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Analysis of P Transposable Element Functions in Drosophila

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Summary

We have made a P-element derivative called Pc[*ry*], which carries the selectable marker gene *rosy*, but which acts like a nondefective, intact P element. It transposes autonomously into the germline chromosomes of an M-strain Drosophila embryo and it mobilizes in *trans* the defective P elements of the *singed-weak* allele. Frameshift mutations introduced into any of the four major open reading frames of the P sequence were each sufficient to eliminate the transposase activity, but none affected signals required in *cis* for transposition of the element. Complementation tests between pairs of mutant elements suggest that a single polypeptide comprises the transposase. We have examined transcripts of P elements both from natural P strains and from lines containing only nondefective Pc[*ry*] elements, and have identified two RNA species that appear to be specific for autonomous elements.

Introduction

Much has been learned about the frequency, mechanism, and regulation of transposition of many procaryotic transposable elements (for reviews see Shapiro, 1983), largely because these elements often carry selectable markers (e.g., antibiotic resistance) and because they can be manipulated both in vitro and in vivo. In contrast, the molecular biology of metazoan transposable elements has been limited almost exclusively to structural description of the different elements and their transcripts. Progress in this field has been hampered by inherent difficulties in studying a multigene family, and by lack of useful assays for detecting transposition of an element within the genome.

P elements, the family of mobile genetic elements that are responsible for the phenomenon of P-M hybrid dysgenesis in Drosophila melanogaster, are of particular interest because their mobility has been shown to be under genetic control (for reviews see Engels, 1983; Bregliano and Kidwell, 1983). When the elements are quiescent they are said to be in the P cytotype (Engels, 1979b), the cellular environment of P-strain flies. P cytotype is apparently determined by the P factors themselves (Engels, 1979a). P factors, which are defined by genetic assays, are presumed to correspond to a fully functional subset of the biochemically defined P elements (O'Hare and Rubin, 1983). Flies lacking functional P elements are called M-strain flies, and are said to possess the M cytotype. Hybrid dysgenesis occurs when P-strain males are crossed to M-strain females, introducing functional P elements into the M cytotype. The offspring of such a cross (dysgenic hybrids) show a series of genetic aberrations, all of which are confined to the germ line of the dysgenic hybrid. These may include chromosomal rearrangements, visible and lethal mutations, male recombination, and a high level of gonadal sterility. In the reciprocal cross, between an M-strain male and P-strain female, or in a P × P cross, the P elements are maintained in the P cytotype, and no hybrid dysgenesis is observed.

A number of P elements have been cloned (Rubin et al., 1982) and sequenced (O'Hare and Rubin, 1983). Some share a conserved 2.9 kb structure and are believed to encode genes for transposition and cytotype determination. Others are smaller, defective elements, missing sequences internal to the terminal inverted repeats that flank the element. The 2.9 kb element has been shown to supply a *trans*-acting function required both for its own transposition (Spradling and Rubin, 1982) and for the transposition of defective nonautonomous P elements (Rubin and Spradling, 1982). These observations led to the development of the P-element-mediated gene-transfer system (Spradling and Rubin, 1982; Rubin and Spradling, 1982).

A genetic approach to dissecting the functions encoded by P elements requires that one be able to examine a single element of defined structure, rather than the heterogeneous population of defective and nondefective P elements found in the genomes of natural P strains. Additionally, the element needs to be marked genetically, so that its presence and location in the genome of a living fly can be easily detected, independent of its ability to produce the symptoms of hybrid dysgenesis.

We have made a P-element derivative which carries the wild-type *rosy* gene, but which acts in several respects like the nondefective 2.9 kb P element. When introduced into an M-strain fly, this element continues to transpose autonomously within the genome, and elicits certain other characteristics of hybrid dysgenesis. By mutagenizing the P element in vitro, and assaying its activity in vivo, both singly and in combination with other P elements, we have tentatively identified the regions of the element encoding the transposase function. In addition, we have examined the pattern of poly(A)$^+$ transcripts encoded by P elements of natural P strains, and by our P element marked with *rosy* in transformed flies. We find two RNA species that appear to be derived from autonomous elements, one of which may be the mRNA for transposase.

Results

Construction of an Autonomous P Element Marked with *rosy*

We constructed a genetically marked, autonomous P element (see Experimental Procedures for details of this construction) from the P-*rosy* vector Carnegie 20 (Rubin and Spradling, 1983) and the prototypical 2.9 kb autono-

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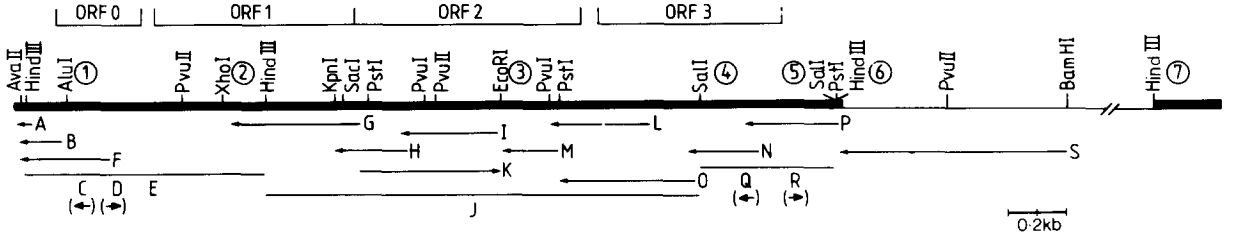

Figure 1. Map of Pc[(*ry*)B] Element and Derivatives

The heavy line represents P-element sequences. A portion of the 7.2 kb Hind III fragment containing the *rosy* sequences (thin line) is shown. Numbered restriction sites have been altered in one or more derivatives of the element: in Pc[*ry*] sites 5, 6, and 7 have been removed. A new Pvu I site has been generated at the position of site 5. The four frame-shift mutations were introduced onto this element as described in Experimental Procedures. In P[(*ry*)Alu] (ORF 0 frame-shift mutant), site 1 has become an Eco RI site by the addition of an 8 bp linker. (The positions of other Alu I sites are not shown.) P[(*ry*)Xho] (ORF1 mutant), P[(*ry*)R1] (ORF2 mutant) and P[(*ry*)Sal] (ORF3 mutant) are 4 bp insertions at sites 2, 3, and 4, respectively. The four open reading frames (ORF0–ORF3) of the P element, as determined by O'Hare and Rubin (1983), are indicated above the map. The extents of the hybridization probes (A–S) used in the course of this study are indicated below the map. All except E and J are single-stranded probes synthesized from M13 clones by the procedure described by O'Hare et al. (1983). Probes E and J were made by nick translation of pBR322 subclones. The arrows indicate the orientation of the single-strand probes, from 5′ to 3′. Probes C, D, and E cover the same Hind III–Hind III fragment with E being double-stranded and C and D single-stranded probes of the indicated orientations. Similarly, probes Q and R cover the same Sal I–Sal I fragment in opposite orientations.

mous P element of pπ25.1 (Spradling and Rubin, 1982; O'Hare and Rubin, 1983). We positioned the *rosy* (*ry*) gene in a noncoding region of the 2.9 kb P element, in a manner that we anticipated would be least likely to perturb element-encoded functions required in *cis* or in *trans*. The resultant plasmid contains the first 2884 of the 2907 bp of the complete 2.9 kb P element unrearranged (Figure 1). It lacks 23 bp of the 31 bp right-hand inverted repeat and has instead a 7.2 kb sequence containing the *rosy* gene, followed by nucleotides 2684 to 2907 of the P element, which supply an intact right-hand terminus.

The marked P element, called Pc[(*ry*)B], and a derivative, Pc[*ry*], (modified by the removal of certain nonessential restriction nuclease cleavage sites flanking the *rosy* gene (see Figure 1), were tested for their ability to transpose without the aid of a helper element from plasmid DNA to the germ-line chromosomes when injected into M-strain ry^- embryos. Of 40 surviving fertile adults, five yielded at least one transformed ry^+ offspring. This rate (12%) is comparable to that seen for other P-element constructions transposed with the aid of helper elements (Goldberg et al., 1983; Hazelrigg et al., 1984; Scholnick et al., 1983). By this criterion, Pc[*ry*] and Pc[(*ry*)B] behave as autonomous, nondefective P elements. A line of flies was established from each of the five independent transformation events (Table 1).

We examined the P elements in these lines by DNA blot hybridization for any large structural alterations relative to the parental plasmid. No obvious changes in the structure of the integrated elements were detected (representative digests are shown in Figure 2, lanes A, D, E, F). As expected of P-element-mediated transformation, only the element (as defined by the functional inverted repeats) has integrated; the flanking plasmid sequences are lost.

Table 1. Transformed Lines Containing Marked P Elements

Name[a]	Site of Lesion	Chromosome Location[b]	*rosy*$^+$ Gene Expression[c]
Pc[(*ry*)B]81	none	X	++
Pc[*ry*]2	none	II	++++
Pc[*ry*]7	none	III	N.D.[d]
Pc[*ry*]11	none	III	+++
Pc[*ry*]17c	none	III	++++
P[(*ry*)Alu]18	ORF 0	III	+++
P[(*ry*)Xho]11	ORF 1	II	N.D.
P[(*ry*)Xho]26	ORF 1	II	N.D.
P[(*ry*)Xho]41	ORF 1	X	++
P[(*ry*)RI]8	ORF 2	X	++
P[(*ry*)RI]28	ORF 2	III	+++
P[(*ry*)Sal]1	ORF 3	III	+++

[a] Transformant lines are named according to the conventions of Spradling and Rubin (1983) and Hazelrigg et al. (1984). P refers to a nonautonomous P-element construct and Pc to an autonomous construct. Symbols in brackets refer to the structure of the construct and numbers outside the brackets refer to the particular transformed line. For example, Pc[*ry*]2, Pc[*ry*]7, Pc[*ry*]11, and Pc[*ry*]17c are four independently derived transformant lines bearing the same P-element construction.

[b] Chromosomal location was determined by following sex linkage and segregation against dominantly marked balancers.

[c] The level of *rosy* gene expression was monitored to control for the possibility that inactivation of P functions was due to chromosomal position effects. Expression of *rosy* was determined by titration of the ry^+ eye color in flies fed on allopurinol, an inhibitor of xanthine dehydrogenase (the product of the *rosy* gene) (Keller and Glassman, 1965). +++ is approximately the amount of inhibitor required to titrate a single dose of wild-type Oregon R. rosy$^+$ product. ++ is between 50% and 100% of the wild-type level, ++++ is greater than 100% of the wild-type level.

[d] N.D. = not determined.

Construction of a Helper P Element That Is Incapable of Transposition

When attempting to introduce a mutagenized Pc[*ry*] element into the Drosophila genome, it is necessary to supply a functional transposase activity in *trans* from a helper P element, since a lesion created in the P sequence of Pc[*ry*] might disrupt sequences essential for the production of transposase. A potential problem arises if the mutagenized Pc[*ry*] element and the wild-type helper element both

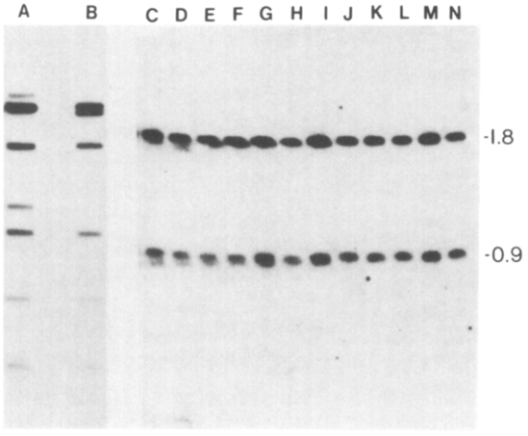

Figure 2. Blot Hybridization of Genomic DNA from Flies Transformed by Autonomous Pc[(*ry*)B] and Its Derivatives

(Left) Comparison of the structure of the Pc[(*ry*)B] element in DNA of the transformant line Pc[(*ry*)B]81 (lane A) and in pPc[(*ry*)B], the parental plasmid (lane B). Both DNAs were digested with Pvu II and Hind III, and probed with radiolabeled pPc[(*ry*)B] plasmid DNA. The extra bands seen in lane A correspond in size to those expected from the endogenous *white* and *rosy* sequences present in the transformation host. The extra band in lane B corresponds to the plasmid vector backbone.

(Right) Pvu II digests hybridized with probe J (see Figure 1). Lane C shows DNA of the parental plasmid pPc[*ry*)]. Lanes D, E, F, G, H, J, K, M, and N show genomic DNA isolated from transformant lines as follows: lane D, Pc[*ry*]2; lane E, Pc[*ry*]11; lane F, Pc[*ry*]17c; lane G, P[(*ry*)Sal]1; lane H, P[(*ry*)Alu]18; lane J, P[(*ry*)Xho]11; lane K, P[(*ry*)Xho]41; lane M, P[(*ry*)RI]8; lane N, P[(*ry*)RI]28. Lanes I and L show parental plasmids pP[(*ry*)Xho] and pP[(*ry*)RI], respectively.

integrate into the fly genome. In such a case the presence of the wild-type element would mask the effect of the mutation. Separating the two elements genetically, while possible, would be extremely tedious.

To circumvent this problem, a modified helper P element was constructed (see Experimental Procedures and Figure 7 for details). It is derived from the autonomous P element of the plasmid pπ25.7 by removal of the last 23 bp of the 31 bp right-hand inverted repeat, leaving the remainder of the element intact. The resultant plasmid, designated pπ25.7wc (for "wings clipped") was tested for its ability to supply the helper function in the transformation procedure, and for its ability to transpose itself, by coinjecting it with the defective recombinant P element pV10 (Rubin and Spradling, 1983), which carries the *rosy* gene.

Of 22 fertile adults developing from injected embryos, three yielded at least one ry^+ offspring, a transformation rate of 14%. All three lines contained the P-element sequences of pV10, but not the pπ25.7wc element (data not shown). The plasmid pπ25.7wc has been used subsequently as a helper to transform nine different independent Drosophila lines in the course of this study and in no case has the P element of pπ25.7wc been found integrated in the genome of the transformed fly.

Establishment of Drosophila Lines Containing Single Mutagenized Pc[*ry*] Elements

Having demonstrated that Pc[*ry*] can autonomously transform Drosophila, we next asked what mutations would destroy its ability to transpose. The four open reading frames (ORFs), found by DNA sequencing (O'Hare and Rubin, 1983) were obvious candidates for P-element-encoded genes. We created frame-shift mutations in each of the ORFs by altering appropriate restriction endonuclease cleavage sites (for details see Experimental Procedures). The four mutagenized Pc[*ry*] elements are designated P[(*ry*)Alu], P[(*ry*)Xho], P[(*ry*)RI], and P[(*ry*)Sal], and carry frame-shift mutations in ORFs 0, 1, 2, and 3, respectively (Figure 1).

By using pπ25.7wc to supply helper function, we could transform Drosophila with each of the different Pc[*ry*] derivatives without regard to the integrity of its transposase gene. For each of the four different Pc[*ry*] mutants we established one or more transformed Drosophila lines. Each line contained a single element, as judged by DNA blot hybridization of the genomic DNA from the transformed flies (see Experimental Procedures). Representative digests of six different lines are shown in Figure 2 (lanes G, H, J, K, M, N). The lesion introduced by modifying the restriction endonuclease cleavage sites in vitro was present in all but two of the Drosophila lines containing the mutagenized elements (not shown). One line containing P[(*ry*)RI] and one containing P[(*ry*)Sal] were found to have regained the ablated restriction site. These lines were not examined further (but see Discussion for speculation on their origins). A summary of the transformed lines is given in Table I.

The Pc[*ry*] Element Continues to Transpose to New Sites within the Genome of the Transformed Fly

A characteristic behavior of P elements is their high level of excision and transposition to new chromosomal sites during dysgenesis (Bingham et al., 1982). One line, Pc[(*ry*)B]81, transformed with an unmutagenized element, and two lines bearing different mutagenized derivatives, P[(*ry*)Xho]41 and P[(*ry*)RI]8 (representing mutations in ORF 1 and ORF 2, respectively), carried the marked P element on the X chromosome. The rates of excision and transposition of the P elements in these three lines were measured by monitoring the appearance of ry^- males and ry^+ females among the F2 offspring of a cross between the transformed male and an ry^- female carrying attached-X chromosomes (see Experimental Procedures). In such a cross, the two X chromosomes of the female segregate as a unit. The only viable offspring are males receiving the free X from their fathers, and females carrying the attached-X from their mothers. Since the Pc[(*ry*)B] element resides on the free X, all the males should be ry^+ and all the females ry^-. Excision of the element from the X in the germ line of the F1 male will produce an ry^- male gamete. Similarly, the transposition of the element to an autosome could produce an ry^+ female gamete.

Seven individual pair matings of Pc[(*ry*)B]81 males and attached-X; ry^- (M) females were carried out. Of the 52 F1 male progeny of this cross examined, 21 showed at least one germ-line excision event, generating 31 ry^- F2 males among a total of 1990 F2 male offspring, a rate of 1.6%.

Eight of the 52 F1 males yielded at least one ry$^+$ F2 female, generating 12 ry$^+$ females from a total of 1132 female offspring, for an adjusted rate of 2.4% (see Experimental Procedures). Four sublines were established from four separate ry$^+$ attached-X females, and were examined by DNA blotting. Each line showed a second band in addition to the one present in the parental Pc[(*ry*)B]81 line, confirming that a transpositional event has resulted in a second integration site on an autosome (Figure 3, lanes 1–6).

Loss of the ry$^+$ phenotype in males does not itself mean the Pc[(*ry*)B] element has precisely excised. From three of the ry$^-$ males we established sublines, (again maintained with attached-X females) and examined the DNA of these lines by blot hybridization (Figure 3, lanes 7–10). One ry$^-$ male had lost the band characteristic of the element integrated in Pc[(*ry*)B]81 and was devoid of sequences hybridizing to the P-element probe (lanes 9 and 10), as expected for a precise excision event. However, two other ry$^-$ males have apparently retained at least some of the P-element sequences (lanes 7 abd 8).

In contrast to the results with the wild-type Pc[(*ry*)B] element, we found no evidence for excision or rearrangement of the mutant P-element derivatives in P[(*ry*)Xho]41 or in P[(*ry*)RI]8, among over 2000 male offspring scored, and no transpositions among about 1000 female progeny scored. This indicates that the ORF 1 and ORF 2 lesions in these two P-element derivatives are each sufficient to eliminate the high levels of excision, rearrangements, and transposition seen with the wild-type P element.

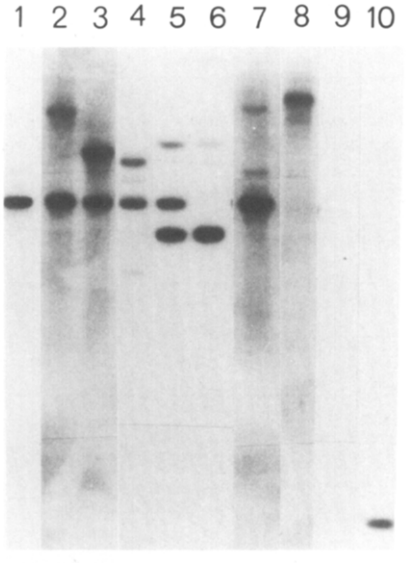

Figure 3. DNA Blot Hybridization of Transpositions and Excisions of Pc[(*ry*)B]

Genomic DNA was digested with Sal I, which cuts once within the element, and hybridized with probe J (see Figure 1). Lane 1, DNA from the parental Pc[(*ry*)B]81 males, which carry a single element on the X chromosome; lanes 2–5, DNA from males and females of four different sublines of Pc[(*ry*)B]81 containing autosomal transpositions. Note that there are one or two new bands in addition to the band present in Pc[(*ry*)B]81 males. In lane 6, the same transposition line as in lane 5 is shown, except that only DNA from attached-X females was used. As expected, the band from the original Pc[(*ry*)B] element on the free X chromosome is not seen, but the putative autosomal bands are present. In lanes 7–9, DNA from males of three different ry$^-$ revertant lines are shown. Lane 10 shows the same revertant as in lane 9, but hybridized with the entire P element in clone pπ25.1. The small band at the bottom of the lane is from host DNA and corresponds to the Drosophila DNA sequences flanking the P element in pπ25.1.

The Wild-Type Pc[*ry*] Element Can Destabilize *singed-weak* while Its Mutagenized Derivatives Cannot

singed weak, (*sn*^{w}) is a hypermutable allele of the singed bristle locus on the X chromosome. It arose in the offspring of a dysgenic hybrid (Engels, 1979b) and its phenotype results from the presence of two small, defective P elements at the *sn* locus (H. Roiha, K. O'Hare, and G. Rubin, unpublished). In the offspring of a P male by M female dysgenic cross in which one parent carries *sn*^{w}, up to 50% of the gametes of the F1 dysgenic hybrids no longer carry the parental *sn*^{w} allele (Engels, 1979b). One or the other of the two defective P elements at *sn*^{w} excises, generating one of two new phenotypes in the F2 offspring: a much more extreme singed bristle (sn^e), and an apparently wild-type bristle (sn$^+$) (H. Roiha et al., unpublished). However, the *sn*^{w} allele is essentially stable when it is maintained in the P cytotype, or in a genome devoid of all other P elements (and therefore M cytotype). Thus the destabilization of *sn*^{w} is an extremely sensitive assay for the presence of transposase activity provided by functional P elements. A single autonomous P element is sufficient to induce *sn*^{w} instability (Spradling and Rubin, 1982).

We tested the ability of Pc[*ry*] and its mutant derivatives of destabilize *sn*^{w}. A male carrying the Pc[*ry*] element is crossed to an *ry*$^-$ M-strain female homozygous for the *sn*^{w} allele. If the transformed line is heterozygous for the chromosome bearing the Pc[*ry*] element, then half of the F1 offspring will receive the Pc[*ry*] element and be ry$^+$, and half will receive the other chromosome and be ry$^-$. These ry$^-$ individuals serve as controls that lack the Pc[*ry*] element but are otherwise of nearly identical genetic composition. The ability of the Pc[*ry*] to destabilize the *sn*^{w} allele in the germ line of the ry$^+$ F1 hybrids was assayed by crossing the F1 *sn*^{w} males to tester females homozygous for *sn*^{3}, a recessive severe allele of the *singed* locus. The phenotypes of the F2 female offspring are exclusively sn^w if no *sn*^{w} destabilization has occurred, but if the Pc[*ry*] element is capable of providing transposase activity some are sn$^+$ or sn^e (see Experimental Procedures for details of the assay).

All four wild-type Pc[*ry*] lines tested were able to induce the destabilization of *sn*^{w} to *sn*^{e} (Table 2) (while *sn*$^+$ derivatives were observed in these experiments, they were not scored separately because of the difficulty in reliably distinguishing the sn^w and sn$^+$ phenotypes). Of the F1 ry$^+$ flies tested, 35%–70%, depending on the transformed line, showed evidence of destabilizing *sn*^{w} by the criterion of having at least one *sn*^{e} offspring. None of the tested ry$^-$ siblings did so, confirming that the presence of the

Table 2. *singed-weak* Destabilization by Pc[*ry*] and Its Derivatives

Strain	*F1* Progeny[a]		F2 Progeny	
	Total No. Tested	No. Showing Germ-line *sn*^{w} Instability	Total	*sn*[e]
P[(*ry*)Alu]18	25	0	1627	0
P[(*ry*)Xho]11	42	0	2357	0
P[(*ry*)Xho]26	13	0	761	0
P[(*ry*)Xho]41	18	0	1864	0
P[(*ry*)RI]8	19	0	2505	0
P[(*ry*)RI]28	19	0	1514	0
P[(*ry*)Sal]1	40	0	2509	0
Pc[(*ry*)B]81	17	6	1290	22 (3.4 ± 1.9%)[b]
Pc[*ry*]2	20	14	1618	131 (8.1 ± 3.6%)[b]
Pc[*ry*]11	20	13	1672	28 (1.7 ± 0.4%)[b]
Pc[*ry*]17c	24	8	1242	32 (2.6 ± 1.0%)[b]
P[(*ry*)Xho]41 + P[(*ry*)RI]28[c]	25	0	5249	0
P[(*ry*)Xho]41 + P[(*ry*)Sal]1[c]	25	0	5136	0
P[(*ry*)Xho]41 + P[(*ry*)Alu]18[c]	24	0	5470	0
P[(*ry*)RI]8 + P[(*ry*)Sal]1[c]	25	0	5319	0
P[(*ry*)RI]8 + P[(*ry*)Alu]18[c]	25	0	6075	0
P[(*ry*)Sal]1 + P[(*ry*)Alu]18[c]	17	0	3352	0
π_2[d]	25	25	2388	277 (11.6 ± 2.4%)[b]
ry$^-$ siblings[e]	16	0	975	0

Tests were conducted as described in Experimental Procedures.

[a] Individual males either homozygous or heterozygous for the chromosome bearing the marked P elements were crossed to *sn*^{w}; *ry* (M) females. The F1 offspring were examined for evidence of *sn*^{w} destabilization in their germ lines by crossing them to flies homozygous for *sn*^{3} (see Experimental Procedures).

[b] Standard errors were determined by the method of Engels (1979c).

[c] Strains containing pairwise combinations of mutagenized elements were generated as described in Experimental Procedures.

[d] π_2 is a strong, naturally occurring P strain, serving as a positive control.

[e] The offspring that did not inherit the chromosome bearing the Pc[ry] element from crosses involving transformed lines heterozygous for the element, serving as negative controls.

Pc[*ry*] element was required for *sn*^{w} destabilization to occur.

In contrast to the wild-type Pc[*ry*] elements, none of the mutant derivatives of Pc[*ry*] showed any evidence of including *sn*^{w} instability (Table 2). This indicates that each of the four different ORF lesions in the elements P[(*ry*)Alu], P[(*ry*)Xho], P[(*ry*)RI], and P[(*ry*)Sal] is sufficient to reduce by at least 30-fold, and most likely totally eliminate, *sn*^{w} destabilizing activity.

Complementation Tests between Pairs of Mutant P Elements Fail to Restore the *sn*^{w} Destabilizing Activity

The four open reading frames of the nondefective 2.9 kb P element could encode several different polypeptides, or could be spliced together by RNA processing to produce a single protein. If the activity responsible for destabilizing *sn*^{w} resides in a single polypeptide, then the four different frame-shift lesions in the four ORFs would all be affecting the same molecule, and no complementation would be expected between pairs of mutants. If, on the other hand, there are two or more different subunits encoded by the ORFs, then the presence of two different mutant P elements within the same genome should restore some level of transposase activity. That is, some combination of mutant elements should induce the destabilization of *sn*^{w}, where neither element alone could do so.

By the appropriate genetic crosses, we generated flies carrying all six pairwise combinations of the four frame-shift mutants of Pc[*ry*], and tested the stability of the *sn*^{w} allele in these lines. Some 2000 gametes bearing *sn*^{w} were scored for each pair, but in no case was any evidence found for destabilization of *sn*^{w} (Table 2). This strongly suggests that all four ORFs contribute to a single polypeptide involved in the destabilization of *sn*^{w}.

Pc[*ry*]-Transformed Drosophila Lines Are of the M Cytotype

The P cytotype is only found in fly strains containing multiple autonomous P elements. This has led to the proposal that autonomous P elements ultimately can determine the cytotype of the fly (Engels, 1979a) when present in sufficient numbers and for several generations. A single autonomous P element in an M-strain genome

Table 3. Test of the Cytotype of Pc[*ry*]2 Flies

Cross[a]	Male Parent	Female Parent	F1 Offspring: No. Tested	F1 Offspring: No. Showing Germ-line *sn*^{w} Instability	F2 Offspring: *sn*^{e}	F2 Offspring: Total
I	*y sn*^{w}/Y; *ry* (M)	*Pc[ry]2/Pc[ry]2*	16	6	15	1808
			10	2	3	738
			27	12	26	2642
			53	20 (39%)	44	5188 (1.7 ± 0.4%)[b]
II	*sn*^{w}/Y π_2 (P)	*Pc[ry]2/Pc[ry]2*	10	8	33	690
			7	3	10	416
			10	9	68	1150
			27	20 (74%)[c]	111	2256 (9.8 ± 2.3%)[b]
III	*sn*^{w}/Y π_2 (P)	*ry/ry* (M) control	4	4	20	248
			4	4	10	266
			2	2	7	124
			10	10 (100%)[c]	37	638 (11.6 ± 2.7%)[b]

[a] For each cross, the cytotype of three individual females was tested by carrying out three separate pair matings.
[b] Rates are determined by doubling the number of *sn*^{e} scored, as only half the F2 offspring carry the original *sn*^{w} locus. Standard are errors determined by the unbiased method of Engels (1979c).
[c] The difference in crosses II and III in the fraction of F1 offspring showing no *sn*^{w} instability is possibly significant (P = 0.086) by the simple Fisher's exact test.

remains in its active state: the element in Pc[*ry*]B]81 continues to transpose and excise from the X chromosome.

We asked whether Pc[*ry*] elements were capable of eventually establishing the P cytotype. Females from a homozygous stock of Pc[*ry*]2, which had been maintained for approximately 12 generations, were crossed to either *sn*^{w} (M) males or *sn*^{w} π_2 (P) males, and the germ-line instability of *sn*^{w} in the F1 progeny was measured (Table 3). If the female is of the P cytotype, neither the M male × P female, nor the P male × P female crosses should yield dysgenic offspring, and the *sn*^{w} allele should be stable. In fact, the *sn*^{w} allele was unstable in both crosses, indicating that the females had the M cytotype. However, there was a suggestion that maternally derived Pc[*ry*] elements might be able to repress partially *sn*^{w} destabilization. Crosses II and III (Table 3) compare the abilities of true M-strain females and Pc[*ry*]2 females to activate the elements of P-strain *sn*^{w} π_2. *sn*^{w} was destabilized in the germ lines of ten out of ten F1 progeny from the cross to the true M-strain females, while seven of 27 F1 progeny from the cross to Pc[*ry*] females showed no evidence of *sn*^{w} instability.

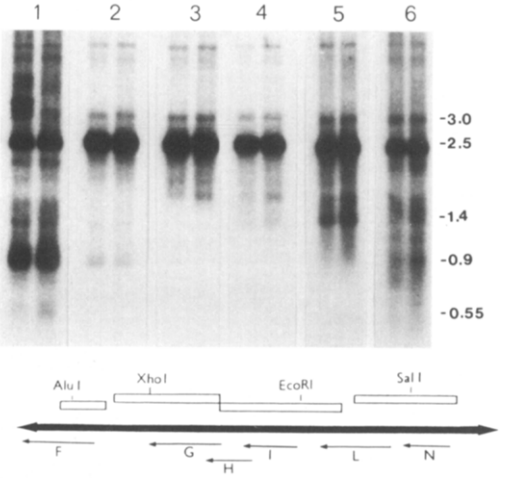

Figure 4. RNA Blot Hybridization to P-Element Transcripts in Dysgenic and Nondysgenic Embryos

Each pair of lanes has embryonic poly(A)$^+$ RNA from π_2(P) male × M female dysgenic hybrid embryos (left), and π_2 × π_2 nondysgenic embryos (right). Approximately 2 μg of RNA was applied per lane of a formaldehyde agarose gel (1.5%). Probes used were: lane 1, probe F; lane 2, probe G; lane 3, probe H; lane 4, probe I; lane 5, probe L; lane 6, probe N. The location and orientation of probes used in relation to the 2.9 kb P element and its ORFs are indicated.

Transcripts of P in Flies Containing Pc[*ry*] Are a Subset of Those Found in Natural P Strains

Poly(A)$^+$ RNA from dysgenic (P male × M female) embryos and from nondysgenic (P × P) embryos of the P strain π_2 were blotted onto nitrocellulose and hybridized with a number of single-strand probes derived from M13 subclones of the 2.9 kb P element (Figure 4). A complex pattern of bands, ranging in size from 0.5 kb to greater than 4 kb, appeared when the blots were probed with fragments from either the left- or right-hand side of the element (lanes 1 and 6). The most prominent species were 2.5, 1.4, and 0.9 kb. In some RNA preparations, a major 0.55 kb species was also seen. Probes derived for more internal segments of the 2.9 kb element hybridized primarily to the 2.5 kb transcript, but not to the majority of the smaller transcripts (lanes 3 and 4). It is likely that the smaller RNA species arise from transcription of deleted P

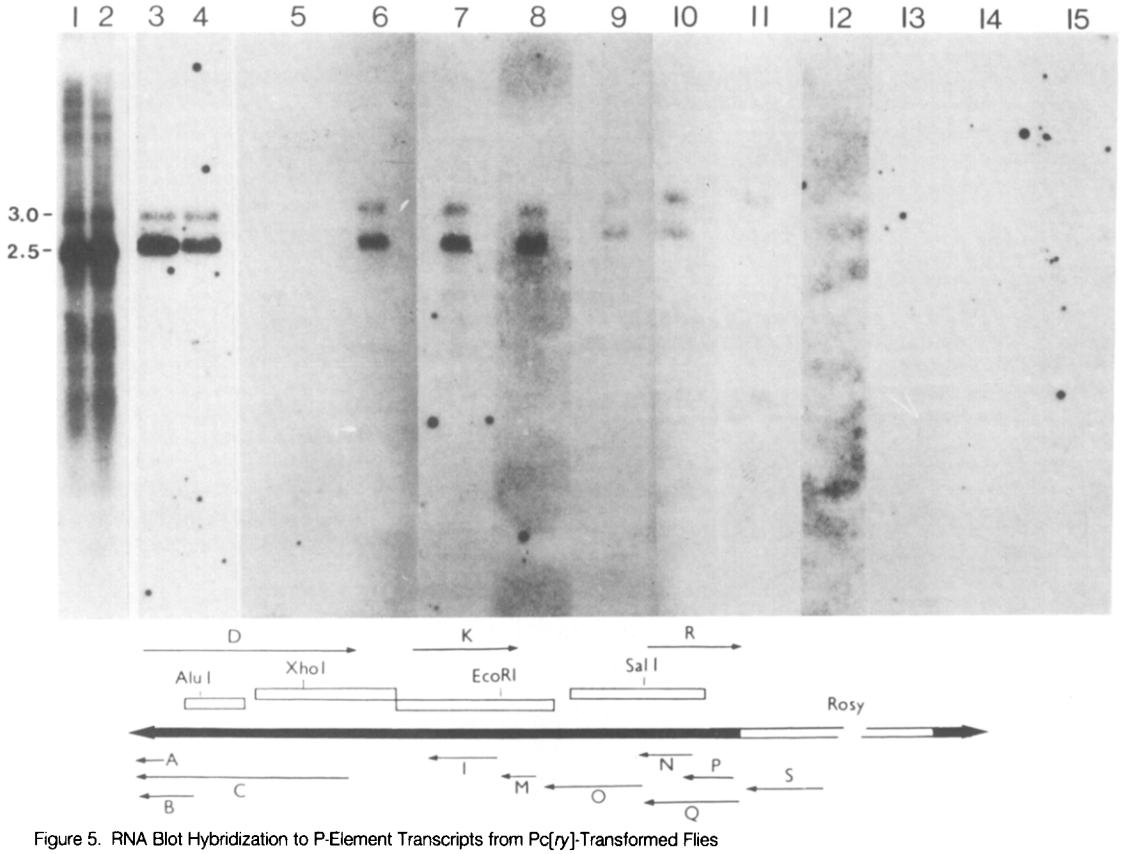

Figure 5. RNA Blot Hybridization to P-Element Transcripts from Pc[*ry*]-Transformed Flies

Lanes 1 and 2 have approximately 2 μg of π_2 male × M female and π_2 × π_2 RNA, respectively. Lane 3, embryonic poly(A)$^+$ RNA from line Pc[*ry*]2. Lanes 4–15, embryonic poly(A)$^+$ RNA from line Pc[*ry*]17c. Lanes 3 and 4 have approximately 10 μg of RNA each; lanes 5–15 approximately 5 μg each. Single-stranded probes used: Lanes 1 and 2, probe N; lanes 3 and 4, probe B; lane 5, probe A; lane 6, probe C; lane 7, probe I; lane 8, probe M; lane 9, probe O; lane 10, probe Q; lane 11, probe P; lane 12, probe S; lane 13, probe D; lane 14, probe K; lane 15, probe R. The location and orientation of the probes in relation to the Pc[*ry*] element are shown.

elements (see Discussion). There were no obvious differences in the transcriptional pattern of P elements in the quiescent (P × P) and active (P male × M female) states. Minor differences in the intensity of a few bands are apparent in Figure 4, but were not reproducible. A similarly complex pattern was found in RNA from another P strain called Harwich, which also displayed a prominent 2.5 kb band and a series of smaller hybridizing RNA species (data not shown).

In contrast, the pattern of transcripts from two strains carrying the Pc[*ry*] autonomous element was relatively simple. (Figure 5, lanes 3 and 4). The signal was much reduced compared to that of the natural P strains, but the only detectable bands corresponded to the 2.5 kb major transcript, and a 3.0 kb minor transcript also found in the authentic P-strain RNA. Based on the intensity of the hybridization signal compared with that seen for the transcript of the *white* locus by O'Hare, Levis, and Rubin (1983), we estimate that these transcripts represent about 0.001% of poly(A)$^+$ RNA. The transcripts were not detected by a probe hybridizing to the first 52 bases of the P element (lane 5), nor to a probe derived from the flanking sequences on the *rosy* gene fragment (lane 12). In addition, a probe derived from the right-hand 350 bases of the element preferentially hybridized to the 3.0 kb species, although the 2.5 kb transcript did hybridize faintly. The two transcripts appeared otherwise coextensive. The relative intensity of the two transcripts is much closer in our Pc[*ry*] lines than in the natural P strains. No transcripts were detected when probes from the opposite strand of the P element were hybridized to blots of the same RNAs (lanes 13–15).

A uniformly labeled single-strand probe covering 162 bases from the left side of the element, and detecting both the 2.5 and 3.0 kb transcripts in the RNA blots described above (Figure 5, lanes 3 and 4), was hybridized to poly(A)$^+$ RNA from both π_2 embryos and P male × M female dysgenic embryos. The resulting hybrids were digested with S1 nuclease and subjected to gel electrophoresis (Figure 6). A fragment about 75 nucleotides long resisted digestion (lanes C and D), suggesting that the P transcripts have their 5′ ends approximately at nucleotide 87. This region is just 28 bases from a TATACA sequence (O'Hare and Rubin, 1983). Another fragment approximately 95 bases long was faintly visible in the original autoradiograph, possibly indicating a minor start around nucleotide 67.

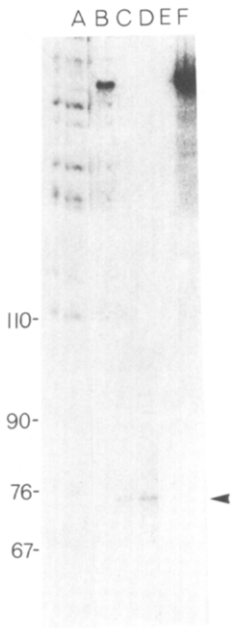

Figure 6. Nuclease S1 Analysis of P-Element Transcripts

Probe B (see Figure 1) was annealed with poly(A)$^+$ RNA from π_2 male × M female dysgenic embryos (lane C), with RNA from π_2 × π_2 nondysgenic embryos (lane D), and with total RNA from M-strain Drosophila embryos (lane E). Samples were digested with S1 nuclease, and resolved on a 5% urea-acrylamide sequencing gel. Lane A, size markers; lanes B and F, untreated probe. The arrow indicates a protected band of approximately 75 bp, which is seen in lanes C and D.

Discussion

The Pc[*ry*] Element Acts in Several Respects Like the Autonomous 2.9 kb P Element

We have shown by a number of criteria that Pc[*ry*] behaves as a wild-type, nondefective P element. First, it autonomously transposes upon injection into an M-strain embryo. Second, it continues to excise and transpose within the resident genome as long as it is maintained in the M cytotype. Third, it destabilizes the nonautonomous P elements responsible for the *singed-weak* (*sn*^{w}) mutation. Thus Pc[*ry*] appears to be a good model element for studying the behavior of P elements and their role in hybrid dysgenesis. Nevertheless, there is the formal possibility that the introduction of the *rosy* fragment introduces artifacts peculiar to our construction. This limitation in interpreting our results must be kept in mind, even if not explicitly stated in the following discussion.

We have mutagenized the Pc[*ry*] element in the four open reading frames of the P sequence, in each case eliminating the transposase activity associated with the element, but not affecting signals required in *cis* for transposition. Combining pairs of mutants in a single genome failed to restore the transposase activity. This crude genetic map of the transposase gene agrees well with the transcriptional map, which shows two RNA species, each covering all four reading frames. Finally, we have shown that an intact right-hand inverted repeat is required in *cis* for a P element to be transposable, but is not needed to produce a functional transposase.

Transposition and Excision of Pc[*ry*]

The wild-type Pc[*ry*] elements are able to transpose between sites within the genome. About 2.4% of the germ cells exposed for one generation to the single X-linked Pc[(*ry*)B] element acquire an autosomal element. This transposition rate is not far from that estimated for individual P elements in dysgenic crosses (Bingham et al., 1982). While the acquisition of a wild-type *rosy* gene by an autosome is a fairly rigorous test of the marked P element's integrity (requiring intact inverted repeats, transposase gene, and *rosy* gene), the loss of ry$^+$ from males of the Pc[(*ry*)B]81 line may have any one of several causes. It is not surprising to find some ry$^-$ males where an element-internal deletion is responsible for the phenotypic change, as the majority of defective nonautonomous P elements in natural P strains such as π_2 appear to be consequences of just such deletions of sequences internal to the 2.9 kb element (O'Hare and Rubin, 1983). That two of the three ry$^-$ males examined here (Figure 3) retained some P sequences suggests that the rate of true excision of Pc[(*ry*)] elements may be well below the 1.6% rate of reversion to ry$^-$ detected. Smaller P elements have been shown to be capable of both imprecise (Searles et al., 1982) and precise (Rubin et al., 1982; O'Hare and Rubin, 1983) excision at rates as high as 1%.

When internal deletions do occur in P elements, they are probably consequences of the transposase activity, perhaps from abortive attempts at transposition, and not simply from random mutational events. If point mutations or deletions of the *rosy* sequences in the wild-type Pc[(*ry*)B] occur independently of the action of the transposase, one would expect a similar frequency of ry$^-$ males appearing in lines P[(*ry*)Xho]41 and P[(*ry*)RI]8. However, these two X-linked mutant Pc[*ry*] derivative lines did not yield any ry$^-$ males among a combined total of some 4300 males scored.

Where Is Transposase Encoded?

A frame-shift mutation in any one of the four open reading frames renders the Pc[*ry*] incapable of destabilizing *sn*^{w} (Table 2). Moreover, X-linked elements with mutations in ORFs 1 and 2 were directly tested and found to be defective in excision and transposition. The absence of detectable transposase activity from the mutant elements does not appear to be a trivial consequence of their specific location within the genome; the level of expression of the *rosy* gene carried by the mutant Pc[*ry*] element in each line is at least 50% of that measured for a single wild-type *ry*$^+$ allele at its normal site (Table 1). Ideally, several lesions in each ORF should be tested, to exclude chance alterations in functions required in *cis*. We have, however, searched the P sequence for potential RNA splice donors and acceptors, and there do not appear to be any obvious signals at the modified sites. Taken to-

gether with our failure to observe complementation between mutations in different ORFs, these results suggest that each of the four ORFs contribute to a single polypeptide having transposase activity. That we find long RNA transcripts embracing the greater portion of all four reading frames (Figure 5) is consistent with this view.

Where Is Cytotype Encoded?

If all four ORFs contribute to transposase, where will the cytotype-determining factor be encoded? Assuming cytotype is indeed determined by a protein, one might expect this protein to recognize some of the same DNA sequences as does the transposase. For example, both might bind to the element's inverted terminal repeats. If so, a protein structure that shares certain domains with the transposase would not be surprising. Conceivably, differential RNA transcription or splicing patterns could generate both transposase and repressor from different portions of the same ORFs. The two transcripts we detected may correspond to transposase and repressor messages.

It appears that unmodified Pc[*ry*]-containing strains are generally of the M cytotype. When Pc[*ry*]2 females were crossed to P-strain *sn*^{w} males, the germ lines of the offspring usually sustained conversions to *sn*^{e}, indicating the crosses had been dysgenic. However, the number of F1 progeny showing no evidence of *sn*^{w} instability was consistently higher when the female parent was Pc[*ry*]2 than when the female parent lacked all functional P elements, suggesting that a low level of cytotype switching may be occurring.

It is possible that, in creating Pc[*ry*], we have inadvertantly altered the element's ability to determine cytotype. For example, if cytotype factors require for their expression an extrachromosomal P-element circle, as suggested by Engels (1981), then the additional 7.2 kb of *rosy* sequence within the element may hamper this aspect of its behavior. Alternatively, it may be that the few elements in Pc[*ry*]2 are insufficient to switch cytotype completely from M to P. There is some evidence supporting this explanation in the work of Engels (personal communication), who finds that flies of the P cytotype invariably contain multiple copies of the intact 2.9 kb P element. It will be necessary to determine the dependence of cytotype on the number of Pc[*ry*] elements to test the latter hypothesis. Such experiments should define conditions for testing mutant P elements for their ability to switch cytotype from M to P.

The Transcripts of P Elements

The two wild-type Pc[*ry*] transformed lines whose poly(A)$^{+}$ RNA was examined showed the same two transcripts of 2.5 and 3.0 kb, which comigrated with transcripts found in natural P strains. The other, smaller RNA species observed in the natural P strains, some of which are quite prominent, do not appear at all in the Pc[*ry*]-containing strains, suggesting that they are transcripts of defective elements, and not relevant to the expression of hybrid dysgenesis. Indeed, the pattern of hybridization of the probes to the smaller transcripts (Figure 4) is reminiscent of the structure of the smaller, internally deleted P elements that predominate in naturally occurring P strains. That transcripts exist, covering the greater portion of all four reading frames, is consistent with the mutant analysis, and is good cicumstantial evidence that either the 2.5 or 3.0 kb species encodes the transposase activity. We have preliminary evidence from S1 nuclease analysis that RNA splicing joins at least ORFs 1 and 2.

We examined the embryonic RNA of dysgenic hybrids because genetic evidence indicated that hybrid dysgenesis does occur at this stage (see Engels, 1983). We detected no reproducible differences between the transpositional patterns of P elements in dysgenic and nondysgenic embryos. This was somewhat surprising in that the simplest class of models predicts that the transposase message will be present only in the dysgenic hybrids, and the transcript for the cytotype-determining factor will be expressed only in the P-strain embryos. Unfortunately, as the Pc[*ry*]-transformed lines all were functionally dysgenic, we were unable to compare the transpositional pattern of this element in the quiescent (P) and active (M) states. Manifestations of hybrid dysgenesis appear to be limited to the germ line. The pole cells, which become the gonial cells, comprise less than 1% of the embryonic tissue. Therefore, it is formally possible that the relevant P-element transcripts are exceedingly rare, and that the ones we have identified are not related to the mechanisms of P-element transposition.

It is significant that no transcript hybridized to the first 52 bases of the element and that the nuclease S1 protection experiment identified a possible transpositional start site around nucleotide 87. These data, in conjunction with the structure of the P element (O'Hare and Rubin, 1983), argue against a retrovirus-like mechanism of transposition, which would require full-length RNA transcripts.

Evidence for Possible Homologous Recombination between Plasmids Injected into Drosophila Embryos

On two separate occasions, we found integrated Pc[*ry*] mutant derivatives that no longer carried the mutation present in the original plasmid. Contamination of the plasmid preparation with wild-type Pc[*ry*] seems an unlikely possibility, since it occurred in two separate plasmid preparations. Two differently marked plasmids appear to have contributed sequence information to the final integrated P element. In each case, the final integrated P elements had the *rosy* sequences from Pc[*ry*], but had acquired a wild-type restriction nuclease cleavage site, presumably from the pπ25.7wc helper plasmid. We suggest that recombination or gene conversion between the mutant Pc[*ry*] and the wild-type sequence of the pπ25.7wc helper is responsible. Rubin and Spradling (1983) have reported the integration of an unusual P element, which appears to be a tetramer of the original recombinant P element present in the injected plasmid. It seems possible that it too was

generated by recombination between molecules of the plasmid after injection into the Drosophila embryo.

The Wings-Clipped P Element

In using the P-element-mediated transformation system to introduce a particular nonautonomous construct into the germ line of the fly, heretofore it has been necessary to risk the cointegration of the helper element into the genome as well. Such double integration events can produce unstable transformants in which the genomic position of the transferred gene is not constant (Hazelrigg et al., 1984). These difficulties, which complicate further characterization of transformants, are avoided when transformation is mediated by the transposition-deficient helper element in pπ25.7wc. Moreover, pπ25.7wc can be microinjected into embryos of a transformed line already containing a nonautonomous recombinant P element and the resident P element can thereby be transiently mobilized to alter its position within the genome (Levis, Hazelrigg, and Rubin, unpublished).

Experimental Procedures

Drosophila Strains

ry^{506} (M strain) was originally obtained from W. Bender. *y* sn^3 v/y^+Y, the P strain π_2, sn^w π_2 (P strain), and *y* sn^w (M strain), were obtained from W. Engels. *y* sn^w (M) was made homozygous for ry^{506} in our laboratory. The second-chromosome balancer stock *CyO;* ry^{506} and the third-chromosome balancer stock *TM3,Sb Ser ry/*ry^{506} were obtained from B. Wakimoto. The *TM3* carries an EMS-induced mutation of *ry*. The attached-X stock *C(1)DX,y f;* ry^{506} was obtained from A. Spradling. A description of the standard alleles and balancer chromosomes may be found in Lindsley and Grell (1968). Flies were reared on standard cornmeal, molasses, and yeast medium at 25°C.

Nucleic Acid Purification and Analysis

Plasmid preparation, adult Drosophila DNA preparation, DNA blots, and nick translation of plasmids were performed as described in Levis et al. (1982). Small-scale fly DNA extractions (used in Figure 3) were done according to McGinnis et al. (1983). RNA extraction, RNA blots, and single-stranded probe synthesis were essentially as described by O'Hare et al. (1983), except that 0–24 hr embryos were the source of RNA. The M13 clones used to generate most of the single-stranded probes shown in Figure 1 came from the sequencing study of O'Hare and Rubin (1983). Probes C, D, Q, R, and S are from M13 clones made in the course of this study. Probes E and J are nick translations of fragments cloned into pBR322 by Spradling and Rubin (1982). For S1 nuclease digestion analysis, 4 μg of poly(A)$^+$ RNA and 5 × 10^4 cpm (specific activity ca. 10^8 cpm/μg) of uniformly labeled probe B (see Figure 1) were hybridized at 65°C in 0.3 M NaCl, 20 mM Tris, pH 7.5, 2 mM EDTA for 6 hr. The samples were diluted to 200 μl in S1 digestion buffer (0.3 M NaCl, 50 mM sodium acetate, 5 mM $ZnSO_4$, 20 μg/ml denatured carrier DNA), and digested with 1000 U of S1 nuclease (BRL) for 2 hr at 37°C. The digested samples were ethanol-precipitated and analyzed on a urea 5% acrylamide gel.

Titration of *rosy* Gene Activity

The relative levels of xanthine dehydrogenase, the *ry* gene product, in the transformed lines were determined according to the method of Keller and Glassman (1965). The ry$^+$ phenotype of the eyes was titrated by increasing amounts of allopurinol in the fly media (from 40–200 μg/ml) until the concentration was reached at which half of the newly eclosed flies had phenotypically ry$^-$ eyes.

Plasmid Constructions

Plasmids constructions are diagrammed in Figure 7. The P-element vector Carnegie 20 (Rubin and Spradling, 1983) was digested with Sal I and partially with Hind III, and the large vector fragment was gel-purified. A purified 2.5 kb Hind III–Sal I fragment from plasmid pπ25.1 was ligated into the Carnegie 20 fragment, yielding a plasmid denoted pPvc1. The P element in this construct lacks approximately 400 bp from the right-hand end. The sequences corresponding to this region of the P element were isolated and altered as follows: pπ25.1 was digested with Ava II, yielding a 2 kb fragment, one end of which lies within the 31 bp repeat, 23 bp in from the end of the element. The ends were repaired with DNA polymerase I (Klenow fragment), and blunt-ligated to a 10 bp Sal I linker. Following digestion with Sal I, the 0.4 kb Sal I fragment was gel-purified and cloned into the Sal I site of pPvc1. The correct orientation was identified by further restriction digests, and the resulting P element on this plasmid is named Pc[(*ry*)B] (see Figure 1). In a series of partial digests, end repairs, and religations, the two Hind III sites and the Sal I site closest to the *ry* fragment in Pc[(*ry*)B] were removed. The resulting P element is called Pc[*ry*] (See Figure 1).

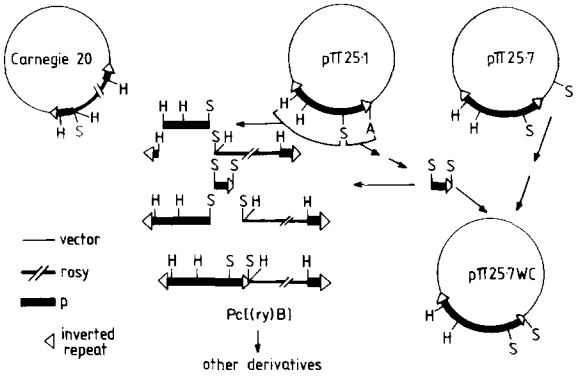

Figure 7. Construction of pPc[(*ry*)R] and pπ25.7wc

Selected restriction sites are indicated as follows. Sal I (S). Hind III (H). Ava II (A). See Experimental Procedures for description of steps.

The four frame-shift mutant derivatives of Pc[*ry*] were generated as follows: for ORFs, 1, 2, and 3, and 5′ overhangs generated by Xho I, Eco RI, and Sal I, respectively, were filled in with DNA polymerase I (Klenow fragment), thus introducing 4 bp insertions that would cause a polypeptide to terminate prematurely at stop codons 4, 4, and 10 amino acids, respectively, downstream from the original endonuclease cleavage sites. For ORF 0, an 8 bp Eco RI linker was ligated to an Alu I site at position 140, which resulted in a frame-shift that would terminate a polypeptide four amino acids from this site. To avoid the extraneous production of unwanted mutations, each of these lesions, except the ORF 3, Sal I lesion, was first created in plasmid pπ25.1. Restriction fragments bearing the mutations were then used to replace the corresponding fragment in pPc[*ry*]. All the frame-shift mutations except the Sal I were confirmed by subcloning into M13 and sequencing by the dideoxy chain termination method (Sanger et al., 1980). The filled Sal I site creates a new Pvu I site, whose existence was confirmed by digestion.

The wings-clipped plasmid pπ25.7wc was made by digesting pπ25.7 with Sal I, gel-purifying the vector fragment, and ligating in the 0.4 kb Sal I fragment described above.

P-Element-Mediated Transformation

Transformation of ry^{506} M-strain embryos was essentially as described by Rubin and Spradling (1982). The helper P-element plasmid pπ25.7wc was employed instead of pπ25.1. Embryos were coinjected with 50 μg/ml of helper plasmid and 300 μg/ml of the plasmid bearing the Pc[*ry*] mutant derivative. Plasmids bearing the wild-type Pc[*ry*] and Pc[(*ry*)B] elements were injected without helper at 250 μg/ml. Generally, about 10% of the surviving fertile adult flies yielded transformed (ry$^+$) offspring.

Testing the Ability of pπ25.7wc to Transpose

Plasmids bearing pπ25.7wc and pV10 (Rubin and Spradling, 1983) were coinjected into M-strain ry^{506} embryos in approximately equimolar ratios, at 300 μg/ml each, so as not to bias the integration of one element over the other. Three ry$^+$ transformants were obtained from 22 surviving fertile adults.

Stocks were established by inbreeding both ry$^+$ and ry$^-$ offspring from each of the transformed flies to retain any of the helper elements that might have integrated into a fly genome without the concomitant integration of the element containing the *ry*$^+$ gene. DNA blot hybridization confirmed the presence of the pV10 and the absence of pπ25.7wc elements in these lines.

Establishment of Transformed Lines

Individual transformed flies were crossed to either *CyO; ry*^{506} or *TM3,Sb Ser ry/ry*^{506}, and the segregation of ry$^+$ from the dominant markers on the balancer chromosomes (curly wings for *CyO* and stubble bristles for *TM3*) was used to identify the chromosome bearing the element. Southern blots confirmed the integrity of the transposed elements and determined their number. All the lines originally contained a single element. Most lines were maintained both as homozygotes and as heterozygotes over the appropriate balancer chromosome. Elements on the X were maintained both as homozygotes and with *C(1)DX, y f/Y; ry*^{506} females. The mutagenized Pc[*ry*] elements were stable, but the wild-type Pc[*ry*] elements continued to transpose in their host genomes. No attempt was made to maintain these lines with single elements, with the exception of the X-linked element line Pc[(*ry*)B]81. While transpositions to new sites could not be picked up phenotypically, excisions (loss of ry$^+$) were detected at a low frequency in all the wild-type Pc[*ry*] lines maintained on heterozygotes, suggesting that multiple copies of the element within a single genome were not very prevalent in the population.

Transposition and Excision Assay of X-Lined Elements

Single Pc[(*ry*)B]81 males were mated to several virgin attached-X females of the stock *C(1)DX, y f/Y; ry*^{506}. The offspring were scored for the presence of any ry$^+$ females, which would indicate that a transposition had occurred in the previous generation. If such females comprised a significant fraction of the offspring, the vial was discarded. Otherwise, several F1 males (putative dysgenic hybrids) from each set of parents were crossed separately to a few virgin *C(1)DX, y f/Y; ry*^{506} females, and the offspring were scored for their ry phenotype. The presence of ry$^-$ males or ry$^+$ females in the F2 generation indicates an apparent loss or transposition (respectively) of the Pc[(*ry*)B] element, in the germ line of the F1. The offspring of each F1 male were scored separately. Rare y$^+$ ry$^+$ females were not scored, as these probably were derived from a breakdown of the attached-X chromosome. The actual frequency of transpositions of Pc[(*ry*)B] to new sites is estimated to be approximately 9/4 the number obtained by counting ry$^+$ females, since half the autosomal transpositions will have segregated to ry$^+$ males with the free X, and be phenotypically undetectable, and any transpositions to new sites on the free X of the male would similarly go undetected. This factor is used to determine the rates presented.

Lines P[(*ry*)Xho]41 and P[(*ry*)RI]8 were maintained in mass culture with *C(1)DX, y f/Y; ry*^{506} females. Flies were examined each generation for any ry$^-$ males or ry$^+$ females.

The *singed-weak* Destabilization Assay

Single ry$^+$ males from the transformed lines carrying autosomal inserts of Pc[*ry*] or its derivatives were crossed to *y sn*^{w}*; ry*^{506} (M-strain) virgins. The ry$^+$ F1 (putative dysgenic hybrid) males carry the *y sn*^{w} X chromosome, and a Pc[*ry*] element on an autosome. For controls, ry$^-$ males (having no P element in their genomes) were also selected. The F1 males were crossed to the tester stock *y sn*^{3} *v*. All F2 females were scored for their sn phenotype. The offspring of such F1s were scored separately, but totals are combined in Table 2. Such females are heterozygous for *sn*^{3} and the possibly destabilized *sn*^{w}. *sn*^{3} acts as a null allele, and permits the scoring of any changes of *sn*^{w} to *sn*$^+$. (*sn*$^+$ revertants were also detectable, but were not scored separately.) Occasional singed, vermillion females were observed, but were not scored as they were apparently a consequence of nondisjunction in the tester cross.

For testing the X-linked elements in lines Pc[(*ry*)B]81, P[(*ry*)RI]8 and P[(*ry*)Xho]41, the same crosses were performed, except that F1 ry$^+$ virgin females were collected and mated singly to *y sn*^{3} *v/y*$^+$Y males. All F2 offspring were scored, but the final frequency of *sn*$^+$ gametes reported is twice the measured rate, since only half the X chromosomes of the F1 actually carried the *sn*^{w} allele. The tests for P cytotype (Table 3) using females of the Pc[*ry*]2 line, were performed in a similar fashion.

Complementation Test between Pairs of Mutants

All pairwise combinations of Pc[*ry*] mutants but one were constructed by combining an X-linked mutant with one of the third chromosome. However, the presence of two *ry*$^+$-bearing elements within one genome had to be inferred by selection against the dominantly marked third-chromosome balancer. The general scheme is illustrated by the following example. From the cross *P[(ry)RI]8/P[(ry)RI]8; TM3/ry* ♀ × *+/Y; P[(ry)Sal]1/P[(ry)Sal]1* ♂, male offspring of genotype *P[(ry)R1]8/Y; P[(ry)Sal]1/TM3* are obtained. These are crossed singly to virgin *y sn*^{w}*; ry* females. All female offspring of this cross not carrying the balancer are of genotype *P[(ry)RI]8/y sn*^{w}*; P[(ry)Sal]1/ry*, and are putative dysgenic hybrids if the two mutants can complement each other. The germ lines of these flies are examined for evidence of *sn*^{w} destabilization by crossing them to *y sn*^{3} *v/y*$^+$*Y* males as described above. The combination of the ORF 0 and ORF 3 mutants (both of which reside on the third chromosome) was achieved as follows: from the cross *y sn*^{w}*/y sn*^{w}*; TM3/ry* ♀ × *+/Y; P[(ry)Alu]18/P[(ry)Alu]18* ♂, male offspring of genotype *y sn*^{w}*/Y; TM3/P[(ry)Alu]18* are obtained. These are crossed to females *+/+; P[(ry)Sal]1/P[(ry)Sal]1*. Female offspring of this cross not carrying the *TM3* balancer have the genotype *y sn*^{w}*/+; P[(ry)Alu]18/P[(ry)Sal]1*, and are putative dysgenic hybrids. The germ lines of these offspring were assayed for evidence of *sn*^{w} destabilization as described above.

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References

Bingham, P. M., Kidwell, M. G., and Rubin, G. M. (1982). The molecular basis of P-M hybrid dysgenesis: the role of the P element, a P-strain-specific transposon family. Cell *29*, 995–1004.

Bregliano, J. C., and Kidwell, M. G. (1983). Hybrid dysgenesis determinants. In Mobile Genetic Elements, J. A. Shapiro, ed. (New York: Academic Press), pp. 363–410.

Engels, W. R. (1979a). Hybrid dysgenesis in Drosophila melanogaster: rules of inheritance of female sterility. Genet. Res. Camb. *33*, 219–236.

Engels, W. R. (1979b). Extrachromosomal control of mutability in Drosophila melanogaster. Proc. Nat. Acad. Sci. USA *76*, 4011–4015.

Engels, W. R. (1979c). The estimation of mutation rates when premeiotic events are involved. Environmental Mutagenesis *1*, 37–43.

Engels, W. R. (1981). Hybrid dysgenesis in Drosophila and the stochastic loss hypothesis. Cold Spring Harbor Symp. Quant. Biol. *45*, 561–565.

Engels, W. R. (1983). The P family of transposable elements in Drosophila. Ann. Rev. Genet. *17*, 315–344.

Goldberg, D., Posakony, J., Maniatis, T. (1983). Correct developmental expression on a cloned alcohol dehydrogenase gene transduced into the Drosophila germ line. Cell *35*, 59–73.

Hazelrigg, T. H., Levis, R., and Rubin, G. M. (1984). Transformation of *white* locus DNA in Drosophila; dosage compensation, *zeste* interaction, and position effects. Cell *36*, 469–481.

Keller, E. C., and Glassman, E. (1965). Phenocopies of the *ma-1* and *ry* mutants of Drosophila melanogaster: inhibition in vivo of xanthine dehydrogenase by 4-hydroxypyrazolo(3,4-d)pyrimidine. Nature *208*, 202–203.

Levis, R., Bingham, P. M., and Rubin, G. M. (1982). Physical map of the *white* locus of Drosophila melanogaster. Proc. Nat. Acad. Sci. USA *79*, 564–568.

Lindsley, D., and Grell, E. (1968). Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. *627*.

McGinnis, W., Shermoen, A. W., and Beckendorf, S. K. (1983). A transposable element inserted just 5′ to a Drosophila glue protein gene alters gene expression and chromatin structure. Cell *34*, 75–84.

O'Hare, K., and Rubin, G. M. (1983). Structures of P transposable elements and their sites of insertion and excision in the Drosophila melanogaster genome. Cell *34*, 25–35.

O'Hare, K., Levis, R., and Rubin, G. M. (1983). Transcription of the *white* locus in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. USA *80*, 6917–6921.

Rubin, G. M., and Spradling, A. C. (1982). Genetic transformation of Drosophila with transposable element vectors. Science *218*, 348–353.

Rubin, G. M., and Spradling, A. C. (1981). Vectors for P element mediated gene transfer in Drosophila. Nucl. Acids Res. *11*, 6341–6351.

Rubin, G. M., Kidwell, M. G., and Bingham, P. M. (1982). The molecular basis of P-M hybrid dysgenesis: the nature of induced mutations. Cell *29*, 987–994.

Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H., and Roe, B. A. (1980). Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. *143*, 161–178.

Scholnick, S. B., Morgan, B. A., and Hirsh, J. (1983). The cloned *dopa decarboxylase* gene is developmentally regulated when reintegrated into the Drosophila genome. Cell *34*, 37–45.

Searles, L. L., Jokerst, R. S., Bingham, P. M., Voelker, R. A., and Greenleaf, A. L. (1982). Molecular cloning of sequences from a Drosophila RNA polymerase II locus by P element transposon tagging. Cell *31*, 585–592.

Shapiro, J. A., ed. (1983). Mobile Genetic Elements. (New York: Academic Press), 688 pp.

Spradling, A. C., and Rubin, G. M. (1982). Transposition of cloned P elements into Drosophila germ line chromosomes. Science *218*, 347.

Spradling, A. C., and Rubin, G. M. (1983). The effect of chromosomal position on the expression of the Drosophila xanthine dehydrogenase gene. Cell *34*, 47–57.