Genetic instability in *Drosophila melanogaster*: *P*-element mutagenesis by gene conversion

(mobile elements/transcriptional control)

PAMELA K. GEYER*, KAREN L. RICHARDSON*, VICTOR G. CORCES*†, AND M. M. GREEN‡

*Department of Biology, The Johns Hopkins University, 34th and Charles Streets, Baltimore, MD 21218; and ‡Department of Genetics, University of California, Davis, CA 95616

ABSTRACT We report the molecular characterization of several *P* element-induced mutations and their revertants at the yellow (*y*) locus of *Drosophila melanogaster*. One of the mutants analyzed, y^{76d28}, results from the insertion of a *P* element into the 5'-transcribed, untranslated portion of the *y* gene. Sequence analysis of several revertants of y^{76d28} shows that *P* excision occurs imprecisely. These events result in insertion of additional ATG codons in the *y* locus mRNA but are without phenotypic effect. In addition, we describe the molecular structure of *P*-associated mutations induced in a near wild-type revertant of y^{76d28} that carries an internally deleted 0.4-kilobase *P* element in the 5' noncoding region. Sequence analysis of two of these mutants demonstrates that they arose as a result of the integration of a larger *P* element at the exact location as in the parental stock without the 8-base-pair additional duplication associated with *P* insertions. The phenotype of these *y* alleles is dependent on the size and orientation of the integrated *P* element. We infer that *P*-element replacement in these mutants has occurred by a recombination/gene conversion mechanism.

Several classes of transposable elements have been identified in *Drosophila melanogaster* that collectively account for 10% of the genome (1). Molecular analyses of mutations at a number of loci have indicated that the major cause of spontaneous mutations in *D. melanogaster* is insertion of a transposable element into a gene altering its expression (2–5). Mechanisms by which these elements transpose and exert their mutagenic effects depend on the properties of a given element.

One of the best characterized elements in *D. melanogaster* is the *P* element. Complete *P* elements are 2.9 kilobases (kb) long and contain short 31-base-pair (bp) terminal inverted repeats flanking four open reading frames that encode a transposase function (6). *P*-element transposition is restricted to germ-line cells as a result of a tissue-specific splicing event required for the production of transposase (7). In addition, *P*-element movement is influenced by the presence of a repressor protein, thought to be produced from the *P* mRNA in which the germ-line-specific splice has failed to occur (8). *P* elements form a heterogeneous family of elements originating from the autonomous 2.9-kb *P* factor by internal deletions (6).

Insertion sites of *P* elements have been found to be clustered in target genes (9–12). In several cases, these sites are located in the 5' end, either upstream of the transcribed region or in the transcribed, untranslated portion of the gene (9–12). Mechanisms by which these *P* insertions affect gene expression are not well established. In at least one case, transcriptional interference has been implicated (13).

We have used the yellow (*y*) locus as a model system to study the mobilization and mutagenic effects of *P* elements. The *y* gene is required for production of the normal brownish–black pigmentation of larval and adult cuticle structures. This gene encodes a single 1.9-kb RNA that is expressed during the late embryo–early larval stages and in mid-to-late pupal stages (12, 14, 15). The spatial and temporal expression of this gene is controlled by a complex set of cis-linked regulatory sequences. Two enhancer-like elements located in the 5' flanking sequences are required for wing and body pigmentation. A bristle-specific control region is located within the intron, whereas a fourth region that is linked to the "TATA box" is required for expression in larval structures (16). Here we describe the characterization of the *P*-induced *y* mutation y^{76d28} and several of its reversions. Additionally, we describe *P*-associated *y* mutations that were generated in crosses involving an *MR* element. *MR* elements are genetically mappable loci that produce unstable mutations resulting from the insertion of *P* elements (12, 17, 18). Our experiments elucidate mechanisms by which *P* elements influence *y* gene expression. In addition, our studies suggest that *P*-element integration can occur by a recombination/gene conversion mechanism.

MATERIALS AND METHODS

***P*-Element Mutagenesis.** *P*-induced *y* mutations analyzed throughout this study were obtained by using the original *P* mutagenesis system described by Hiraizumi (18). This system exploits *P*-element mobilization and integration under control of an *MR* element. Several different *MR* elements have been isolated. All are equivalent except in their association with gonadal dysgenesis (19). We have used either a second chromosome element [e.g., *MRh12* or *MRnl* (20, 21)] or a third chromosome element [*MRTx(3)1* (19)] to generate revertants of y^{76d28} and other mutations.

Molecular Analysis of *y* Alleles. *D. melanogaster* DNA was prepared as described (15). Construction of genomic libraries in EMBL3 and the subsequent analysis were carried out by using standard procedures (22). RNA was prepared from late pupae by using the NaDodSO$_4$/phenol technique (23). RNA gel blot analysis of purified RNA was performed as reported (24). DNA sequencing was done as described by Maxam and Gilbert (25).

RESULTS

Isolation of *y* Mutants and Revertant Stocks. The mutant y^{76d28} arose as a single yellow female in the progeny of a cross between a y^+ male whose germ line carried mobilized *P* elements and a female of the genotype Maxy/y^-Basc (21). Maxy is a multiple recessive marker X chromosome carrying y^1, and y^-Basc is a balancer chromosome containing a deletion of *y*. A yellow female could only arise as a result of

†To whom reprint requests should be addressed.

a mutation in the paternal X chromosome. In y^{76d28} flies, pigmentation of all adult cuticular structures is tan. This indicates that *y* gene expression is not affected in a tissue-specific manner but is reduced in all cell types to levels intermediate between wild-type flies and flies carrying a deficiency for the *y* gene. Revertants of y^{76d28} were obtained by crossing homozygous y^{76d28} females to males carrying the *MR-Tx(3)1* element. Since mobilization of *P* elements occurs in the germ line of the F_1 progeny, revertants will only be obtained in the F_2 generation. Thus, wild-type flies were isolated from the progeny of a cross between F_1 males and y^-ac^- females (Fig. 1). Revertants were obtained at a frequency of 1 in 500 chromosomes scored. The molecular analysis of three revertants, y^{+3}, y^{+13}, and y^{+13-11}, is described below. Additional mutations derived from y^{+13-11} were obtained in a similar manner. Homozygous y^{+13-11} females were crossed to *MR-Tx(3)1* males, and the resulting F_1 males were crossed to y^-ac^- females. In this case, yellow flies were selected. Additional mutations arose at a frequency of about 1 in 100. Two phenotypic classes were obtained among the progeny of these crosses. The phenotype of one class was indistinguishable from that of a null allele and these flies are designated y^1; in the second class pigmentation was reduced relative to wild type in all tissues, as was seen in y^{76d28}, and are called y^2. Fig. 1 shows the mutants that were analyzed here.

Analysis of y^{76d28}. As a first step in determining the molecular basis of the y^{76d28} phenotype, Southern analysis of genomic DNA was undertaken. The wild-type restriction pattern of the *y* gene is shown in Fig. 2A. Genomic DNA isolated from y^{76d28} was digested with the restriction endonucleases *Bam*HI and *Hind*III, transferred to nitrocellulose, and hybridized with *y* sequences. The results of this experiment demonstrate that the pattern of restriction fragments obtained from y^{76d28} DNA differs from wild type only by the replacement of the 0.7-kb *Hind*III–*Bam*HI fragment with a band of 1.6 kb (Fig. 3), suggesting that there is an insertion of 0.9 kb. This *Hind*III–*Bam*HI fragment contains the 5′ end of the *y* gene (Fig. 2A) and is the region identified to be a hot spot for *P*-element integration (9). A genomic λ library was then prepared from these flies, the mutant *y* gene was isolated, and DNA sequence analysis was performed. The insertion into y^{76d28} is a 1.1-kb *P* element integrated 76 bp downstream from the mRNA cap site in the 5′-transcribed, untranslated portion of the *y* gene (Fig. 2A). A restriction map
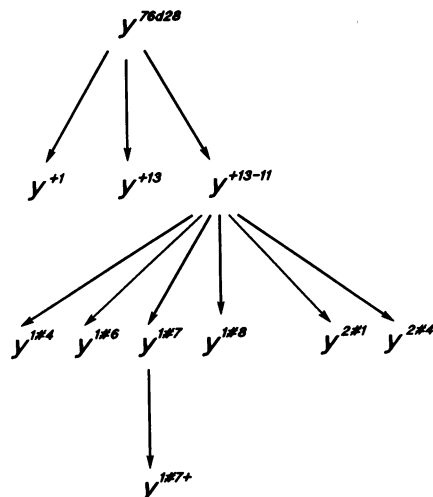

FIG. 1. Pedigree of *P*-induced alleles and revertants of *y*. The origin of *y* revertants and additional mutants is shown here. The arrows represent crosses of the parental flies to an *MR*-containing strain, *Tx(3)1*. The flies indicated in these crosses were all obtained in the F_2 generation.

of this *P* element is shown in Fig. 2B. This *P* element contains residues 1–808 fused to residues 2560–2904, as described by O'Hare and Rubin (6). This defective element possesses all transcription initiation and termination signals as well as sequences required in cis for transposition. The discrepancy between the predicted and observed size of the insertion in y^{76d28} is due to the presence of a *Hind*III site in the *P* DNA 200 bp upstream of the *Bam*HI site in the *y* locus. Thus, digestion of y^{76d28} genomic DNA with these two enzymes produces two fragments, one of 1.6 kb and one of 0.2 kb. Only the larger fragment is retained on nitrocellulose and is, therefore, the only new band observed. The 1.1-kb *P* element is integrated into the *y* locus so that its direction of transcription is opposite to that of the *y* gene. RNA analysis was done to determine the structure of the *y* mRNA produced in this mutant. Poly(A)$^+$ RNA was isolated from three stages of pupal development, electrophoresed on a formaldehyde/1.5% agarose gel, transferred to nitrocellulose, and hybridized with either a mixture of a *y* cDNA probe and a probe containing the *Drosophila ras2* gene (Fig. 4A) or the *y* cDNA probe alone (Fig. 4B). Hybridization to the *ras2* gene serves as a control for the amount of RNA loaded per lane (26). Two *y* mRNA transcripts of 3.0 kb and 1.9 kb are present. The size of the larger transcript corresponds to an RNA that is initiated at the normal promoter, reads through the *P* sequences, and terminates at the correct site. The second RNA is wild type in size, suggesting that *P* sequences are not present in this transcript. The temporal appearance of both transcripts is similar to that of wild-type RNA in that accumulation is maximal on day 8 of development. The amount of both RNAs is greatly reduced relative to wild-type levels, which probably accounts for the fact that pigmentation in these flies is tan.

Revertant Alleles of y^{76d28}. A high frequency of reversion of y^{76d28} was found among the progeny of males bearing y^{76d28} and a paternally inherited *MR* element. To assess genetically the extent to which the putative y^+ revertants restored wild-type function, eight revertants were tested for their ability to evoke the curled wing phenotype of the divers (*dvr*) mutation. The *dvr* mutant phenotype is manifested only with the concurrent presence of a mutation or deletion of the *y* gene (ref. 27; M.M.G., unpublished observation). Seven y^+ revertants, including y^{+1} and y^{+13}, failed to elicit the *dvr* phenotype, whereas one, y^{+13-11}, did show curly wings. Closer examination of y^{+13-11} males showed pigmentation is slightly lighter than wild type. Thus, this assay provides a sensitive measure with which to judge *y* gene function. Genomic Southern analysis was performed to determine the molecular basis for these two classes. DNA was isolated from y^{+1}, y^{+13}, and y^{+13-11}, digested with *Hind*III plus *Bam*HI, and analyzed by Southern hybridization. The restriction pattern generated from y^{+1} and y^{+13} was indistinguishable from that of wild-type DNA (data not shown). However, DNA fragments obtained in the digest of y^{+13-11} DNA showed a replacement of the wild-type 0.7-kb *Hind*III–*Bam*HI fragment with a 0.9-kb band, suggesting an insertion of 0.2 kb (Fig. 3). The molecular structure of y^{+1}, y^{+13}, and y^{+13-11} was determined from DNA sequence analysis of *y* DNA isolated from each revertant. The sequence information obtained in these studies is summarized in Fig. 5. Each revertant contains residual *P*-element DNA in the 5′ untranslated region. A similar result was observed from DNA sequence analysis of the revertant $y^{1#7+}$ that was obtained from $y^{1#7}$ (Fig. 5). The revertant y^{+13} contains 4 bp of *P* DNA plus the 8-bp insertion duplication, y^{+1} contains 20 bp of the *P* element 31-bp repeats plus the 8-bp duplication, and y^{+13-11} contains a *P* element of 405 bp plus the 8-bp duplication. The *P* element in y^{+13-11} is inserted in the same orientation as in y^{76d28}, thus in the opposite transcriptional orientation relative to *y*. This defective *P* element fuses

Genetics: Geyer *et al.*

Proc. Natl. Acad. Sci. USA 85 (1988) 6457

A

P element

Sal I Hind III Bgl II Hind III Hind III Bam HI Cla I Cla I Eco RI Bgl II

$y^{1\#8}$

500 bp

B

Hind III Xho I / Hind III Pst I Eco RI Pst I Sal I

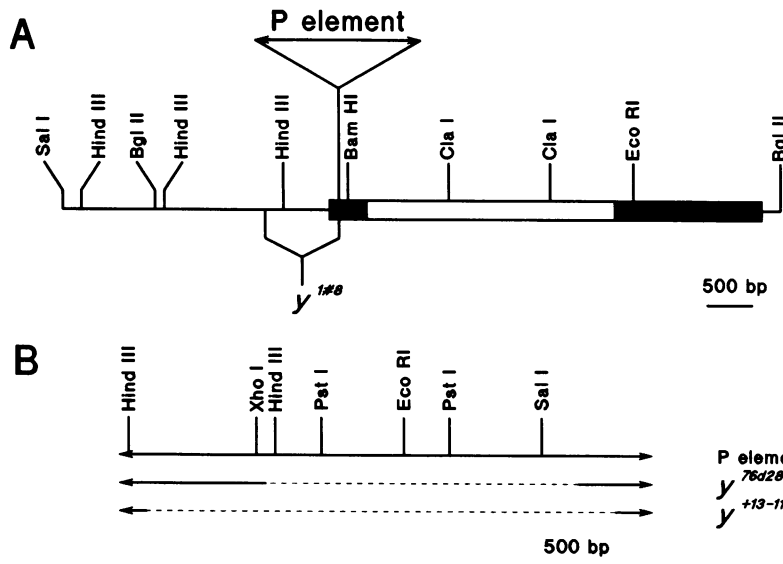500 bp

P element y^{76d28}
y^{76d28}
y^{+13-11}

FIG. 2. Restriction map of the *y* gene and *P* elements associated with the alleles y^{76d28} and y^{+13-11}. (*A*) *y* Gene. Solid boxes, exons; open box, intron. The *P* element in y^{76d28} and y^{+13-11} is inserted 76 bp from the transcription initiation site, indicated by the triangle. These elements are positioned in the opposite transcriptional orientation relative to the *y* gene. Indicated below the line is the position of the endpoints of the deletion in $y^{1\#8}$ at position −712 and position +81 relative to the *y* cap site. (*B*) Restriction map of the *P* elements in various *y* mutants and the prototypic *P* element (6). The *P*-element sequences deleted in the two derivative elements are shown by dashed lines. The deletion endpoints in the y^{76d28} *P* DNA are 808 bp and 2560 bp and in the y^{+13-11} *P* DNA the deletion endpoints are 226 bp and 2723 bp. This numbering system is as described by O'Hare and Rubin (6).

residue 226 to residue 2723 and contains transcription initiation signals. Here again, the discrepancy between the predicted and observed size of the insertion in y^{+13-11} is due to the presence of a *Hind*III site in the *P* DNA 200 bp upstream of the *y Bam*HI site. To determine the effect of the residual 0.4 kb of *P* DNA on the structure of the *y* mRNA, analysis of late pupal poly(A)$^+$ RNA was performed as described above. The results are shown in Fig. 4. As seen in RNA isolated from y^{76d28}, y^{+13-11} produces two *y* mRNA species, one of 1.9 kb and one of 2.3 kb, which is the size predicted for a hybrid RNA composed of *P* and *y* sequences. The 1.9-kb RNA accumulates at a higher level than in y^{76d28} and is close to wild-type levels explaining the restoration of pigmentation in these flies.

The *y* Gene in y^{+13-11} Is a Hot Spot for *P* Insertion. The revertant y^{+13-11} was tested to determine whether the presence of the 0.4 kb of *P* DNA would increase the

frequency of *P* mutagenesis of the *y* gene by an *MR* element. As a control, parallel mutagenesis was carried out by using y^{+1} and y^{+13} males that have 4 and 20 bp of *P* DNA remaining. In each case, revertant males carrying a paternally inherited *MR* element were crossed to females bearing a deletion of the *y* gene. Mutations were scored in the resulting females. Four separate experiments were carried out, two with y^{+13-11}, one with y^{+1}, and one with y^{+13} flies. In the first experiment with y^{+13-11} males, 93 *y* mutations (86 y^1-like and 7 y^2-like) were recovered among 7608 X chromosomes scored. In a second experiment, 78 mutations (64 y^1-like and 14 y^2-like) were found among 5216 X chromosomes scored. No *y* mutations were found in 6947 X chromosomes derived from y^{+1} and only 1 *y* mutation was found in female progeny

$y^{2\#1}$ y^{+13-11} y^{76d28} wt

A 6 7 8 6 7 8 6 7 8 6 7 8 kb

◄ 2.3
◄ 1.9
◄ 1.6

B

◄ 3.0
◄ 2.3
◄ 1.9

Ore R y^{76d28} y^{+13-11} $y^{2\#1}$ $y^{2\#4}$ $y^{1\#4}$ $y^{1\#6}$ $y^{1\#8}$

kb   kb
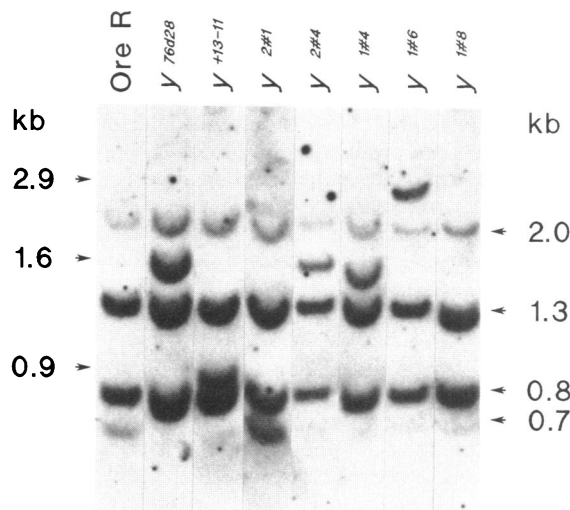
2.9 ►

◄ 2.0

1.6 ►

◄ 1.3

0.9 ►

◄ 0.8
◄ 0.7

FIG. 3. Southern analysis of genomic DNA from *P*-induced and revertant alleles. Five micrograms of total DNA from Oregon R, the *P*-induced allele y^{76d28}, a phenotypic revertant y^{+13-11}, and mutants derived from y^{+13-11} ($y^{2\#1}$, $y^{2\#4}$, $y^{1\#4}$, $y^{1\#6}$, and $y^{1\#8}$) were digested with *Hind*III plus *Bam*HI, electrophoresed on a 1% agarose gel, and blotted onto nitrocellulose. The filter was then probed with ^{32}P-labeled DNA from the *y* locus. The numbers at the sides indicate the size of the hybridizing fragment. The 2.0-kb band present in all lanes corresponds to the upstream *Hind*III fragment that spans the *Sal* I site indicated in Fig. 2. It should be noted that the intensity of the 0.7-kb band in the Oregon R lane is artificially low.
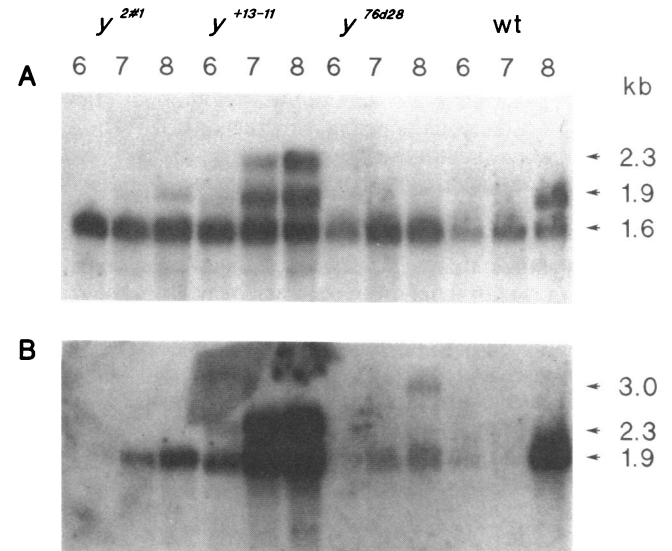
FIG. 4. Analysis of *y* mRNA. Ten micrograms of poly(A)$^+$ RNA isolated from 3 days of pupal development of $y^{2\#1}$, y^{+13-11}, y^{76d28}, and Canton S (wild type, wt) were electrophoresed on a 1.5% agarose/formaldehyde gel, blotted onto nitrocellulose, and hybridized with either a mixture of [^{32}P]DNA fragments containing a *y* cDNA and *ras2* gene (*A*) or only the *y* cDNA (*B*). The numbers at the top of the lanes refer to the number of days the flies developed after embryos were collected. The *y* gene hybridizes to a 1.9-kb RNA in Canton S and to extra transcripts of 3.0 and 2.3 kb in y^{76d28} and y^{+13-11}, respectively. The 1.6-kb RNA corresponds to the ras transcript that is expressed at approximately constant levels during *D. melanogaster* development and controls for the amount of RNA loaded per lane (26).
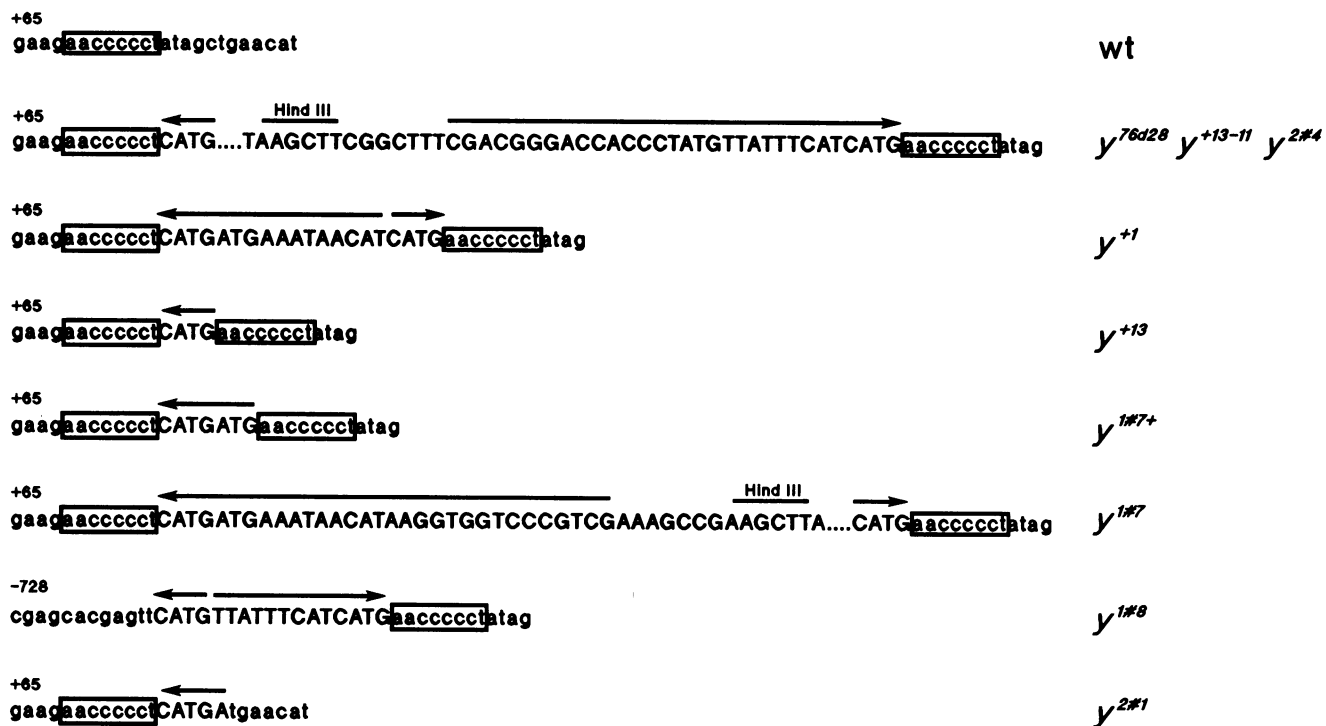
FIG. 5. Sequence structure of *y* alleles. Shown are the endpoints of *P* insertions obtained from sequence analysis of y^{76d28}, y^{+13-11}, $y^{2#4}$, y^{+1}, y^{+13}, $y^{1#7+}$, $y^{1#7}$, $y^{1#8}$, and $y^{2#1}$ DNA. These flies carry 1154 bp, 405 bp, 1154 bp, 20 bp, 4 bp, 7 bp, 1154 bp, 16 bp, and 5 bp of *P* DNA, respectively. The endpoints of the *P* elements inserted in y^{76d28} and y^{+13-11} are the same. For $y^{1#8}$ and $y^{2#1}$, *y* sequences are deleted along with the insertion of *P* DNA. The upper nucleotide sequence is that of Canton S illustrating the site of insertion of all of the *P* elements. The number at the left refers to the distance of that nucleotide relative to the start site of transcription of the *y* gene. The boxed areas show sequences duplicated upon *P*-element insertion. Lowercase letters are sequences of the *y* gene and uppercase letters are from the *P*-element insertion. The arrows indicate DNA of the 31-bp repeats.

of y^{+13} in 7104 chromosomes scored. The results from the control experiment reflect the expected frequency of *P*-element mutagenesis of a wild-type *y* gene that occurs at about 1 in 10,000 (21). Thus the y^{+13-11} gene is 100 times more prone to *P* mutagenesis than wild-type *y* genes. This frequency is an underestimate of the total number of events occurring at the locus, since some excisions of *P* DNA will not change the phenotype.

Genomic DNA of eight mutations induced in y^{+13-11} (six y^1 and two y^2) was isolated and characterized by Southern analysis as described above. These results are shown in Fig. 3. In two of the mutants, $y^{1#8}$ and $y^{2#4}$, the restriction pattern is consistent with deletion of the *P* element from the 0.7-kb *Hind*III–*Bam*HI fragment. The DNA restriction pattern from $y^{1#8}$ shows complete deletion of the 0.9-kb band present in y^{+13-11}, whereas that of $y^{2#1}$ shows a restoration of this band to wild-type size. In the remaining mutants, $y^{1#4}$, $y^{1#6}$, and $y^{2#4}$, the 0.9-kb band is replaced with new fragments of ≈1.5 kb, 2.9 kb, and 1.6 kb, respectively. A similar analysis of another mutant, $y^{1#7}$, indicated that additional sequences were also present in this region (data not shown). To understand the origin of these mutants, genomic λ libraries were constructed from flies representing each type of event, $y^{2#1}$, $y^{2#4}$, $y^{1#7}$, and $y^{1#8}$. The results of DNA sequence analysis of the mutant *y* genes are summarized in Fig. 5. They indicate that *y*' mutations are produced in two ways. In $y^{1#8}$, *P*-element excision produces a deletion of sequences upstream of the insertion site. The deleted DNA includes the TATA box and transcription initiation site, which explains the resulting null phenotype (Fig. 2*A*). In $y^{1#7}$, a *P* element replaced the one already present in y^{+13-11}. Sequence analysis of this insert revealed no additional duplication of either *P* or *y* DNA, as would be expected if this second element was inserted into the existing *P* element in y^{+13-11} by a conventional transposition event. The *P* element in-

serted in $y^{1#7}$ is identical to the one found in y^{76d28} but is in the opposite orientation.

The y^2 alleles are also produced in two ways. In $y^{2#4}$, the *P* element present in y^{+13-11} was replaced with a *P* element identical in sequence and orientation to that found in y^{76d28}. The mutant phenotype of $y^{2#1}$ flies results from imprecise excision of *P* DNA. In this case, along with deletion of the 8-bp duplication created by the initial *P* insert, 5 bp of *y* DNA were removed. Left behind are 5 bp of the 31-bp terminal repeats. Analysis of RNA was done to determine how this deletion affects the production of *y* mRNA. Poly(A)$^+$ RNA was isolated from late pupae and analyzed as described above. Results from this experiment are shown in Fig. 4. These flies accumulate a 1.9-kb RNA with a wild-type development profile but at a greatly reduced level.

DISCUSSION

Effect of *P*-Element Integration on *y* Gene Expression. Here, we present the molecular characterization of the *P*-induced mutation y^{76d28}, several revertants of this allele, and *P*-associated mutations obtained from the revertant y^{+13-11}. These mutations were created by an *MR* element, and in each case, they result from the insertion of a *P* element. Our results demonstrate that both size and orientation of the integrated *P* element are important in conferring a mutant phenotype. This conclusion is based on a comparison of the structure of *P* DNA found in three phenotypically distinct flies, y^{76d28}, the revertant y^{+13-11}, and the null allele $y^{1#7}$, which was derived from y^{+13-11}. In each case, a *P* element is inserted at the same nucleotide in the 5'-transcribed untranslated region of the *y* gene. In y^{76d28} and y^{+13-11}, the inserted *P* element is in the opposite transcriptional orientation relative to the *y* gene. RNA analysis showed that both flies produce a 1.9-kb RNA whose size and developmental expression are indistinguish-

able from wild type. In addition, they both accumulate a second transcript. The size of these larger transcripts suggests that they are hybrid P-y mRNAs that are correctly initiated and terminated at y sequences. The origin of the 1.9-kb mRNA can be explained by alternative hypotheses. It could result either from initiation within *P* sequences or, alternatively, *P*-element sequences could be spliced out of the larger RNAs due to the recognition of cryptic donor and acceptor sites near the *P*-element ends. In support of the second model, an *Mbo* II fragment containing sequences located upstream of the *P* element insertion site hybridized to both y mRNAs (data not shown), indicating that the normal y promoter is being used. In addition, a putative donor splice junction can be found close to the 31-bp repeat (CATAAG/CT) and an acceptor splice site is present in the region of the 8-bp duplication (CCTATAG/CT) (28). The amount of the 1.9-kb RNA that is accumulated depends on the size of the inserted *P* DNA. Near normal levels of RNA accumulate in y^{+13-11}, which carries a 0.4-kb element, whereas barely detectable levels accumulate in y^{76d28}, which has a 1.1-kb *P* element integrated. Thus, deletion of 0.7 kb of *P*-element sequences in y^{+13-11} either increases the stability of the hybrid RNA or removes DNA that affects the rate of y gene transcription. Reversing the orientation of the 1.1-kb element in y^{76d28}, as occurred in $y^{1\#7}$, inhibits y protein production since the phenotype is that of a null allele. Although RNA accumulation in these flies was not measured, it is doubtful that any 1.9-kb y mRNA is made. Transcription of this gene most likely results in the accumulation of aberrant products as a result of improper splicing of the mRNA at *P* sequences or by termination of transcription in *P* DNA.

Revertants of several *P*-induced mutations at a number of different loci have been isolated (9–13). These experiments have demonstrated that excision of *P* elements often occurs imprecisely, as we have observed for reversions of *P* mutations at the y gene. In two revertants of y^{76d28}, y^{+1} and y^{+3}, and one revertant of $y^{1\#7}$, $y^{1\#7+}$, imprecise excision occurred within the 31-bp inverted repeats leaving behind 4, 20, and 7 bp, respectively. A consequence of these excision events is the addition of an ATG upstream of the normal one. These additional initiation codons reside in sequences that fit the consensus sequence for translation initiation sites (29), and, therefore, might be functional. However, initiation at these ATG codons terminates within 4 amino acids. Since the phenotype of these flies is indistinguishable from wild type, this suggests that translation is still beginning at the normal site. It is interesting that in the mutant $y^{2\#4}$, excision of the *P* element left behind 5 bp of the inverted repeat but deleted the 8-bp duplication and 5 bp of y DNA. In this case, the first in-frame termination codon encountered is past the normal ATG. Analysis of late pupal mRNA in these flies shows that the resulting transcript accumulates at a reduced level relative to wild type. It is possible that this low level of y mRNA present results from a greater turnover of RNA as a consequence of faster release from the polysomes, since initiation beginning at the additional ATG would make only a small peptide. Alternatively, this deletion may remove sequences required for proper transcription of the y gene. In either case, translation initiation also occurs at the normal ATG since some y protein is produced.

Integration of *P* Elements by Gene Conversion. Characterization of y mutations obtained from the revertant y^{+13-11} shows an unusual mechanism by which *P* sequences integrate. In four of the six cases analyzed, additional DNA was present at the site of the original insertion. Sequence analysis of two of these mutations clearly demonstrated that a *P* element is inserted exactly where the old one resided. Since no duplication of *P* or y DNA was found, the *P* element is

probably inserted by recombination/gene conversion. The *P* element in y^{+13-11} is unstable only in the presence of an *MR* element, suggesting that production of double-strand breaks by the *P*-encoded transposase stimulates these events. Our studies demonstrate that a large percentage of events activated by *MR* elements are due to *P*-element replacement, not just excision. This suggests that a subset of *P* mobilization at other loci, which are interpreted to result from an internal deletion of *P* DNA, may actually be caused by the replacement of the larger *P* elements with a smaller one. *P*-element replacement similar to that observed here was suggested to occur in the vestigial locus in *D. melanogaster* (13).

Exactly the same 1.1-kb element was inserted in two y mutations derived from y^{+13-11}. This *P* element does not represent the major class present in the genome of these flies (data not shown). This suggests that either these sequences are preferentially mobilized or that this element is closely linked to the y gene and thereby acts as the source of DNA that replaces the 0.4-kb *P* element present in y^{+13-11}. The replaced 1.1-kb *P* element is identical to the *KP* element that has been linked to repression of *P* movement (30). Preferential replacement of *P* elements with this sequence may be a way in which flies acquire the ability to repress *P* mobilization.

1. Rubin, G. M. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. (Academic, New York), pp. 329–361.
2. Zachar, Z. & Bingham, P. M. (1982) *Cell* **30**, 529–541.
3. Bender, W., Akam, M., Karch, F., Beachy, P. A., Peifer, M., Spierer, P., Lewis, E. B. & Hogness, D. S. (1983) *Science* **221**, 23–29.
4. Kidd, S., Lockett, T. J. & Young, M. W. (1983) *Cell* **34**, 421–433.
5. Campuzano, S. L., Carramolino, L., Cabrera, C. V., Ruiz-Gomez, M., Villares, R., Boronat, A. & Modolell, J. (1985) *Cell* **40**, 327–338.
6. O'Hare, K. & Rubin, G. M. (1983) *Cell* **34**, 25–35.
7. Laski, F. A., Rio, D. C. & Rubin, G. M. (1986) *Cell* **44**, 7–19.
8. Rio, D. C., Laski, F. A. & Rubin, G. M. (1986) *Cell* **44**, 21–32.
9. Chia, W., Howes, G., Martin, M., Meng, Y. B., Moses, K. & Tsubota, S. (1986) *EMBO J.* **5**, 3597–3605.
10. Tsubota, S. & Schedl, P, (1986) *Genetics* **114**, 165–182.
11. Searles, L. L., Greenleaf, A. L., Kemp, W. E. & Voelker, R. A. (1986) *Mol. Cell. Biol.* **6**, 3312–3319.
12. Kelley, M. R., Kidd, S., Berg, R. L. & Young, M. W. (1987) *Mol. Cell. Biol.* **7**, 1545–1548.
13. William, J. A., Pappu, S. S. & Bell, J. B. (1988) *Mol. Cell. Biol.* **8**, 1489–1497.
14. Biessman, H. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7369–7373.
15. Parkhurst, S. M. & Corces, V. G. (1986) *Mol. Cell. Biol.* **6**, 47–53.
16. Geyer, P. K. & Corces, V. G. (1987) *Genes Dev.* **1**, 996–1004.
17. Roiha, H., Rubin, G. M. & O'Hare, K. (1988) *Genetics* **119**, 75–83.
18. Hiraizumi, Y. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 268–270.
19. Green, M. M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1036–1040.
20. Green, M. M. (1979) *Mutat. Res.* **59**, 291–293.
21. Green, M. M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3490–3493.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
23. Spradling, A. C. & Mahowald, A. P. (1979) *Cell* **16**, 589–598.
24. Geyer, P. K., Spana, C. & Corces, V. G. (1986) *EMBO J.* **5**, 2657–2662.
25. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
26. Mozer, B., Marlor, R., Parkhurst, S. M. & Corces, V. G. (1985) *Mol. Cell. Biol.* **5**, 885–889.
27. Sakharov, V. V. (1937) *Drosoph. Inf. Serv.* **8**, 81.
28. Snyder, M., Hunkapiller, M., Yuen, D., Silvert, D., Fristrom, J. W. & Davidson, N. (1982) *Cell* **29**, 1027–1040.
29. Kozak, M. (1986) *Cell* **44**, 283–292.
30. Black, D. M., Jackson, M. S., Kidwell, M. G. & Dover, G. A. (1987) *EMBO J.* **6**, 4125–4135.