesis observed among the daughters of these crosses therefore indicates that the *H(hsp/CP)* transgenes can induce gonadal dysgenesis when they are combined with *P*-element targets for the transposase to attack. The fact that they do not do so in the daughters of P females demonstrates that the P cytotype can repress the transposase activity encoded by the *H(hsp/CP)* transgenes. Other experiments have demonstrated that the P cytoype represses *sn*^{w} mutability induced by the *H(hsp/CP)* transgenes. Thus, these transgenes could become useful genetic tools in efforts to elucidate the mechanistic nature of the P cytotype.

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