

Genetic Transformation of *Drosophila* with Transposable Element Vectors

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Efficient methods for the transfer of genetic information into the germ line cells of multicellular organisms will be essential for continued progress in understanding the genetic control of development. Many attempts have been made over the past few years to develop such methods. DNA segments introduced into

might be solved by the use of vectors that can catalyze the specific integration of defined sequences into the host genome at high frequency. While several virally derived vectors capable of propagating exogenous sequences in cultured vertebrate cells have been described (8), none of them has been shown to repro-

Summary. Exogenous DNA sequences were introduced into the *Drosophila* germ line. A *rosy* transposon (*ry*¹), constructed by inserting a chromosomal DNA fragment containing the wild-type *rosy* gene into a P transposable element, transformed germ line cells in 20 to 50 percent of the injected *rosy* mutant embryos. Transformants contained one or two copies of chromosomally integrated, intact *ry*¹ that were stably inherited in subsequent generations. These transformed flies had wild-type eye color indicating that the visible genetic defect in the host strain could be fully and permanently corrected by the transferred gene. To demonstrate the generality of this approach, a DNA segment that does not confer a recognizable phenotype on recipients was also transferred into germ line chromosomes.

some cultured mammalian cells may be expressed and, in a small fraction of the cells, converted into a form that is heritable over many generations (1). DNA introduced directly into the nucleus of cultured cells by microinjection results in a higher frequency of gene transfer (2). DNA microinjected into fertilized eggs has been shown in several cases to persist during embryonic development (3). By means of this approach mice have been obtained in which the injected DNA was inherited in the next generation and expressed into RNA and protein (4). DNA sequences introduced by these transformation procedures became associated with high molecular weight DNA (5) and in some cases became integrated into host chromosomes (6, 7) by unknown mechanisms. These techniques are limited, however, by the frequent rearrangement of the transferred sequences during the process of transformation and by their subsequent instability (5, 7).

The problems of low efficiency, sequence rearrangement, and instability

might be solved by the use of vectors that can catalyze the integration of an exogenous DNA in a predictable, and hence potentially controllable, manner. We report here the development of a system of gene transfer based on the properties of a *Drosophila* transposable element, the P element, which substantially overcomes these difficulties. This method permits the efficient transfer of specific DNA segments, without rearrangement, into the germ line of a multicellular organism and results in their stable inheritance in future generations. Moreover, this transfer is potentially susceptible to precise experimental control.

Experimental Design

As described in the accompanying article (9), cloned 3-kilobase (kb) P elements frequently transpose from plasmid sequences into the chromosomes of developing germ line cells after microinjection into *Drosophila* embryos lacking such elements (M embryos). The behavior of 3-kb P elements is compatible with the hypothesis that they code for a *trans*-acting product that acts specifically at

the termini of both intact and internally deleted elements to catalyze transposition. By analogy to prokaryotic transposable elements, we refer to this *trans*-acting product as a transposase, although its detailed mechanism of action is unknown. It is further hypothesized that a second element-coded product, a repressor, accumulates in cells containing intact P elements, preventing transposition. Embryos from element-free strains would therefore lack repressor, thus explaining the high frequency of transposition of P elements after injection into such embryos.

Since cloned 3-kb P elements can transpose from plasmids and stably integrate into chromosomal DNA, a DNA segment containing a gene of interest might be introduced into the *Drosophila* germ line using the 3-kb P element as a vehicle. This approach would require the identification of a suitable insertion site within the element in order not to disrupt sequences required for transposition. Alternatively, DNA's could be ligated into an internally deleted P element to produce a defective transposon. If transposase were supplied from an intact 3-kb P element co-injected with the defective transposon, integration of the element and its associated foreign DNA might occur. One advantage of using a transposition-defective vector would be that, in the absence of a complementing complete element, the integrated transposon should be completely stable. For this reason we chose the latter approach.

Transfer of the Gene Encoding Xanthine Dehydrogenase

The marker we used to test the gene transfer scheme outlined above was the *rosy* (*ry*⁺) gene, the structural locus for the enzyme xanthine dehydrogenase (XDH). We chose this gene for several reasons. First, a cloned DNA segment thought to contain the entire *ry*⁺ gene was available (10). Second, both visible and selectable phenotypes are conferred by this gene; individuals homozygous for appropriate mutant *rosy* alleles have an easily recognized abnormal eye color and are unable to grow on a medium containing high levels of purine. Third, normal eye color can be restored by the production of as little as 5 percent of the wild-type XDH activity (11). Finally, *rosy* gene function is not cell autonomous; restoration of a normal eye color does not require proper function of the gene in the cells of the eye (12). Thus the eye color phenotype conferred by the *rosy* gene provides an easily scored and

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sensitive marker that maximizes our chances of detecting gene transfer events.

The construction of the *rosy* transposon, *ry1*, is diagrammed in Fig. 1. An 8.1-kb Sal I restriction fragment thought to contain the *ry*⁺ gene (10) was inserted into the Xho I site within the 1.2-kb P element cloned on the plasmid p6.1 (13). The two resulting plasmid constructs, *pry1* and *pry3*, differ only in the orientation of the 8.1-kb Sal I fragment relative to the P element sequences; both contain the putative *ry*⁺ gene flanked by the termini of the P element.

The DNA sequences contained within the 1.2-kb P element are not sufficient to encode any of the putative protein products of the 3-kb P element (14). We would therefore expect that transposition of these *rosy* transposons would require the production of the P element-specific transposase by other P elements. Two basic protocols for providing such complementing transposase activity are illustrated in Fig. 2. In the first protocol, plasmids containing the *rosy* transposon are injected into embryos derived from a cross between males containing P elements and females lacking P elements. The P elements are known to transpose at high rates in the developing germ line of such dysgenic embryos (15) and we would therefore expect high levels of transposase activity to be present. This protocol has the disadvantage, however, that mutations and chromosomal rearrangements are also induced in such dysgenic embryos (15). In the second protocol, a mixture of plasmid DNA's containing the *rosy* transposon and the 3-kb P element was injected into embryos lacking P elements [M cytotype (15)]. We have shown that the 3-kb P element carried on the plasmid *pπ25.1* (14), when injected into an M cytotype embryo, can produce sufficient transposase activity to catalyze its own transposition from the injected plasmid DNA into chromosomal sites (9). We reasoned that the transposase produced by the 3-kb P element might act on the *rosy* transposon to cause its transposition into the genome when the DNA's were co-injected. As a control, we also investigated the ability of plasmids containing partially deleted P elements to promote transfer of the *rosy* transposon. When carrying out such co-injections we mixed an excess of the *rosy* transposon with the 3-kb element DNA to favor transfer of the *rosy* transposon (see Table 1). All injected embryos were homozygous for *rosy* mutations to permit phenotypic screening for successful gene transfer events.

Both protocols (Table 1) resulted in

successful transfer of a functional *rosy* gene or genes as assayed by the appearance of flies with wild-type eye color among the G1 progeny of the injected embryos. In the case of those injected embryos that gave *ry*⁺ progeny, the percentage of their G1 progeny that were

rosy⁺ varied between 0.4 and 39 (Table 2). Some of the adult flies that developed from injected embryos themselves displayed wild-type or near wild-type eye color phenotypes. For example, 17 out of the 40 fertile G0 adults in experiments 3, 4, and 5 showed some evidence of *rosy*

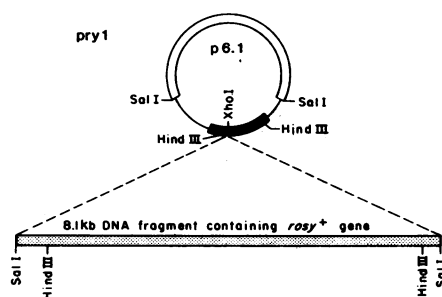


Fig. 1. Construction of *pry1*. An 8.1-kb Sal I DNA fragment containing the *rosy*⁺ gene (10) was inserted into the single Xho I site in the plasmid p6.1 (13). (The junctions between these two DNA's, labeled X/S, are not cleaved by either Sal I or Xho I.) This construction placed the *rosy* gene in the middle of the 1.2-kb P element, which is indicated by the solid shading, to create the *rosy* transposon *ry1*. The pBR322 vector sequences in p6.1 are indicated by the open bar. The remainder of the DNA sequences in p6.1 are nonrepetitive sequences derived from the *white* locus of *D. melanogaster*.

Fig. 2. Two protocols for gene transfer with the *rosy* transposon *ry1*. The test crosses that were carried out to assay for transfer of a functional *rosy* gene and the corresponding generation numbers (G0, G1, or G2) are indicated. In the first method the plasmid *pry1* was microinjected into a dysgenic embryo derived from a cross between a P father and M mother, both of whom were homozygous for the *rosy* mutation *ry*⁶⁰. To prevent continued hybrid dysgenesis, the progeny in subsequent generations were mated to flies with the P cytotype (15). In the second method a mixture of *pry1* and the plasmid *pπ25.1*, which carries a 3-kb P element (14), were co-injected into homozygous, *rosy* mutant, M cytotype embryos. To avoid introducing a chromosomal source of P elements, M strains were used for the test crosses. Table 1 summarizes the results of these experiments. Method 1 was used in experiment 1 and method 2 in experiments 2 to 6.

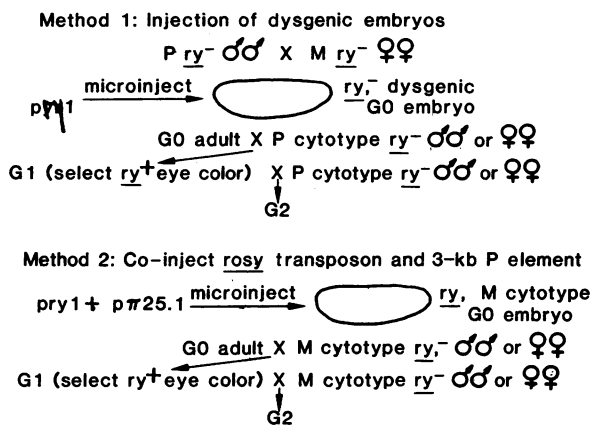


Table 1. Summary of *rosy* gene transfer experiments.

Experiment	DNA's injected*	Host embryos†	Injected (No.)	Fertile adults‡ (No.)	Giving <i>ry</i> ⁺ progeny	
					No.	Percent§
1	<i>pry1</i> + <i>pry3</i>	M/P <i>ry</i> ⁶⁰	147	9	2	22
2	<i>pry1</i> + <i>pry3</i>	M <i>ry</i> ⁶⁰	114	5	1	20
3	<i>pry1</i> + <i>pry3</i>	M <i>ry</i> ⁴²	215	19	11	58
4	<i>pry1</i> + <i>pry3</i>	M <i>ry</i> ⁴²	121	17	5	29
5	<i>pry1</i> + <i>pry3</i>	M <i>ry</i> ⁴²	87	4	2	50
6	<i>pry1</i> + <i>pry3</i>	M <i>ry</i> ⁴²	427	28	0	

*The plasmids *pry1* and *pry3*, which carry the *rosy* transposons *ry1* and *ry3*, were constructed as diagrammed in Fig. 1. Plasmids *pπ25.1* and *pπ25.7* (9, 14) carry the 3-kb P element. These plasmids differ only in the relative orientations of the *Drosophila* DNA insert and the pBR322 vector sequences. Plasmids *pπ25.7Δ* are constructed by deleting various portions of the 3-kb P element carried on *pπ25.7*. The data from four different deletions have been pooled in experiment 6. DNA's were injected in 5 mM KCl, 0.1 mM sodium phosphate (pH 6.8) at the following concentrations. In experiment 1, *pry1* and *pry3* were each at 500 μg/ml. In experiment 2: *pry1* and *pry3* were each at 500 μg/ml, and *pπ25.1* was 50 μg/ml. In experiment 3, *pry1* and *pry3* were each at 150 μg/ml; *pπ25.1* was at 50 μg/ml. In experiment 4, *pry1* and *pry3* were each at 150 μg/ml; *pπ25.7* was at 50 μg/ml. In experiment 5, *pry1* was at 300 μg/ml; *pπ25.1* was at 50 μg/ml. In experiment 6, *pry1* and *pry3* were each at 150 μg/ml; *pπ25.7Δ* plasmids were at 50 μg/ml. †The cytotype of all injected embryos was M, and the genotype was as indicated. Dysgenic embryos with M mothers and P fathers are designated by M/P. ‡Adults were considered fertile if they gave more than ten progeny. §The percentages indicate the fraction of fertile adults that gave *ry*⁺ individuals among their progeny.

function. Such expression of the injected *rosy* gene occurred more frequently, but not exclusively, in flies that subsequently gave *rosy*⁺ progeny. It is likely that *rosy* expression in G0 adults is due primarily to transcription of the injected plasmid DNA rather than to transposition of the *rosy* transposon into the cells of the injected embryo. In experiment 6 we observed G0 expression under conditions where no germ line integrations occurred.

In the G2 generation the *ry*⁺ phenotype was inherited in the manner expected for a stable dominant marker. To confirm that the inheritance of the *ry*⁺ eye color phenotype was due to the chromosomal integration of the *rosy* transposon, DNA from *ry*⁺ and *ry*⁻ segregants of the three *ry*⁺ lines generated in experiments 1 and 2 were analyzed. DNA was prepared from the host strain, which was homozygous for the *rosy* mutant allele *ry*⁶⁰, and separately from *ry*⁺

and *ry*⁻ G2 progeny of each of the three lines. These DNA's were digested with Sal I, an enzyme that does not cut within the *rosy* transposon sequences. The digests were then fractionated by gel electrophoresis, transferred to nitrocellulose paper, and hybridized with ³²P-labeled pDm2844S8.5, a plasmid carrying the cloned 8.1-kb Sal I fragment containing the *ry*⁺ gene. The band of hybridization corresponding to the *ry*⁶⁰ mutant allele, which contains a 0.9-kb deletion relative to wild type (10) was seen in all DNA samples. The DNA from the *ry*⁺ G2 flies of each line showed one or two additional bands of hybridization. Lines R102 and R113 each had one additional band of greater than 25 kb and 14 kb, respectively, while line R202 had two additional bands of 24 and 16 kb (Fig. 3A). Thus the additional genomic copies of *rosy* DNA segregated with the *ry*⁺ eye color phenotype in each case indicating that these additional *rosy* DNA sequences

are indeed responsible for the *ry*⁺ eye color.

Our studies on the transposition of the 3-kb P element from injected plasmid DNA showed that only the P element sequences, and not the rest of the plasmid, were inserted into the genome of the recipient (9). A similar integration mechanism appears to operate in the case of injected plasmids containing the *rosy* transposon. Sal I digests of DNA's from the *ry*⁶⁰ host, as well as from the *ry*⁺ and *ry*⁻ G2 progeny of the R102, R113, and R202 lines were each hybridized with the plasmid pm12.8, which contains all those DNA sequences present on the injected plasmids, *pry1* and *pry3*, except those of the *rosy* transposon itself (see maps in Fig. 3). All of the DNA samples showed a single, identical band of hybridization corresponding to the 1.5-kb Sal I *Drosophila* DNA fragment carried on pm12.8; none of the G2 flies acquired additional sequences homologous to pm12.8. Thus, only the transposon sequences are stably transferred to the recipient genome. Figure 3B shows the results of this experiment for the R202 line. The fact that lines carrying only a single copy of additional *rosy* sequences have a *ry*⁺ eye color suggests that the *rosy* transposon can integrate in a nonpermuted manner without DNA loss or rearrangement. Consistent with this suggestion is the observation that, when DNA from *ry*⁺ G2 flies is digested with Hind III, fractionated on agarose gels, and hybridized with the cloned 8.1-kb Sal I fragment containing the *ry*⁺ gene, a 7-kb Hind III fragment, identical in size to that contained within the *rosy* transposon, is observed (Fig. 3C).

To determine the chromosomal sites at which *ry1* integration had occurred, chromosomes were prepared from G2 larvae and hybridized to plasmid pDm2844S8.5 sequences (16); chromosomes from the *ry*⁶⁰ host strain were labeled only at 87D (17), the site of the *rosy* gene (Fig. 4A). The R113 strain contained an additional site of hybridization at 87A. Larvae from the R202 strain contained two additional sites, 79E and 84C. Chromosomes from seven additional independent lines derived by transformation of *ry*⁴² M embryos were also studied by *in situ* hybridization. In all, 12 sites of *ry1* integration were observed (Fig. 4).

As in the case of intact P elements (9), the *ry1* transposon integrates at a variety of sites. However, the *rosy* transposon does not appear to integrate at random. Six of the 12 mapped sites of insertion were located in regions 84 to 87, a chro-

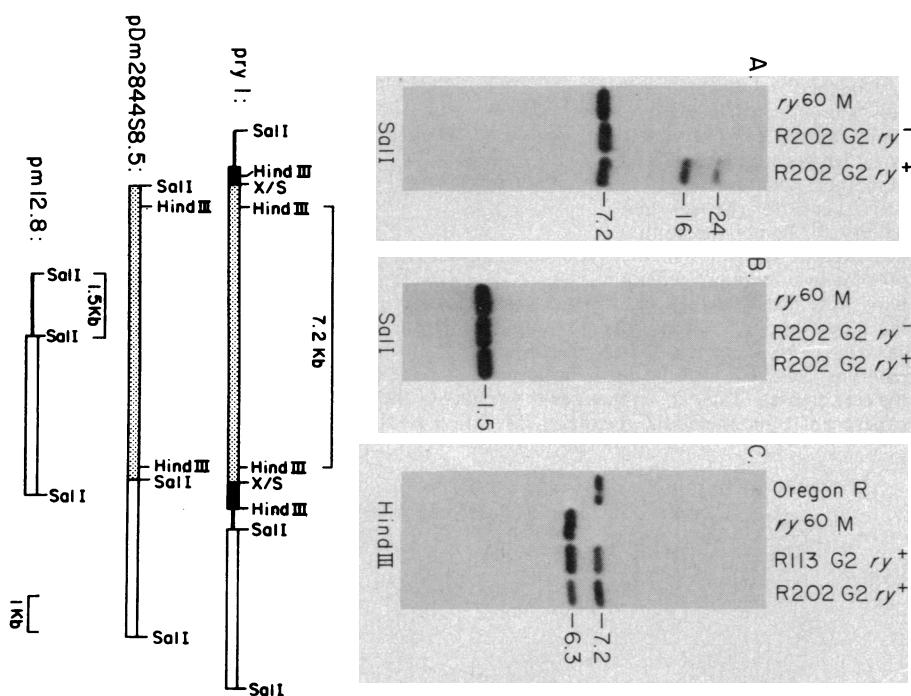


Fig. 3. Analysis of DNA from *rosy* transformant lines. (A) Sal I digests of DNA from the host strain, *ry*⁶⁰ M, and from *ry*⁻ and *ry*⁺ G2 segregants of line R202 were subjected to electrophoresis in adjacent lanes of a 0.4 percent agarose gel, transferred to nitrocellulose paper, hybridized with ³²P-labeled DNA of the plasmid pDm2844S8.5, and autoradiographed. Plasmid pDm2844S8.5 (10) contains the same 8.1-kb Sal I fragment that is carried by the *rosy* transposon. The *ry*⁶⁰ mutant strain carries a 0.9-kb deletion within this Sal I fragment relative to wild type, generating a 7.2-kb Sal I fragment. (B) The same DNA digests as in (A) were fractionated by electrophoresis on a 0.8 percent agarose gel and hybridized with plasmid pm12.8 probe. This plasmid contains all those sequences contained on the injected plasmids *pry1* and *pry3*, except those of the *rosy* transposon itself. (C) Results of hybridization of pDm2844S8.5 sequences to Hind III digests of DNA isolated from the *rosy* wild-type strain Oregon R, the *ry*⁶⁰ M host strain, and *ry*⁺ G2 segregants of transformant lines R113 and R202. The Oregon R strain has a 7.2-kb Hind III fragment identical in size to that contained within the *rosy* transposon. The *ry*⁶⁰ mutant strain has a 0.9-kb deletion within this Hind III fragment. Linear maps representing the circular plasmids *pry1*, pDm2844S8.5, and pm12.8 are shown. Open bars represent pBR322 sequences. The stippled bars represent the 8.1-kb fragment containing the *rosy*⁺ gene, and the solid bars in the *pry1* map indicate sequences of the 1.2-kb P element. The thin lines in the *pry1* and pm12.8 maps represent the same *Drosophila* DNA sequences that are derived from the *white* locus.

mosomal interval surrounding the normal *ry*⁺ gene that comprises less than 5 percent of the genome. This local site preference for insertion was less pronounced, however, in subsequent experiments (18).

Transfer of Unselected DNA

In many cases it may be desirable to transfer DNA segments which do not themselves confer a recognizable phenotype on the recipient strain. As discussed later, the development of P element vectors containing selectable markers should greatly facilitate the detection of such transfers. An alternative approach is to co-inject a transposon containing the DNA of interest along with a transposon which confers a recognizable phenotype on the host strain. Offspring whose phenotype indicates that uptake of the marker plasmid has occurred are then screened to determine whether the desired sequences were also transferred.

A simple variant of this scheme is illustrated in Fig. 5. An 11.1-kb insert consisting of three copies of a 3.7-kb Sal I fragment, containing two *Drosophila* chorion genes and flanking sequences from the 66D region (19), was ligated into the Xho site of p6.1. The resulting plasmid (pch1) carrying the 12.3-kb chorion transposon (ch1) was then injected along with DNA from a 3-kb P element into embryos of the *sn*^w M strain (20). As described (9), embryos in which 3-kb P element coded products function in germ line cells will often yield G1 progeny males in which the *sn*^w phenotype has

Table 2. Summary of the phenotypes of G0 and G1 individuals.

G0 fly	G0 <i>rosy</i> expression*	G1 progeny	
		<i>rosy</i> −	<i>rosy</i> +
R102	−	158	6
R113	−	173	6
R202	−	51	32
R301	+	237	2
R302	+	243	14
R303	+	133	5
R304	−	134	11
R305	−	234	1
R306	+	39	2
R307	−	249	2
R308	−	214	4
R309	−	152	2
R310	−	149	1
R311	−	119	3
R401	+	113	15
R402	+	154	27
R403	−	142	5
R404	+	241	6
R405	−	84	7
R501	−	196	1
R502	+	52	16

*The eye color phenotype of G0 adults was scored either − (unchanged from the *rosy* eye color of the host *ry*⁶⁰ or *ry*⁴² strain) or + (wild-type eye color either partially or completely restored).

mutated to *sn*^c or *sn*⁺. Any P element, whether complete or defective, present in a cell expressing the putative transposase would be expected to be a target for transposition. Thus the observation of *sn*⁺ or *sn*^c G1 males was used to identify G0 individuals in which transposase activity had been present in at least some germ line cells. Therefore, only the progeny of such individuals were tested for the presence of ch1 DNA sequences. Lines were started from single G1 males derived from mutable *sn*^w embryos produced by co-injection of

pch1 and p π 25.1, a plasmid carrying the 3-kb P element. Chromosome preparations from the lines were hybridized to a plasmid probe, p302.77 (19), which contains the same chorion gene segment as pch1 (Table 3). Of 12 strains whose chromosomes were tested by in situ hybridization, one showed labeling by the chorion gene probe at four sites in addition to 66D (Fig. 6). In principle, any DNA segment cloned between the P element termini in p6.1 could be transformed into *sn*^w embryos by means of this approach.

Concluding Remarks

The method of gene transfer described above has significant advantages over previous approaches for transforming the cells of multicellular organisms. Because the process of chromosomal insertion appears to be catalyzed by an enzyme (transposase) which recognizes a specific nucleotide sequence (the P element terminal repeat), transformation occurs with high frequency and does not result in detectable sequence rearrangement. In our *rosy* gene transfer experiments, approximately 8 percent of the injected embryos developed into fertile adults and, on average, 39 percent of these gave progeny containing the *ry*1 transposon. This DNA segment was transferred without apparent sequence rearrangement and was inserted into the recipient genomes in a nontandem and nonpermuted manner. Moreover, since the DNA segment that is transposed from the injected plasmid into the recipient genome is delineated by the terminal repeats of the P element, the exact se-

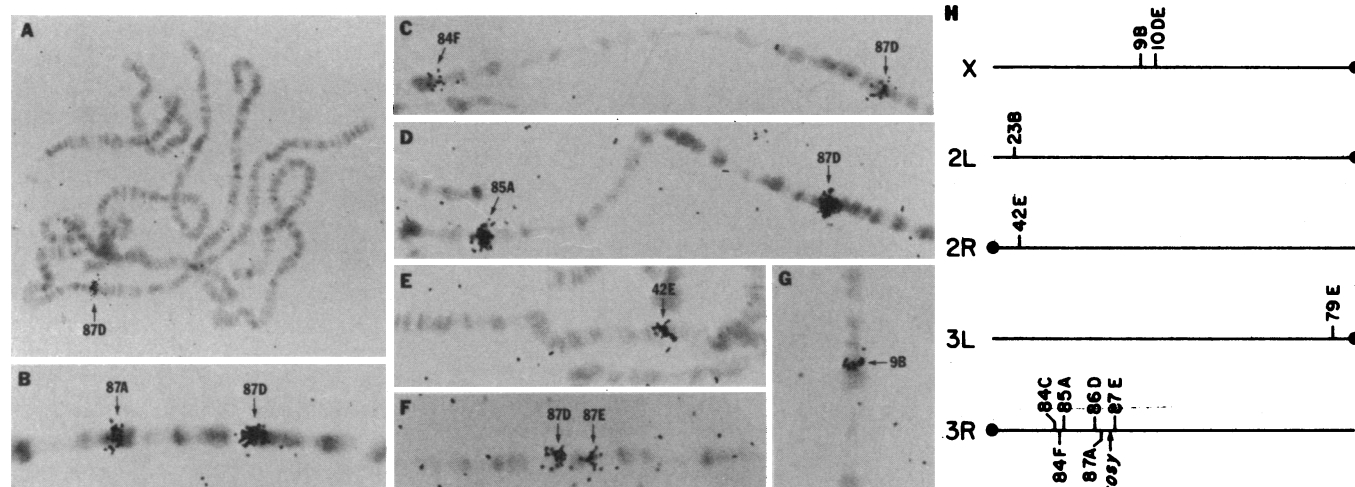


Table 3. Co-injection of pch1 and p π 25.1 into *sn*^w embryos.

Injected	Fertile adults	Adults mutable for <i>sn</i>	G1 lines established	Lines containing ch1*
194	21	3	12	1

*This represents a minimum estimate since only a small fraction of the progeny of the three mosaic flies were tested for the presence of ch1.

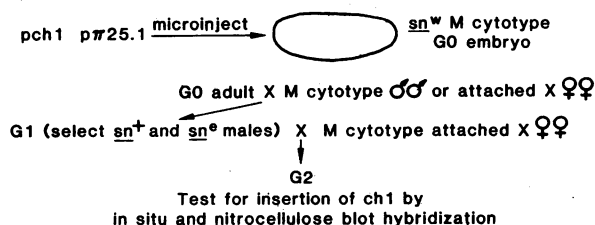


Fig. 5. Protocol for cotransformation of the chorion transposon pch1. The plasmid p π 25.1 carries a 3-kb P element. The test crosses and corresponding generation numbers are indicated [see (9) for details of the *sn*^w assay system].

quences that will be transferred can be predetermined. In contrast, when other gene transfer methods are used (1-8), sequences frequently become reiterated and rearranged.

The transposition reaction apparently requires the production of an enzyme, the P element transposase, not normally found in the recipient. Thus, both the initial transfer and the subsequent stability of the inserted transposon can be controlled by manipulating the supply of transposase. In the future it might be possible to obtain an even greater degree of control over the process of gene transfer. For example, if the transcriptional specificity of the transposase gene were altered by substituting developmentally specific promoter sequences, it might be possible to direct the integration of DNA into the chromosomes of only a particular cell type following delivery by any of a variety of methods.

There is no apparent size limit to the DNA segment that can be transferred. The 9-kb and 12-kb transposons we constructed transpose at high rates even though they are several times the length of the 3-kb P element. We suspect that the practical size limit of the method will be about 40 kb and will be imposed by the difficulty, by means of currently available methods, of constructing and propagating in *Escherichia coli* circular DNA molecules of greater size.

Two improvements should increase the utility of the gene transfer system. First, a plasmid could be constructed containing a modified P element vector having a wide variety of internal restriction enzyme sites suitable for cloning as well as carrying an appropriate marker to facilitate the transfer of DNA segments that do not themselves confer an easily scored phenotype. Second, modification of the 3-kb P element used to provide complementary transposase activity by

removing its terminal repeats could produce a P element that can provide transposase activity but that cannot itself transpose.

In most instances, genes transferred by the methods outlined above will find themselves at abnormal chromosomal positions. Their activity might be influenced by the particular chromosomal regions in which they are embedded. The methods of gene transfer described in this article allow direct study of the frequency and mechanism of such position effects. The observation that *ry*1 could produce sufficient XDH to cause

wild-type eye pigmentation when inserted at a large number of different chromosomal positions demonstrates that large amounts of normal flanking DNA are not a prerequisite for *rosy* gene function. However, the *rosy* eye color phenotype is not a highly demanding measure of normal *rosy* gene function. Only a few percent of wild-type XDH activity is required for this *rosy*⁺ phenotype (11). Moreover, the gene need not function in the eye itself to produce wild-type pigmentation (12). Thus, further studies on these *ry*⁺ transformants will be necessary to determine if the newly incorporated gene is expressed normally and to what extent it is subject to the appropriate developmental controls.

It is known from classical genetic and cytogenetic studies that transposition of chromosome segments within *Drosophila* euchromatin only affects the function of genes located very close to or at the ends of the rearrangement (21). However, the distance on a molecular scale over which such position effects influence gene expression is not well defined. If enough of the normal flanking sequences are transferred along with the gene of interest, it should provide a buffer against the influence of sequences surrounding the site of integration, allowing the normal developmental regulation of the gene to be directly scrutinized.

The advantages of our transformation methods should be shared by any gene transfer system in which a specific enzyme recognizes a specific target site defining the DNA sequences to be transferred. What are the prospects for the development of similar methods for use in organisms other than *Drosophila*? One possibility would be the direct use of the P element. The ability of P elements to function in other species remains to be tested. If intact P elements cannot be mobilized, modifications of the specificity of P element promoters might allow transposase production in a foreign environment. Even if modified elements will not cross species lines, analogous families of transposable elements are probably widespread in natural populations of numerous organisms other than the fruit fly.

There are two major areas in which vectors derived from the P element or analogous transposable elements may be useful. An immediate goal is the application of genetic transformation methods to the problems of gene regulation in developing eukaryotic organisms. At present, research in this area has been limited by the lack of ways to test developmental properties of genes that have been isolated by recombinant DNA tech-

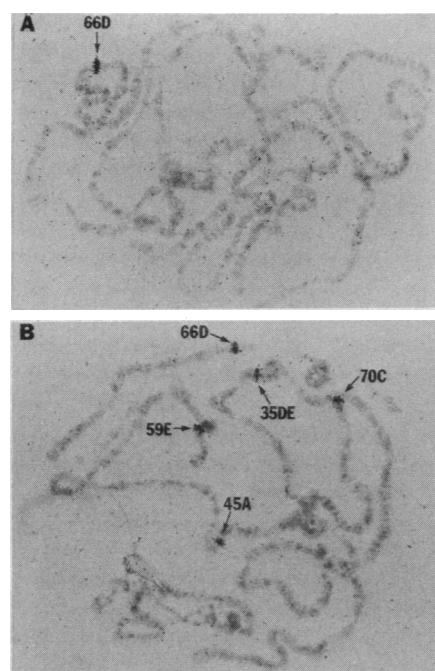


Fig. 6. Chromosomal sites of ch1 insertion as determined by in situ hybridization of p302.77 ³H-cRNA to polytene chromosomes. (A) The *sn*^w M host strain. A single site of labeling is seen at 66D, the location of the chorion genes on chromosome 3 (19). (B) The ch1 transformant line. Four new sites in addition to the 66D site are seen.

niques and that can be subjected to the powerful new techniques of in vitro mutagenesis (22). Transformation provides such an assay. *Drosophila melanogaster* is a widely used model system for developmental studies. Classical genetic studies have revealed the existence of numerous genes that appear to play important roles in the programming and regulation of developmental pathways (23) but whose products are unknown. P element-mediated transformation will greatly facilitate studies on the structure, regulation, and mechanism of action of such genes.

In the long term, transposable element vectors may be of significant use in modifying the genomes of plants and animals for beneficial purposes. They largely overcome the problems of low efficiency, rearrangement, and instability which limit the usefulness of current transformation techniques. They offer the further prospect of bringing the integration process itself under experimental control.

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