Genetic Transformation of *Drosophila melanogaster* with an Autonomous *P* Element: Phenotypic and Molecular Analyses of Long-Established Transformed Lines

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ABSTRACT

Following transformation of a *Drosophila melanogaster* true M strain with an autonomous *P* element, six lines were established and monitored for their molecular and phenotypic properties during a 4-yr period. The number of *P* elements increased with time in all the lines but the rate of increase differed among lines. Furthermore, degenerate elements arose in each of the lines during propagation. By the end of the 4th yr, the total number of elements in every line was similar to that of a very strong P strain.—At the phenotypic level, all of the transformed lines evolved high P activity, but only three developed complete or nearly complete regulatory ability. The other three lines attained only intermediate levels of regulation over the 4-yr period. One of these lines was particularly noteworthy. Although it contained as many as 55 *P* elements per genome (20 of which were potentially complete) and had extremely high P activity potential, it continued to exhibit limited regulatory ability. In addition, when females of this line were maintained at high temperatures, the ability to suppress P activity was even further diminished. A strain with this combination of molecular and phenotypic properties, in an apparently stable configuration, has not been previously described.—The results are discussed in the context of the possible role of degenerate elements in regulating *P* element expression.

QUESTIONS relating to the origin and mode of regulation of mobile genetic elements are currently of considerable interest. With respect to one type of mobile element, the *P* element of *Drosophila melanogaster*, a method has recently been devised that permits the selective introduction of naturally occurring or engineered elements into germline chromosomes (RUBIN and SPRADLING 1982). As a result, it is now possible to address more precisely some of the issues concerning the entry, spread and regulation of this class of element within a virgin genome. To this end, we have followed the development of several *P* transformed lines over the course of several years using both molecular and genetic methods. The results of this analysis form the basis of this report.

P elements constitute one of several classes of mobile elements that are found in *D. melanogaster* (RUBIN 1983). The *P* family is particularly noteworthy because of its association with a constellation of germline abnormalities, collectively known as P-M hybrid dysgenesis (KIDWELL, KIDWELL and SVED 1977) (for more recent reviews see BREGLIANO and KIDWELL [1983]; ENGELS [1983]; O'HARE [1985]; SIMMONS and KARESS [1985]; KIDWELL [1986]). The characteristic features of the syndrome include temperature-dependent sterility, male recombination, chromosomal aberrations, segregation distortion and elevated rates of mutation, nondisjunction and female recombination. Manifestations of P-M dysgenesis arise in the germline of progeny produced from matings between males of a paternally contributing type (P strain) and females of a maternally contributing type (M strain). Appreciable levels of hybrid dysgenesis do not usually occur in progeny from the reciprocal cross or from P × P or M × M crosses. The nonreciprocal nature of this effect implicates specific cytoplasmic and chromosomal interactions as being the underlying cause of the hybrid dysgenesis phenomenon.

Members of the *P* element family exhibit a high degree of sequence homology but are diverse with respect to size. Based on structure, *P* elements can be divided into two basic types, either complete or degenerate. The complete (autonomous) element, sometimes referred to as the *P* factor, is 2.9 kb in length and contains four open reading frames, all of which are used to encode the transposase peptide (KARESS and RUBIN 1984; LASKI, RIO and RUBIN 1986). The smaller, degenerate (nonautonomous) elements are heterogeneous in size and are derived from complete elements by internal deletions. Such deletions occur at high frequencies under dysgenic conditions (VOELKER *et al.* 1984; DANIELS *et al.* 1985). All *P* elements that have been sequenced to date have been shown to possess terminal 31-bp repeats (O'HARE and

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RUBIN 1983). These sequences are thought to contain the recognition sequences for the transposase enzyme. Autonomous *P* elements are capable of catalyzing their own transposition and can also act *in trans* to promote the transposition of degenerate elements (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1982).

D. melanogaster P strains contain multiple *P* element copies (BINGHAM, KIDWELL and RUBIN 1982). However, the proportion of these that are complete can vary from one strain to another (TODO *et al.* 1984; I. BOUSSY, personal communication). All *P* strains manifest a condition known as the P cytotype, in addition to their ability to promote dysgenesis in crosses to M strains. M strains, on the other hand, manifest the M cytotype. On the molecular level, M strains can be classified as either true M or pseudo M (M′). The former completely lack *P* elements while the latter contain *P* elements, although most or all are presumed to be degenerate (KIDWELL 1985; I. BOUSSY, personal communication). The term "cytotype" refers to certain cellular properties inherited by the zygote through the maternally derived cytoplasm (ENGELS 1979; ENGELS and PRESTON 1979). P and M cytotypes are defined on essentially phenomenological grounds since the precise nature of the constituent cellular components remains unknown.

At present the available evidence suggests that the underlying cellular events leading to the dysgenic response are associated with *P* element movements, which include both excisions and transpositions. Such events occur at high frequencies when autonomous *P* elements are introduced into the M cytotype, the susceptible cellular state that permits their expression. In the P cytotype, however, autonomous elements are essentially quiescent because of immunity conferred by this cellular state. Their repression greatly reduces, but does not necessarily abolish, mobilizations within P strains or in crosses between such strains (O'HARE and RUBIN 1983; ENGELS 1984; PRESTON and ENGELS 1984).

Every P strain thus far described has been either a wild-derived isolate or a genetically manipulated derivative of such a strain. These strains may contain as many as 50 or more *P* elements per genome, a high proportion of which usually consists of a diverse array of degenerate elements. Because of this complexity, the molecular properties of naturally occurring P strains and their derivatives are often difficult to characterize. Consequently, this type of strain does not represent ideal material for studies attempting to identify the various components responsible for the characteristic features of the P-M system (*e.g.*, the repression of transposition and the establishment and maintenance of the P cytotype). However, with the advent of an efficient method to transduce natural *P* elements or their engineered derivatives into the *Drosophila* genome (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1982), lines can now be created that initially have well-defined numbers and types of elements. Recently such lines have been successfully employed to identify the major somatic cell and germline *P* element transcripts and their protein products (KARESS and RUBIN 1984; LASKI, RIO and RUBIN 1986; RIO, LASKI and RUBIN 1986).

Using the transformation approach, it should be possible to gain some insight into the regulation of *P* element transposition and the development of the P cytotype. Currently there are two main hypotheses concerning the regulation of transposition, the "two component" model proposed by O'HARE and RUBIN (1983) and the "titration" model proposed by SIMMONS and BUCHOLZ (1985). Although the two models differ mechanistically, both postulate that autonomous elements are essential components in the regulation of transposition. If this is so, then, under the simplest assumptions, M strains that have been transformed with autonomous elements should eventually develop the P cytotype, as has been suggested by O'HARE (1985).

As a first step in addressing this issue, we have generated several lines transformed with an autonomous *P* element from a wild-derived strain. Our aim was to determine whether these lines would eventually manifest the features characteristic of naturally occurring P strains (*i.e.*, P activity potential, formation of degenerate elements, control of transposition and establishment of the P cytotype). Although some of our transformed lines have, in fact, evolved into *bona fide* P strains, others exhibit properties that have not been described previously in wild-derived material.

THE EXPERIMENTAL SYSTEM

The manifestations of P-M hybrid dysgenesis have been correlated with the mobilization of *P* elements in germline cells. Of the many dysgenic traits that have been described (KIDWELL, KIDWELL and SVED 1977; ENGELS 1983), gonadal dysgenesis (GD sterility) is one of the few that is suitable for efficient routine assays of P activity. The condition is characterized by the failure of one or both gonads to develop beyond an early stage, is highly temperature sensitive and is easily scored by dissecting adult flies.

Unknown strains may be characterized in the P-M system by making several reference crosses at 29° to standard tester strains and scoring for GD sterility in the F_1 progeny (*e.g.*, KIDWELL, FRYDRYK and NOVY 1983). Cross A is used to determine the level of activity of a strain's functional *P* elements when they are in a derepressed state (*i.e.*, P activity potential); males of the strain in question are crossed to females of a true M strain, such as Canton-S. Cross A* is used

to determine the ability of a strain to regulate P activity; females of the strain in question are crossed to males of a known strong P strain, such as Harwich. Cross B, which is the reciprocal of cross A, and the intrastrain mating, in which flies from the unknown strain are mated *inter se*, also serve as indices of a strain's ability to suppress *P* element expression. Cross B*, which is the reciprocal of cross A*, is a control for genetic factors other than *P* that might cause a condition similar to GD sterility.

Operationally, strains are characterized according to the following criteria (KIDWELL, FRYDRYK and NOVY 1983). A strain is classified as P if it produces greater than 10% sterility in the cross A progeny and less than 10% sterility in the cross A* progeny. A strain is said to be neutral or Q if it produces less than 10% sterility in both crosses. A strain is M if it produces less than 10% sterility in cross A but greater than 10% sterility in cross A*. In the latter cross, true M strains usually exhibit nearly 100% sterility while pseudo M strains usually show varying degrees of sterility, ranging from 10% to 100%.

MATERIALS AND METHODS

Drosophila strains: ry^{506} is a true M strain, homozygous for a deletion that removes a portion of the *ry* coding element. This strain was used as the injection recipient.

Canton-S is a long-established laboratory strain that is completely devoid of *P* element sequences as determined by Southern blot analysis (classified as true M in the P-M classification system). It was used as the standard M strain in our phenotypic tests.

Harwich-77 is an inbred, strong P strain obtained in 1977 as a subculture from the original Harwich line (KIDWELL and NOVY 1979). It was used as the standard P strain in our phenotypic tests. See text for details concerning the molecular characterization of the *P* element composition of this strain.

Agana is an inbred line collected at Agana, Guam, in the 1960s. It is classified as a moderate P strain on the basis of standard phenotypic tests.

Chepachet 74i is an inbred, weak P strain collected in Chepachet, Rhode Island, in 1974.

w^{1118};*P*[*hspP*;w^-] is a transformed line, provided by F. LASKI and G. RUBIN, that contains a derivative of the *P* element transposon, *P*[*hspP*;w^+] (LASKI, RIO and RUBIN 1986), in a white (w^-) background. The *P*[*hspP*;w^+] transposon carries approximately 10 kb of w^+ DNA, which confers a wild-type eye color phenotype, and a copy of an engineered *P* element that has the heat shock protein 70 promoter fused to the coding sequences of an autonomous element. The derivative *P*[*hspP*;w^-] transposon arose as a white-eyed revertant and is the result of a deletion that has excised some, or all, of the w^+ DNA from *P*[*hspP*;w^+].

Plasmids: pπ25.1 contains an intact 2.9-kb *P* element flanked by genomic sequences from polytene chromosome region 17C. The genomic fragment, obtained from the *D. melanogaster* P strain, π_2 (ENGELS and PRESON 1979), was cloned into the *Bam*HI site of pBR322. A restriction map of pπ25.1 is shown in Figure 1; for further details see SPRADLING and RUBIN (1982) and O'HARE and RUBIN (1983). The *P* element borne by pπ25.1 has been previously shown to be capable of catalyzing its own transposition (SPRADLING and RUBIN 1982) as well as the transposition of other, nonautonomous elements (RUBIN and SPRADLING 1982). The *Xho*I/*Sal*I, *Acc*I/*Acc*I and *Sst*I/*Sal*I *P* element fragments were used as DNA probes in some of our experiments.

pπ25.7 BWC ("both wings clipped") is a derivative of the pπ25.7 WC ("wings clipped") plasmid (KARESS and RUBIN 1984), that was, in turn, derived from pπ25.7 (SPRADLING and RUBIN 1982). (The pπ25.1 and pπ25.7 plasmids differ only in the orientation of the passenger DNA relative to the pBR322 sequences.) The pπ25.7 BWC plasmid, constructed by K. O'HARE, contains a *P* element that lacks 39 bp from its left end and 23 bp from its right end. All the genomic sequences that flank the complete element in the original pπ25.7 plasmid, as well as some of the pBR322 sequences, have been removed in the process of making BWC.

Transformation: Plasmid DNA was introduced into the posterior cytoplasm of syncytial, pre-pole cell embryos by the microinjection technique described by SPRADLING and RUBIN (1982).

Culturing of transformants: All transformants were generated during June and July of 1982, and a separate line was established from each. Details as to how transformants were initially selected are supplied in the text. For the first 9 months, transformed lines were cultured on a standard cornmeal medium in half-pint bottles; thereafter, they were maintained in duplicate vials. Throughout the course of this study, lines were periodically expanded into half-pint bottles for analysis. Cultures were maintained at room temperature (23–25°).

GD sterility tests: The phenotypic characteristics of each transformed line were determined by making the appropriate test crosses (see THE EXPERIMENTAL SYSTEM section for details). For each cross, 15–20 pairs of flies were mated *en masse* in half-pint bottles and immediately placed at 29°. In some instances, a second brood was established by turning parents onto new food 3 days later. Approximately 2 days following the onset of eclosion, F_1 progeny were collected and allowed to mature for 2–3 days at room temperature. Individuals were then taken at random for dissection. Dissected gonads were scored as either normal or dysgenic (either unilateral or bilateral) and the frequency of gonadal sterility was calculated by dividing the number of dysgenic gonads by the total number of gonads scored. In some experiments, only female GD sterility was measured; in others, the frequency of GD sterility for both males and females was calculated.

The regulatory potential of single females from the 88-15-1 transformed line was measured by placing each in a vial with two Harwich males (cross A*) at 29°. Two broods were established. Progeny were harvested and aged as described above. The frequency of GD sterility for each female was determined by dissecting and scoring the ovaries of all F_1 daughters. Only females that produced 10 or more daughters in both broods were used to form the data base.

Southern blot analysis: The method for extraction of genomic DNA from adult flies is described in detail elsewhere (DANIELS and STRAUSBAUGH 1986). Procedures for restriction enzyme digestion, agarose gel electrophoresis, gel blotting, preparation of nick-translated probes and filter hybridization are described by RUSHLOW, BENDER and CHOVNICK (1984).

***In situ* hybridization:** Polytene chromosome spreads from larval salivary glands were prepared as previously described (DANIELS *et al.* 1985). Nick-translated, ^{3}H-labeled pπ25.1 plasmid DNA was used as the probe.

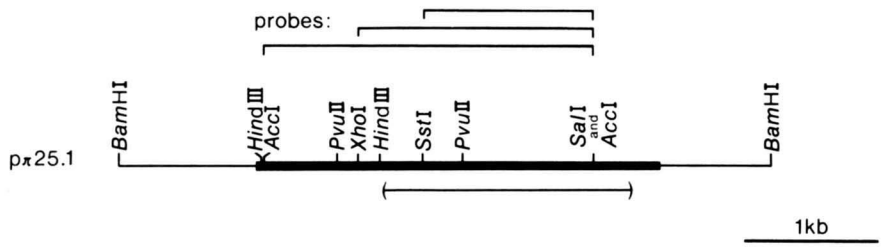

FIGURE 1. Restriction map of pπ25.1. This plasmid contains an autonomous *P* element (thick bar) flanked by genomic sequences (thin bars) from chromosome region 17C. The genomic fragment was cloned into the *Bam*HI site of pBR322 (not shown). For further details see SPRADLING and RUBIN (1982) and O'HARE and RUBIN (1983). The degenerate element in plasmid p6.1 (RUBIN and SPRADLING 1982), which was used as the vector in the construction of the ry^+ transposon, was derived from an intact element by the deletion indicated below the map. The *Xho*I/*Sal*I, *Sst*I/*Sal*I and *Acc*I/*Acc*I fragments were used as probes during this study.

RESULTS

Generation of *P* element transformants: *P* element transformants arose from our early efforts to produce rosy locus transformants via the original *P* element gene transfer technique described by RUBIN and SPRADLING (1982). In these experiments, plasmid DNA containing a ry^+-bearing *P* element transposon was injected into true M, homozygous ry^{506} (brownish eye color) embryos along with pπ25.1 plasmid DNA (see MATERIALS AND METHODS). The pπ25.1 plasmid (Figure 1) contains an autonomous *P* element that provides the *trans*-acting transposase needed for the germline integration of the ry^+ transposon, which is otherwise incapable of catalyzing its own transposition. Flies transformed with the ry^+ transposon were selected on the basis of their wild-type (red) eye color phenotype.

From the initial experiments, 20 ry^+ transformed lines were established, 14 of which were completely stable with respect to their ry^+ phenotype. These stable lines formed the basis of a recent report describing some of the underlying causes of gene expression differences in rosy locus transformants (DANIELS *et al.* 1986). Six of the original 20 transformants, however, displayed signs of instability during propagation or upon outcrossing to laboratory strains (*i.e.*, excision and/or further transposition of the ry^+ transposon). This instability was subsequently shown to be correlated with the presence of complete *P* elements that must have integrated into the genome coincidentally with the ry^+ transposon at the time of embryo injection. These cotransformed lines, which were generated during June and July of 1982, provided the opportunity to analyze the behavior of autonomous elements in a previously *P*-element-free, *D. melanogaster* genome.

***P* elements increase in number in P transformed lines:** Genomic DNA samples from the six P transformed lines were examined at two points in time. The first samples were obtained 4–6 months (Figure 2A) and the second samples 32 months (Figure 2B) following establishment of the lines. DNA was digested with *Pvu*II and probed with the *Xho*I/*Sal*I fragment from pπ25.1 (Figure 1). With this combination, each intact *P* element yields an internally derived, 0.9-kb fragment and a unique fragment of variable size that contains the right portion of the element and its flanking genomic DNA (see Figure 1). The variable fragment is characteristic of a particular element at a given genomic location. The *Xho*I/*Sal*I probe also shares a small region of homology (~170 bases) with the *P* sequences in the ry^+ transposon.

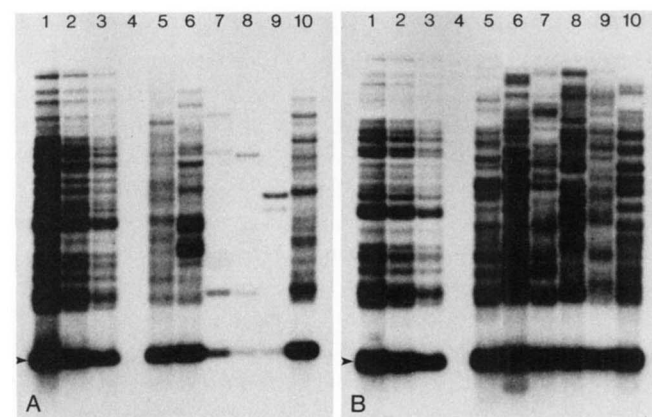

FIGURE 2.—Whole genome Southern blot analysis of the six *P*-transformed lines at two points in time. *A* and *B*, Genomic DNA was prepared by *en masse* extraction of approximately 125 flies. Samples were digested with *Pvu*II and probed with the *Xho*I/*Sal*I fragment from pπ25.1. Lanes 1–3 contain, respectively, 4.0, 2.0, and 1.0 μg of DNA from Harwich-77. Lanes 4–10 contain approximately 4.0 μg of DNA from the injection recipient strain and the six transformed lines as follows: (4) ry^{506}; (5) 88-4-1; (6) 88-15-1; (7) 88-48-4; (8) 88-63-1; (9) 88-72-2; and (10) 88-283-3a. The transformant DNA in (A) was obtained 4–6 months and the DNA in (B) 32 months following the creation of the lines. In every case, the number of *P* elements has increased with time. Arrows indicate the internally derived, 0.9-kb *Pvu*II fragment.

Several months following their establishment, three of six transformed lines contained numerous *P* element sites, while the other three had very few (Figure 2A). The number of complete elements in one line, 88-72-2, was monitored for over a year. For nearly 12 months, this line maintained only a single intact *P* element; thereafter, the number of elements increased rapidly. By month 32, the number of potentially com-

plete elements in all six transformed lines was similar to the number in Harwich-77, judging from the relative intensities of the internally derived, 0.9-kb fragment (Figure 2B), although it should be noted that certain degenerate elements can also produce this fragment.

Degenerate elements arise in lines transformed with autonomous *P* elements: It has been demonstrated that degenerate *P* elements arise from complete elements by the deletion of internal sequences (O'HARE and RUBIN 1983). Such partial excision events occur at high frequencies in the dysgenic state (VOELKER *et al.* 1984). To determine whether degenerate elements have formed in the *P*-transformed lines, genomic DNA was digested with *Sst*I and *Sal*I and probed with the *Sst*I/*Sal*I fragment from pπ25.1 (Figure 1). If only complete elements are present, then a single 1.3-kb fragment will hybridize to the probe. The appearance of other fragments indicates that sequences spanning either the *Sst*I or the *Sal*I site, or sequences between the two sites, have been disrupted or deleted in some elements. Furthermore, the *Sst*I/*Sal*I probe does not hybridize to the *P* element sequences of the ry^+ transposon, which would otherwise appear as a degenerate element and confound the analysis (Figure 1). It should be noted that degenerate elements with deletions that span both the *Sst*I and *Sal*I sites will not be detected.

The results of this analysis, which was again performed at the two points in time, are shown in Figure 3. The DNA samples are from the same preparations as those in Figure 2, except that the 88-63-1 sample is missing in Figure 3A. Four observations are noteworthy: (1) by the 6th month, degenerate elements have arisen in at least two of the lines, 88-4-1 and 88-15-1 (Figure 3A, lanes 2 and 3, respectively); (2) by month 32, all transformed lines contained degenerate elements (Figure 3B); (3) the number of degenerate elements in one line 88-63-1 (Figure 3B, lane 5) far exceeds the number of such elements in the other lines; and (4) between months 6 and 32, there was an overall increase in the number of degenerate elements in all but the 88-4-1 line.

Do lines transformed with autonomous *P* elements behave as bona fide P strains? An extensive phenotypic analysis of the six *P*-transformed lines was begun at month 34. At this time, tests to assess P activity and its regulation were initiated by making the appropriate crosses and scoring GD sterility in the F_1 progeny (see MATERIALS AND METHODS for details of the regime employed). Each line was tested monthly over the 4-month period, May through August 1985 (experiments A through D), and again approximately 1 yr later at month 48 (experiment E). In experiments A through D, frequencies of GD sterility were determined by scoring 50 F_1 individuals; in experiment E,

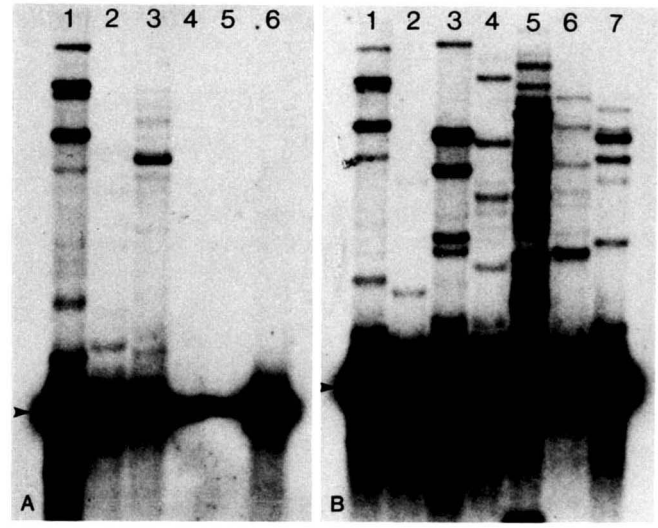

FIGURE 3.—Demonstration that degenerate *P* elements arise in lines transformed with complete elements. *A* and *B*, Genomic DNA samples, from the same preparations as those in Figure 2 were digested with *Sst*I and *Sal*I and probed with the *Sst*I/*Sal*I fragment from pπ25.1. *A*, Analysis of DNA obtained from transformed lines 4–6 months following their creation. Samples are as follows: (1) Harwich-77; (2) 88-4-1; (3) 88-15-1; (4) 88-48-4; (5) 88-72-2; and (6) 88-283-3a. (The 88-63-1 line was not included.) *B*, Analysis of DNA at month 32. Samples are as follows: (1) Harwich-77; (2) 88-4-1; (3) 88-15-1; (4) 88-48-4; (5) 88-63-1; (6) 88-72-2; and (7) 88-283-3a. In both (*A*) and (*B*), the appearance of fragments other than the 1.3-kb fragment (arrows) indicates the presence of degenerate elements.

frequencies were determined by scoring 100 individuals. The results of the phenotypic tests are shown in Table 1. The frequency of GD sterility in F_1 females from crosses A, B and A* was measured in all five experiments; in experiments B, C, D and E, the intrastrain mating was also performed. The frequency of GD sterility in F_1 males from all four tests was measured in experiments C, D and E. Cross B* was performed only in experiment E. In the last experiment, phenotypic tests were also performed on the wild-derived P strains, Agana and Chepachet 74i; the results of these tests are included in Table 1 for comparison. Control crosses with the Harwich-77 and Canton-S tester strains were performed throughout the course of the analysis. The points to be made from the data in Table 1 are as follows:

1. In cross A, all six transformed lines exhibited high levels of P activity, approaching or equaling that of Harwich-77, a very strong P strain. In several cases, the level of cross A sterility differed between the sexes. Although three of the lines (88-4-1, 88-63-1 and 88-72-2) produced nearly equivalent levels of F_1 sterility in females and males, two lines (88-15-1 and 88-283-3a) consistently yielded lower sterility in males than in females. This has been observed in other studies (KIDWELL, KIDWELL and SVED 1977; ENGELS and PRESTON 1979) and may be a characteristic feature of the P-M system. ENGELS and PRESTON (1979) have

TABLE 1

Percent gonadal sterility in the F_1 progeny of crosses to determine the P-M phenotypic characteristics of the *P* transformed lines

		Cross									
		A CS[b]♀ × U♂		B U♀ × CS[b]♂		A* U♀ × H♂		B* H♀ × U♂		Intrastrain U♀ × U♂	
Line (U)	Experiment[a]	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
88-4-1	A	95		0		1					
	B	100		0		8				0	
	C	100	100	0	0	1	0			0	1
	D	99	96	0	2	0	0			0	3
	E	95	99	0	1	3	2	0	0	0	2
88-15-1	A	100		33		63					
	B	100		50		71				43	
	C	100	86	46	38	35	22			66	44
	D	100	77	40	18	65	36			70	30
	E	100	84	38	19	60	46	0	0	51	15
88-48-4	A	80		9		4					
	B	80		2		9				4	
	C	79	93	2	4	6	4			15	3
	D	84	99	1	3	0	0			15	4
	E	82	96	0	0	0	1	0	0	11	5
88-63-1	A	98		21		4					
	B	97		8		9				5	
	C	96	91	5	4	1	4			4	9
	D	98	100	2	13	0	0			2	14
	E	97	98	0	2	0	1	0	0	1	6
88-72-2	A	91		1		18					
	B	92		5		29				18	
	C	95	93	4	16	27	13			7	9
	D	91	94	3	10	15	19			32	7
	E	97	100	0	13	13	16	0	2	7	19
88-283-3a	A	97		4		6					
	B	91		2		16				1	
	C	97	86	3	25	14	11			2	9
	D	96	65	9	16	10	3			2	15
	E	97	92	1	16	11	8	0	1	2	14
Harwich-77	A	100		ND[c]							
	B	100		1						1	
	C	100	100	0	0					2	0
	D	100	100	0	0					2	1
	E	100	100	ND	0					2	2
Agana[d]	E	58		2	2	3	2	0	0	0	0
Chepachet 741[d]	E	20		0	0	0	0	2	2	3	0

[a] Experiments A–D were conducted over the 4-month period May–August 1985 and experiment E in June of 1986.
[b] The frequency of undeveloped gonads in Canton S intrastrain crosses ranges from 0 to 2% in females and 1 to 4% in males.
[c] ND = not determined.
[d] See MATERIALS AND METHODS for a description of this P strain.

suggested that the different developmental programs of the male and female gonads during the temperature sensitive period may account for the frequently higher female sterility. However, one transformed line, 88-48-4, consistently produced higher cross A sterility in males than in females.

2. The cross A*, cross B and intrastrain results reveal that three of the transformed lines (88-4-1, 88-48-4 and 88-63-1) have achieved complete or nearly complete regulation, while the remaining three lines (88-15-1, 88-72-2 and 88-283-3a) showed varying degrees of susceptibility to P activity, with the 88-15-1 line being the most susceptible to the sterilizing action of *P* factors.

3. As mentioned earlier, cross B* serves as a control for genetic factors other than *P* that might cause a type of sterility similar to that produced by P-M dysgenesis. The cross B* results for the transformed lines indicated that the sterility observed in the phenotypic test crosses was caused by the expression of *P* elements and not by some other genetic factor.

4. The phenotypic properties of all six transformed lines remained essentially unchanged over the 14 month test period.

TABLE 2

Comparison of GD sterility in F_1 progeny from consecutive broods of crosses A, B and A* from experiment C[a]

		Cross					
		A		B		A*	
Line	Brood	♀	♂	♀	♂	♀	♂
88-4-1	1	100	100	0	0	1	0
	2	100	100	10	3	12	0
88-15-1	1	100	86	46	38	35	22
	2	100	82	92	69	90	73
88-48-4	1	79	93	2	4	6	4
	2	79	92	4	4	ND[b]	ND
88-63-1	1	96	91	5	4	1	4
	2	98	94	2	10	3	5
88-72-2	1	97	93	3	16	14	13
	2	89	94	5	31	19	12
88-283-3a	1	97	86	3	25	14	11
	2	97	99	14	22	12	13

[a] Experiment C was conducted in July of 1985 at month 36.
[b] ND = not determined.

The 88-15-1 transformed line exhibits unusual properties: At one point during the phenotypic analysis of the transformed lines, we compared the frequency of GD sterility in broods 1 and 2 of crosses A, B and A* in order to determine whether there were any differences between consecutive broods established 3 days apart. Sterility frequencies were calculated by scoring 50 F_1 individuals. The results of this analysis, which was performed as part of experiment C (at month 36), are presented in Table 2. In all cases, little change was noted in the GD sterility frequencies between broods 1 and 2 of cross A. However, with respect to crosses B and A*, one line, 88-15-1, displayed dramatic differences in both male and female sterility between the two broods.

A possible explanation for the 88-15-1 test result is that this line may represent a heterogeneous population with two distinct subpopulations, one with high and the other with low regulatory potential. Such a population would be expected to exhibit an intermediate level of regulation when tested by mass mating in cross A*. Moreover, a bimodal distribution in regulatory potential in the 88-15-1 population might explain the dramatic differences in GD sterility between broods.

In order to resolve this issue, we have examined the regulatory potential of single females from 88-15-1 in consecutive broods of cross A* (see MATERIALS AND METHODS). The results of two such experiments, which were performed at months 41 and 42, are shown in Figure 4. Each rectangle represents the percent GD sterility in the daughters of a single female. Two days and 4 days, respectively, separate the broods in A and B of Figure 4. In both experiments, there was a dramatic shift toward more sterility in

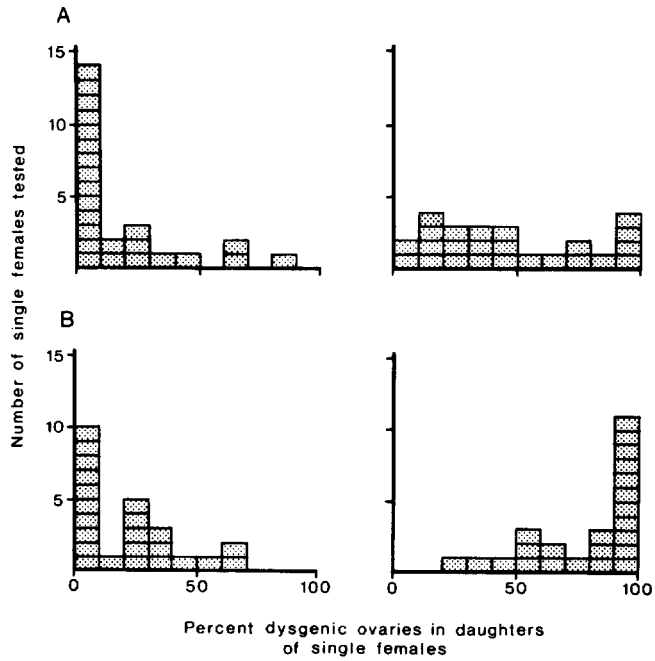

FIGURE 4.—Analysis of the regulatory potential of individual females from 88-15-1 in consecutive broods of cross A*. *A* and *B*, Each rectangle represents the percent sterility in the daughters of a single female. The distributions on the left represent the brood 1 results, those on the right the brood 2 results. *A*, Two days separate broods 1 and 2; 24 females tested. *B*, Four days separate broods 1 and 2; 23 females tested. In both (*A*) and (*B*), there is a dramatic shift toward higher sterility in brood 2. The mean number of daughters scored per single female computed over both experiments was 34.2 in brood 1 and 20.2 in brood 2.

brood 2. When the single fly data were pooled within each brood, the frequency of sterility in broods 1 and 2 of the experiment shown in Figure 4A was, respectively, 16 and 41%, with 22 of 24 females tested showing a shift toward greater sterility in brood 2; in the experiment shown in Figure 4B, the frequency of sterility in broods 1 and 2 was, respectively, 22 and 76%, with all 23 females tested exhibiting higher sterility in brood 2 than in brood 1. It is also apparent from the data in these experiments that most individuals within the 88-15-1 population were capable of producing both dysgenic and nondysgenic progeny and therefore displayed a truly intermediate type of regulatory potential.

The increase in sterility in brood 2 of cross A* suggests that either parental age or the duration of time at 29° has an influence on the regulatory capacity of the 88-15-1 line. In order to distinguish between these two possibilities, the following experiment was performed at month 43. Virgin females from 88-15-1 were collected and aged for different periods of time at either 23° or 29°. Single females were then mated to Harwich-77 males as described in the MATERIALS AND METHODS. A second brood was established 2 days later. The results of this experiment are shown in Figure 5. Each rectangle represents the frequency of cross A* sterility in the daughters of a

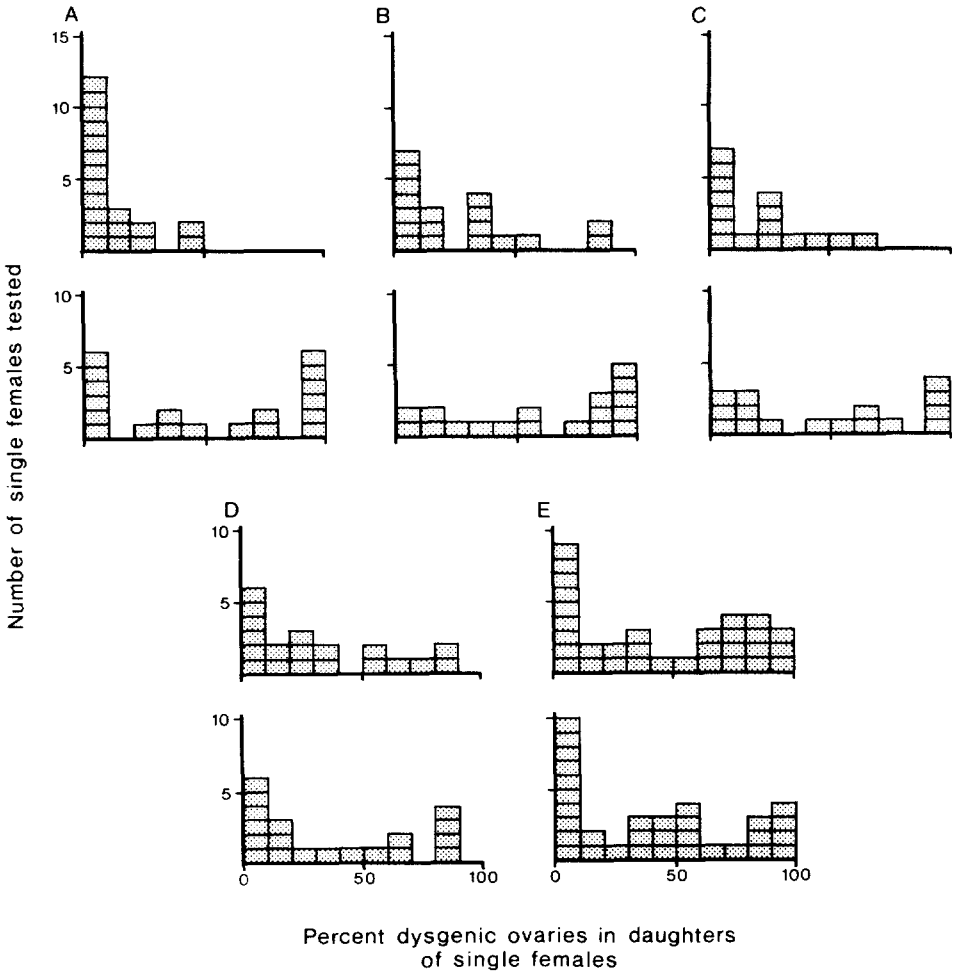

FIGURE 5. Cross A* analysis of individual 88-15-1 females aged for different lengths of time at 23° or 29°. *A–E*, Each rectangle represents the percent GD sterility in the daughters of a single female. In each case, the upper distribution represents the brood 1 results, the lower one the brood 2 results. Females were aged before mating as follows: (*A*) 2 days at 23°; (*B*) 4 days at 23°; (*C*) 6 days at 23°; (*D*) 2 days at 29°; and (*E*) 4 days at 29°. In (*A–C*), there is a shift toward more sterility in brood 2; in (*D*) and (*E*) there is little change in the sterility distributions between broods. The mean number of daughters scored per single female computed over all five experiments was 24.5 in brood 1 and 19.7 in brood 2.

single female. In A, B and C of Figure 5, females were aged, respectively, 2, 4, and 6 days at 23° before being placed together with males at 29°. In D and E of Figure 5, females were aged, respectively, 2 and 4 days at 29° before mating. In those experimental groups in which females were aged at 23°, there was a shift toward increased dysgenesis in brood 2, irrespective of maternal age (Figure 5, A–C); however, when females were aged for a time at 29° before mating, there was very little change in the frequency of sterility between broods (Figure 5, D and E). It appears, then, that maintenance temperature has a pronounced effect on the regulatory capacity of 88-15-1 individuals, although maternal age may also have a slight effect (compare brood 1 in Figure 5A with brood 1 of Figure 5, B and C).

A comparison of the distributions in Figure 4 (months 41 and 42) with those in Figure 5 (month 48) reveals a higher proportion of individuals with the P cytotype (*i.e.*, those that exhibit less than 10% cross A* sterility) within the 88-15-1 population in the latter experiment (see especially Figure 5E). This may indicate a growing capacity of this line to produce individuals with nearly complete resistance to P activity. If this is so, then the progressive shift toward the P cytotype is occurring at a much slower rate than has been observed in experiments in which *P* elements have been introduced into M genomes by chromosomal contamination (M. KIDWELL, unpublished results).

Molecular characterization of two phenotypically distinguishable *P*-transformed lines: The two transformed lines that differed the most at the phenotypic level, 88-4-1 and 88-15-1, were subjected to a molecular analysis at month 44 in an attempt to determine whether there were differences in the *P* element complements of these two lines. Our analysis consisted of estimating the total number of elements in each line and calculating the proportion of complete and degenerate elements. However, because of the complex and heterogeneous nature of the *P* element profiles, only very rough estimates were possible in most cases.

As a first step in the analysis, we partially characterized the *P* element constitution of the highly inbred Harwich-77 strain so that it could be used as a standard of comparison. This strain exhibits very high P activity potential and its individual members show little variation in their *P* element profiles. This homogeneity was demonstrated by the highly uniform *P* element hybridization patterns of DNA samples from

small groups of flies (1–20 flies per group; data not shown). An estimate of the total number of elements within Harwich-77 individuals was obtained by *in situ* hybridization of pπ25.1 DNA to polytene chromosomes from larval salivary glands. In this analysis, 60 to 65 sites of hybridization were detected, although not all sites were present in every nucleus. Since *P* elements vary with respect to size (RUBIN, KIDWELL and BINGHAM 1982; O'HARE and RUBIN 1983), the sites of sporadic hybridization probably represent the location of smaller elements that have less affinity to the probe than do larger ones. Our estimate of 60 to 65 elements per genome for Harwich-77 is slightly higher than the estimate of 30 to 50 elements per genome previously reported for most P strains (BINGHAM, KIDWELL and RUBIN 1982). (Genome is used here in the strict sense of the word to mean the haploid genetic complement.) Although it is not possible to determine with certainty whether an element at a particular site is present in a homozygous or hemizygous configuration by *in situ* analysis, if one assumes essentially total homozygosity in the highly inbred Harwich-77 strain, then the genotype may contain as many as 130 elements. Furthermore, the sites of hybridization in Harwich-77 are not uniformly distributed throughout the genome: the *X* chromosome and chromosome *3R* each have 17 to 20 sites, whereas chromosomes *2L*, *2R* and *3L* each have approximately eight sites. Chromosome *4* has a single site.

To assess the degree of uniformity of 88-4-1 and 88-15-1 lines, genomic DNA was prepared from single flies and examined by Southern blot analysis; the BWC plasmid was used as probe (data not shown). Although there were similarities in the *P* element hybridization patterns among members of each line, individual differences were also apparent, indicating that the transformed lines are not as homogeneous as Harwich-77. Consequently, estimates of the total number of elements in these lines by Southern blot analysis of DNA obtained by mass extraction (approximately 100 adults used) will only include those elements that are present in a significant proportion of the population. An initial attempt to make such an estimate of the total number of elements within the two transformed lines, using Harwich-77 as a control, was performed by digesting DNA with both *Bam*HI and *Bgl*II, restriction enzymes that lack sites within the *P* element. Such a digest yields a single band for each homozygous and hemizygous element within the genome. However, the majority of bands in the analysis were greater than 6 kb in size and resolution was poor, even in low percentage agarose gels. Moreover, our estimate of the number of elements in Harwich-77 was 40, much less than the number estimated from the *in situ* analysis. We, therefore, decided to analyze the *P* element

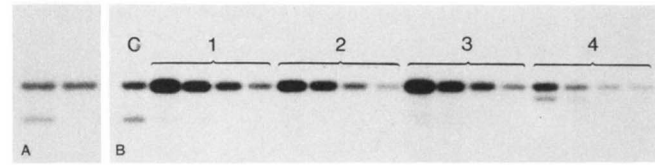

FIGURE 6.—Estimation of the number of intact *P* elements in transformed lines and wild-derived P strains. *A* and *B*, Genomic DNA was digested with *Acc*I and probed with the BWC plasmid. *A*, DNA samples from two transformed lines, both of which contain only one 2.4-kb *Acc*I *P* fragment, are shown. The lane on the left contains DNA obtained from the 88-72-2 transformed line at month 6. At this point in time, all members of this line possessed a single intact *P* transposon on chromosome *3*; the inserted chromosome was maintained against a third chromosome balancer. Two fragments are evident. One is the 2.4-kb internally derived fragment. The other contains the leftmost *P* sequences and the adjacent chromosomal DNA. The 88-72-2 DNA was used as the reference standard against which comparisons were made in (*B*). The lane on the right contains an equivalent amount of DNA from the w^{1118}; *P*[*hspP*;w^{-}] transformant (MATERIALS AND METHODS). In this line, a single engineered *P* transposon is inserted into chromosome *3* and maintained against a third chromosome balancer. The intensity of the 2.4-kb band is identical in the two transformed lines. *B*, Lane C contains approximately 2 μg of the reference DNA described above. This lane is followed by four groupings, each of which consists of a series of DNA dilutions. The groups are as follows: (1) Harwich-77, (2) 88-4-1, (3) 88-15-1, and (4) Agana. Each dilution series consists of samples with 1/10, 1/20, 1/40 and 1/80 the amount of DNA applied in lane C. Only the 2.4-kb *Acc*I fragment is shown in each lane. By matching the 2.4-kb band in the reference sample with a band of corresponding intensity in the dilution series, or by aligning the reference sample between two dilutions, it is possible to obtain a rough estimate of the number of potentially intact elements per genotype. For example, the band in the reference sample has an intensity greater than the band in the 1/80 dilution of the Harwich-77 DNA, but slightly less than the band in the 1/40 dilution. From this comparison, we estimate that the Harwich-77 P strain has approximately 50 intact elements per genotype or 25 per genome.

complements of the transformed lines by independently assessing the numbers of intact and degenerate elements.

The number of intact *P* elements was determined as follows. Genomic DNA from Harwich-77, 88-4-1, 88-15-1 and Agana (see Table 1) was digested with *Acc*I, which has two restriction sites within the intact *P* element (see Figure 1). Such a digest produces a 2.4-kb internal fragment for every intact element. Dilutions of digested DNA from each sample were then compared against a reference sample of DNA from a strain containing only one intact element. By selecting the 2.4-kb band in the dilution series that corresponds in intensity to the band in the reference sample, it is possible to assess the approximate number of complete elements in the experimental sample. By this method, we estimate that Harwich-77, 88-4-1, 88-15-1 and Agana have, respectively, 50, 30, 40 and 15 intact elements, or approximately 25, 15, 20 and 7 intact elements per genome (Figure 6). It should be mentioned that degenerate elements with deletions either to the right or to the left of the 2.4 kb *Acc*I fragment will be included in this estimate. The BWC

plasmid was used as probe in this analysis.

The number of degenerate elements in the samples listed above was determined by a strategy similar to the one outlined in Figure 3. Genomic DNA was digested with *Acc*I and probed with the *Acc*I fragment from pπ25.1 (see Figure 1). With this combination, an element with a deletion between the two *Acc*I sites or one with a deletion that removes one or the other of the *Acc*I sites will produce a single fragment that differs in size from the 2.4-kb fragment produced from an intact element. Degenerate elements with deletions that span the *Acc*I interval will not be detected in this analysis. By counting the number of fragments that are larger or smaller than the 2.4-kb fragment, a rough estimate of the number of degenerate elements per genome can be obtained. From this analysis, we estimate that Harwich-77, 88-4-1, 88-15-1 and Agana have respectively 40, 40, 35 and 35 degenerate elements per genome (Figure 7). It is important to note, however, that the analysis is complicated by the diversity and number of degenerate elements within these lines. In autoradiograms, such elements often produce very faint bands that are difficult to resolve. Consequently, multiple exposures are sometimes needed in order to obtain accurate estimates of these numbers. The best estimates are made from autoradiograms produced without the use of intensifying screens.

The results of the molecular analysis suggest that there is very little that distinguishes the two transformed lines, 88-4-1 and 88-15-1, with respect to their *P* element complements. Both lines contain similar numbers of intact and degenerate elements, although the ratio of intact to degenerate elements seems to be slightly higher in 88-15-1 than in 88-4-1. Finally, we note from the gel blotting analysis of Harwich-77 that the combined number of intact (25) and degenerate (40) elements per genome is in good agreement with the total number of elements per genome (60–65) estimated from the *in situ* analysis.

Do ry^+ transposons or their deletion derivatives proliferate in *P*-transformed lines? Since the progenitors of the six *P*-transformed lines were initially selected as ry^+ transformants, we were interested in determining whether ry^+ transposons proliferate in a manner similar to that of *P* element transposons. In many respects, the ry^+ transposon (RUBIN and SPRADLING 1982) (see also DANIELS *et al.* [1985, 1986]) can be viewed as a modified *P* element. It contains 1.1 kb of *P* element DNA, including the terminal repeats, which are thought to possess the recognition sequences for the transposase molecule. Moreover, it has been demonstrated that the ry^+ transposon exhibits major characteristics of *bona fide P* elements. In the dysgenic state, it can excise in both a precise and imprecise way (DANIELS *et al.* 1985), as has been

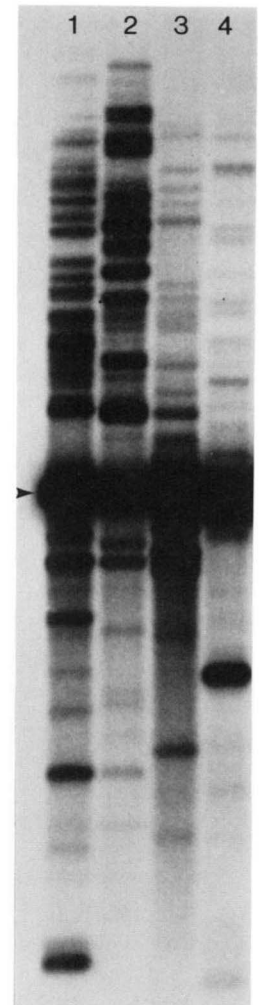

FIGURE 7.—Estimation of the number of degenerate *P* elements in the samples shown in Figure 6. Genomic DNA was digested with *Acc*I and probed with the *Acc*I fragment from pπ25.1. With this combination, each degenerate element produces a single band that differs in size from the 2.4-kb one (see text). Samples are as follows: (1) Harwich-77, (2) 88-4-1, (3) 88-15-1 and (4) Agana. The autoradiogram in this figure was produced with the aid of an intensifying screen in order to enhance contrast for photographic reproduction; therefore, many of the fainter bands visible in autoradiograms made without the use of an intensifying screen are not apparent.

demonstrated for *P* element transposons (O'HARE and RUBIN 1983; VOELKER *et al.* 1984); it can also transpose to new genomic locations in the presence of autonomous *P* elements (CLARK and CHOVNICK 1985; DANIELS *et al.* 1986).

The number of ry^+ transposons in *P* transformed lines was estimated at months 9 and 44 (data not shown) by an analysis similar to that described by DANIELS *et al.* (1985). Nine months after transformation, five of the six lines contained only a single complete ry^+ transposon; the remaining line contained two copies, one of which was a deletion derivative. At month 44, line 88-4-1 contained one complete, and one probable deletion derivative; line 88-15-1 contained one complete and two probable deletion deriv-

atives. Overall, the results of the analysis indicate that ry^+ transposons are relatively less amplified than are *P* element transposons in cotransformed lines.

DISCUSSION

In this report, we describe the transformation of a *D. melanogaster* true M strain with an autonomous *P* element. Six independently transformed lines were established and subjected to phenotypic and molecular analyses at various points over the course of a 4-yr period. On the molecular level, all six lines showed increases in the number of *P* elements over time, although differences were noted in the rate at which this occurred. By month 42 the number of elements in every line approached that of Harwich-77, a very strong P strain. In addition, degenerate elements arose in each of the lines at some point during their propagation. On the phenotypic level, all of the transformed lines presently exhibit high P activity potential, but only three have developed the P cytotype. The remaining three lines manifest intermediate regulatory capabilities. In experiments in which *P* elements have been introduced into a *P*-element-free genome by chromosomal contamination, the combination of high P activity and intermediate regulatory ability has only been observed as an unstable transient condition. Lines with these properties evolved to characteristic P or M strains in a period of less than 15 generations (M. KIDWELL, unpublished results). However, in our transformation experiments, lines with this phenotypic combination were essentially stable over more than 25 generations. The 88-15-1 is particularly noteworthy in this regard (Table 1). This line exhibits extremely high P activity potential and contains nearly as many complete *P* elements per genome (~20) as does Harwich-77 (Figure 6). Yet, it still has not developed complete regulation after 4 yr, long after the onset of rapid *P* element proliferation. A strain with these molecular and phenotypic properties, in an apparently stable configuration, has not been reported before. The existence of 88-15-1 raises questions, therefore, as to whether autonomous elements alone are sufficient for the development of the P cytotype.

Two other laboratories have obtained results with some similarities to those documented in this report. W. ENGELS and his colleagues (personal communication) followed the evolution of a transformed line that was initiated with a single autonomous *P* element. For more than 45 generations, the number of elements in this line was monitored by *in situ* hybridization; cytotype was periodically examined by measuring sn^w hypermutability in cross A*. Although there was a rapid and dramatic increase in the number of elements between generations 15 and 25, the first detectable increases in regulatory potential did not appear until much later (approximately 15 to 20 generations after the onset of *P* element proliferation). Moreover, the switch from M to P cytotype occurred gradually, taking approximately 25 generations before full P cytotype was established at about generation 50. We note that three of our transformed lines achieved complete, or nearly complete, regulatory potential at some point prior to generation 60; however, the remaining three lines still manifest intermediate levels of regulation at generation 200. In a separate set of experiments, SIMMONS (1986) constructed a number of isogenic lines with chromosomes extracted from a wild population. Several of her lines exhibited intermediate levels of GD sterility in cross A*, as well as nontrivial levels of sterility in cross A, indicating the presence of functional *P* elements and the absence of full *P* element regulation. It was also observed that such lines were often stable in this configuration over an extended period of time.

In light of our results and those from other laboratories, it now appears that there is a growing body of evidence that suggests that autonomous *P* elements may not be directly responsible for the establishment of the P cytotype. If this is so, then what factors might be responsible? A possible answer to this question was first suggested by SAKOYAMA *et al.* (1985). They examined a Q strain isolated from a natural population on Chichi Jima, an island 1000 km south of Tokyo. This strain manifested full P cytotype but apparently lacked any complete *P* elements. This observation led them to speculate that the P cytotype of this strain might be maintained by a functional repressor produced either by the independent action of certain kinds of degenerate elements or by the coordinate action of specific combinations of such elements. This notion that certain classes of degenerate elements might be responsible for the suppression of P activity is further supported by two other studies. D. BLACK and his colleagues (personal communication) have isolated a particular type of degenerate element, called the KP element, which is prominent in many European M strains. This element has a large internal deletion and is apparently capable of efficiently suppressing P activity. In a separate set of experiments, H. ROBERTSON and W. ENGELS (personal communication) have found that certain types of mutated elements, specifically those with deletions or perturbations in ORF3 (KARESS and RUBIN 1984; LASKI, RIO and RUBIN 1986), are capable of conferring regulatory potential, whereas autonomous elements alone appear unable to do so. From this they postulate that the repressor molecule is a truncated transposase and that it is a major component in establishing P cytotype. This is, in part, supported by the observation that viable transcripts are produced from at least some degenerate elements (O'HARE and RUBIN 1983;

LASKI, RIO and RUBIN 1986). Furthermore, there is no evidence at present to suggest that any molecule other than the transposase is encoded by the autonomous element in germline cells, although alternate processing of the transposase transcript still cannot be completely ruled out as a possible way by which a repressor molecule might be generated (LASKI, RIO and RUBIN 1986; RIO, LASKI and RUBIN 1986).

If certain types of degenerate elements are primarily responsible for establishing complete regulation, what are the consequences when only autonomous elements are introduced into a naive genome? We propose the following hypothetical sequence of events: When an autonomous *P* element is introduced into the permissive cellular environment of a *P*-element-free genotype, no constraints on its expression exist and it is free to make transposase. Transposase is, in turn, involved in two important processes. It can promote the transposition of an element to other chromosomal locations; it is also an essential component in the degenerative process, which results in the formation of elements with internal deletions (VOELKER *et al.* 1984; DANIELS *et al.* 1985). Thus, in this initial stage, two different forces with quite different consequences may act in concert to establish a potentially dynamic situation. On the one hand, the number of autonomous elements may be augmented by transposition events, while, on the other, functional elements may be inactivated by the excision process that results in the deletion of internal sequences. These deletions occur apparently at random throughout the element (DANIELS *et al.* 1985). At some point in time, certain elements with the capacity to produce repressor function may be formed by the excision process. Only when these repressor-producing elements arise will the evolution of complete regulatory potential (P cytotype) be possible. If such elements are not formed early on, then the continued availability of transposase may lead to a proliferative phase in which the number of chromosomal elements rapidly increases over a short period of time.

In such a scenario, the variability in the regulatory potential observed in our transformed lines can be explained by proposing that efficient repressor-producing elements have formed in those lines that have developed the P cytotype (*i.e.*, 88-4-1, 88-48-4 and 88-63-1) and that they have not formed in those that still manifest incomplete regulation (*i.e.*, 88-15-1, 88-72-2 and 88-283-3a). At present, the basis for the partial suppression observed in the latter, as well as in many pseudo M lines (KIDWELL 1985; SIMMONS and BUCHOLZ 1985), is not understood. It may be that such lines are able to produce some repressor, but at very low concentrations; alternatively, they may produce repressors that are weak or unstable. If a variety of elements with different deletions or perturbations in ORF3 are capable of providing repressor function in the form of truncated transposase, as proposed by H. ROBERTSON and W. ENGELS, then repressors may constitute a heterogeneous population of molecules. Consequently, some repressors may have slightly different properties than others, *e.g.*, some may be more stable or function more efficiently. This might explain the apparent thermolability of the regulatory potential of the 88-15-1 line; possibly it can only make a weak, temperature-sensitive repressor.

As indicated earlier, the progenitors of our *P*-transformed lines were initially selected as ry^+ transformants. At two points during the analysis, we examined ry^+ transposon copy number and found that the transposons did not undergo the rapid proliferation observed for *P* transposons. No more than one intact ry^+ transposon and one or two deletion derivatives were observed in any of the lines examined. In other experiments, lines have been constructed with as many as a dozen intact ry^+ transposons per genotype (A. CHOVNICK, unpublished results). Although these produce very high levels of XDH, they are nevertheless perfectly viable, indicating that XDH toxicity is not the basis for the difference in *P* element and ry^+ transposon proliferation. Although the precise reason for this difference remains unclear, we speculate that perhaps it may be related to transposon size. Intact *P* elements are 2.9 kb in length, whereas the ry^+ transposon is more than three times longer. Other differences in the behavior of the two kinds of transposons have also been observed and these, too, may be related to the difference in size. For example, under dysgenic conditions, the proportion of excision events that are imprecise has been estimated at 35% for the *P* element transposon (VOELKER *et al.* 1984), while the estimate for the ry^+ transposon is more than twice this figure (DANIELS *et al.* 1985). It has been suggested that the degenerative process may somehow be associated with transposition (*e.g.*, O'HARE and RUBIN 1983), although there is no direct evidence on this point at present. If degenerate elements arise as the result of abortive or aberrant transposition, then it is possible that the larger size of the ry^+ transposon may increase the likelihood of its degeneration, thereby preventing extensive proliferation of such elements within the genome.

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