Copyright © 1998 by the Genetics Society of America

Y-Linked Male Sterile Mutations Induced by *P* Element in *Drosophila melanogaster*

Ping Zhang and Rebecca L. Stankiewicz

Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06269

Manuscript received April 10, 1998
Accepted for publication June 26, 1998

ABSTRACT

The *Y* chromosome in *Drosophila melanogaster* is composed of highly repetitive sequences and is essential only in the male germ line. We employed *P*-element insertional mutagenesis to induce male sterile mutations in the *Y* chromosome. By using a combination of two modifiers of position effect variegation, adding an extra *Y* chromosome and increasing temperature, we isolated 61 *P(ry*$^{+}$*)* elements in the *Y* chromosome. Six of these *Y*-linked insertions (approximately 10%) induced male sterile mutations that are mapped to two genes on the long and one on the short arms of the *Y* chromosome. These mutations are revertible to the wild type in a cell-autonomous and germ-line-dependent manner, consistent with previously defined *Y*-linked gene functions. Phenotypes associated with these *P*-induced mutations are similar to those resulting from deletions of the *Y* chromosome regions corresponding to the male fertility genes. Three alleles of the *kl-3* gene on the *Y* long arm result in loss of the axonemal outer dynein arms in the spermatid tail, while three *ks-2* alleles on the *Y* short arm induce defects at early postmeiotic stages. The recovery of the *ms(Y)* mutations induced by single *P*-element insertions will facilitate our effort to understand the structural and functional properties of the *Y* chromosome.

THE *Y* chromosome in *Drosophila melanogaster* is entirely heterochromatic and accounts for approximately 12% of a normal male genome (Gatti and Pimpinelli 1992). Drosophila males lacking a *Y* chromosome, *i.e.*, *X*O males, have normal appearance, but are completely sterile (Bridges 1916a,b). The functions of the *Y* chromosome are essential only in the male germline cells, because males developed from *X*O embryos that were transplanted with *XY* germ-line cells produced mature sperm (Marsh and Wieschaus 1978). Despite the large size of the *Y* chromosome, only six *Y*-linked male fertility genes have been identified in near saturation mutagenesis, with four on the long arm and two on the short arm (Kennison 1981; Hazelrigg *et al.* 1982; Gatti and Pimpinelli 1983). In addition to the male fertility genes, the *Y* chromosome contains the *bb* locus, which encodes the rRNA genes (Ritossa 1976), and the *Su(Ste)* locus, which interacts with the *X*-linked *Ste* locus (Livak 1990).

Male sterile mutations in the *Y* chromosome are associated with various defects at different stages of spermatogenesis, which were shown by using deletions of each of the six fertility genes (Hardy *et al.* 1981). Deletion of either the *Y*-linked *kl-3* or *kl-5* gene results in loss of the outer arm dyneins in the sperm flagellar axoneme (Hardy *et al.* 1981). The presence of the *kl-2*, *kl-3*, and *kl-5* genes is correlated with the syntheses of three high-molecular-weight polypeptides with similar electrophoretic mobility to Chlamydomonas dynein heavy chains (Goldstein *et al.* 1982). Moreover, Gepner and Hays (1993) cloned a genomic DNA fragment by PCR and mapped it into the *Y*-linked *kl-5* region. Its sequence is capable of encoding a portion of a dynein β-heavy chain polypeptide, suggesting that the *kl-5* gene encodes a dynein subunit.

The Drosophila *Y* chromosome is associated with unusual properties. Though only six genes essential for spermatogenesis are located on the *Y* chromosome, *Y*-linked male sterile mutations have been isolated at exceptionally high frequency after EMS or X-ray treatment. Mutability of *Y*-linked loci to male sterility is as much as 100 times of *X*-linked loci to male sterility (see Lindsley and Tokuyasu 1980; Fuller 1993). It is unclear why the *Y* chromosome is hypersensitive to the mutagens. Cytogenetic studies showed that three of the fertility genes, *kl-5*, *kl-3*, and *ks-1*, are unusually large in physical sizes up to several megabase pairs (Gatti and Pimpinelli 1983; Bonaccorsi *et al.* 1988). The chromosome regions corresponding to these genes contain largely simple satellite and other repetitive sequences (Bonaccorsi and Lohe 1991). Peculiar nuclear structures known as giant lampbrush loop-like structures are present in the primary spermatocytes and are associated specifically with the *Y*-chromosomal regions containing the *kl-5*, *kl-3*, and *ks-1* fertility genes (Bonaccorsi *et al.* 1988). It has been proposed that the nuclear structures in the primary spermatocytes are essential for male fertility (Hennig 1985; Bonaccorsi *et al.* 1988; Gatti and Pimpinelli 1992).

With the advent of efficient genetic methods to isolate *P* elements located in Drosophila heterochromatin

Corresponding author: Ping Zhang, Department of Molecular and Cell Biology, U-131, University of Connecticut, 354 Mansfield Road, Storrs, CT 06269. E-mail: ping@uconnvm.uconn.edu

(Zhang and Spradling 1994; Roseman *et al.* 1995; Wallrath and Elgin 1995), it is now possible to mutagenize and isolate male sterile mutations in the *Y* chromosome of *D. melanogaster* by using single *P*-element insertional mutagenesis (Cooley *et al.* 1988). Marker genes in the *P* element, either ry^+, w^+, or y^+, are suppressed by position effect variegation (PEV), when the transposons are located in the heterochromatic *Y* chromosome. Previous studies reported the recovery of genetically marked *P* elements in the *Y* chromosome (Berg and Spradling 1991; Karpen and Spradling 1992). Despite variation of $rosy^+$ eye color in individual males, the *Y*-linked *P* elements were identified at low frequency by expression of the ry^+ marker in regular *XY* males. The physical locations of these *P* elements are restricted to the subtelomeric regions of the *Y* chromosome (Zhang and Spradling 1994). Further studies (Zhang and Spradling 1994) have shown that *P* insertions into the *Y* chromosome could be recovered at higher frequency under conditions where PEV is suppressed by adding an extra *Y* chromosome, a strong and broad PEV suppressor (Spofford 1976; Henikoff 1992; Lloyd *et al.* 1997). Moreover, the *Y*-linked *P* elements isolated by suppressing PEV are distributed throughout many regions of the *Y* chromosome. In this article we report the isolation and genetic characterization of male sterile mutations in the *Y* chromosome that are induced by single *P* elements.

MATERIALS AND METHODS

Drosophila strains: Flies were cultured on standard cornmeal and agar media at 22°, unless stated otherwise. Six stocks with *X-Y* reciprocal translocations were obtained from the Bloomington Stock Center: *T(1;Y)V24, y* y^+ *w f* B^S; *T(1;Y;3)W27, y* y^+ *w f* B^S; *T(1;Y)E15, y* y^+ *w f* B^S; *T(1;Y)F12, y* y^+ *w f* B^S; *T(1;Y)V8, y* y^+ *w* B^S; and *T(1;Y)W19, y* y^+ *w f* B^S. We constructed a strain, *C(1)RM, y v/C(1;Y)1, y; TMS, Sb P[*ry^+ *Δ2-3](99B)/Dr*, to facilitate a genetic screen described below. Three enhancer-trap lines, which express *lacZ* in the adult male germ line, were obtained from the Bloomington Stock Center: cue^2, *ms(2)21D*, and $Rb97D^1$.

The *Y95-2* chromosome is a wild-type *Y* chromosome except that it carries a *P(lacZ,* ry^+*)* element [Mlodzik and Hiromi 1992; hereafter called *P(*ry^+*)*] within the h11-13 region of the *Y* long arm (Zhang and Spradling 1994). Phenotypically, the ry^+ gene in *Y95-2* is silent due to strong position effect variegation.

For the separable components of translocations (Ts, Translocation segregant) current nomenclature adopted by FlyBase is used throughout this report.

A genetic screen to isolate *Y*-linked *P* elements: To recover male sterile *Y* chromosomes from a genetic screen shown in Figure 1, the *P(*ry^+*)* element in *Y95-2* was activated by a transposase source (*Δ2-3*) in the female germ lines. The targeted *Y* chromosome was kept as a nonessential element throughout the *P*-element mutagenesis. F_1 females, *C(1)RM, y v/Y95-2; TMS, Sb* $\Delta 2\text{-}3/ry^{506}$, were produced in bottles from a cross between F_0 *y/Y95-2;* ry^{506}/ry^{506} males and *C(1)RM, y v/0; TMS, Sb* $\Delta 2\text{-}3$*/Dr* females. The F_1 females were virgins because the sibling males were sterile *X*O. Five F_1 females were crossed to five *C(1;Y)1, y/0;* ry^{506}/ry^{506} males in vials. Under conditions where PEV was suppressed by an extra *Y* chromosome in F_2 males of *C(1;Y)1, y/Y*;* ry^{506}/ry^{506} (*Y**: a mutagenized *Y95-2* chromosome after exposure to the transposase source), new insertions were detected and recovered in individual F_2 males expressing the $rosy^+$ eye color. The F_2 males also enabled the recovery of a male sterile *Y* chromosome because they had an extra wild-type *Y* chromosome to provide essential functions for spermatogenesis. Up to two F_2 males of *C(1;Y)1, y/Y*;* ry^{506}/ry^{506} expressing the $rosy^+$ eye color were collected from a vial and crossed singly to *C(1)RM, y v/0;* ry^{506}/ry^{506} females in vials to produce two types of progeny (F_3). When a *P(*ry^+*)* element is inserted in heterochromatin, $rosy^+$ eye color was enhanced in the F_3 females, *C(1)RM, y v/Y**, by the extra *Y* chromosome. If the new *P(*ry^+*)* element is located in the *Y* chromosome, the F_3 females but not the males expressed the $rosy^+$ eye color. If an insertion is located in the autosomal heterochromatin, however, level of $rosy^+$ gene expression varies greatly among the individual F_3 males because of lack of an extra *Y* chromosome to suppress PEV. Eye color variegation among the individual facets is not expected, since ry^+ gene expression is not cell autonomous in the eye (Rushlow and Chovnick 1984; Rushlow *et al.* 1984).

Male fertility test: To identify *ms(Y)* mutations, each of the *Y** chromosomes carrying a newly transposed *P(*ry^+*)* element was tested in *XY** males for male fertility. A genetic cross between *C(1;Y)1, y/Y*;* ry^{506}/ry^{506} males and *y/y;* ry^{506}/ry^{506} females was used to produce *y/Y*;* ry^{506}/ry^{506} males whose fertility was tested. At least five *y/Y*;* ry^{506}/ry^{506} males of 1–3 day age were mated individually to five females in vials and their progeny were counted to determine male fertility. Similarly, in the genetic complementation analysis involving the *T(1;Y)* chromosomes, two young males of *Ts(1;Y)/ms(Y)* were mated to five females in a vial to examine their fertility.

Fluorescence *in situ* hybridization: Physical locations of the *P(*ry^+*)* elements in the *Y* chromosome were determined by using fluorescence *in situ* hybridization to the metaphase *Y* chromosome, which was carried out as described previously (Zhang and Spradling 1994). Two DNA clones, pMC1872 (Casadaban *et al.* 1983) and Carnegie 20 (Rubin and Spradling 1983), which encompass over 90% of the *P(*ry^+*)* element, were used as the *in situ* probes to detect the element inserted into the *Y* chromosome.

Examination of male sterile phenotypes by phase-contrast microscopy: To study mutant phenotypes in spermatogenesis by phase-contrast microscopy, testes of 1 to 3-day-old males were dissected out in PBS and transferred to a drop of PBS on a glass slide. The testes were torn open, squashed under the weight of a coverslip (Kemphues *et al.* 1980), and examined for defects in the primary and secondary spermatocytes, and young spermatids.

Staining of testes with X-gal and DAPI: To examine the nuclear shape and distribution of the postmeiotic germ-line cells, testes were dissected out from 1- to 3-day-old males in PBS. The germ-line nuclei were labeled with X-gal staining (Montell *et al.* 1992) by using three enhancer-trap lines, cue^2, *ms(2)21D*, and $Rb97D^1$, which express the *lacZ* gene in the adult male germ lines (Castrillon *et al.* 1993). Sterile *X/ms(Y)* males carrying each of the enhancer-trap *lacZ* constructs were produced from crosses between *C(1)DX/ms(Y)* females and *XY* males from each of the three enhancer-trap lines. After X-gal staining, the testes were stained with 4′,6-diamidino-2-phenylindole (DAPI) at 0.5 μg/ml in PBS for 5 min and mounted to a slide with the DAPI staining solution.

Examination of spermatid postelongation defects by electron microscopy: Testes were prepared as described in Tokuyasu *et al.* (1972) and as modified by Hardy *et al.* (1981) with the following exceptions. Testes were dissected in phosphate buffer containing 2 mm $MgCl_2$ (pH 7.4) and then placed

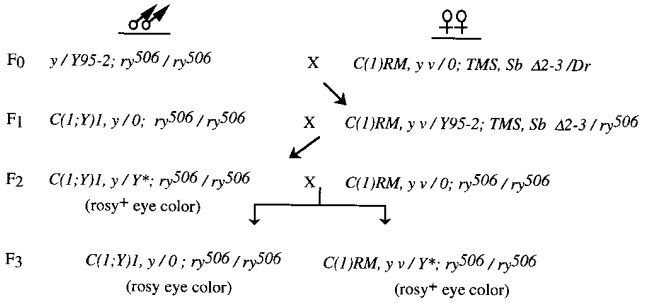

Figure 1.—A genetic screen to isolate single *P* elements in the *Y* chromosome. A genetically silent *P(ry*$^+$*)* element in *Y95-2* was used as a starting transposon to increase local transposition activity in the *Y* chromosome. A balancer, *TMS*, was used to suppress recombination between *Sb* and *Δ2-3 (99B)*. *Y** represents a mutagenized *Y* chromosome. For details see materials and methods.

immediately into 2% glutaraldehyde made in the same buffer. The tissues were embedded in a 1:1:2.4 mixture of Araldite 6005, SPI-pon 812, and dodecenyl succinic anhydride, respectively, followed by mixing with 1.5% (v/v) DMP-30 [2,4,5, tri(dimethylaminomethy)phenol]. Sections of 70–100 nm were viewed at 100 kV accelerating voltage on a Zeiss EM910 transmission electron microscope with a single tilt stage. Tilt was determined by the view which gave clearly illustrated dense cylinders located in the central and nine peripheral singlets.

RESULTS

Isolation of *Y*-linked male sterile mutations induced by single *P* elements: Two steps were taken to recover *Y* chromosomes that carry *P*-induced male sterile mutations. First, using a genetic screen, we isolated *Y* chromosomes that have acquired new *P(ry*$^+$*)* elements through single *P*-element insertional mutagenesis (Cooley *et al.* 1988). Second, we identified *Y*-linked male sterile mutations among the *P(ry*$^+$*)*-carrying *Y* chromosomes isolated in the screen.

The genetic screen (Figure 1) shared several features that were employed in a previous experiment designed to isolate *P* elements in Drosophila heterochromatin (Zhang and Spradling 1994). A *P(ry*$^+$*)* element located on the long arm of the *Y95-2* chromosome (see materials and methods) was used as the starting transposon. Because this *P(ry*$^+$*)* element is subject to strong PEV, its *ry*$^+$ marker gene fails to express even in the presence of an extra *Y* chromosome, which suppresses PEV (Spofford 1976). This genetically silent *P(ry*$^+$*)* element allowed simple phenotypic detection, *i.e.*, rosy$^+$ eye color, of newly transposed *P(ry*$^+$*)* elements into different *Y* chromosomal regions, where PEV is less strong than *Y95-2*. Moreover, starting with this element in the *Y95-2* chromosome produced more new *Y*-linked insertions because of *P*-element local transposition activity.

Previous studies suggest that an extra *Y* chromosome alone is insufficient to suppress PEV on the *ry*$^+$ gene located within heterochromatin (Zhang and Spradling 1994). Increasing fly culture temperature allows higher levels of the *ry*$^+$ gene expression, consistent with a typical PEV response (Spofford 1976). Therefore, we expected to recover more heterochromatic *P(ry*$^+$*)* insertions in the *Y* chromosome by increasing the incubation temperature. However, at 27°, the *P(ry*$^+$*)* element in *Y95-2* expresses its *ry*$^+$ gene in approximately 5% adults carrying an extra *Y* chromosome, interfering with our genetic detection using rosy$^+$ eye color. We found empirically that *Y95-2* does not express rosy$^+$ phenotype at 25.5°. As a result, we carried out the genetic screen at 25.5°, instead of 22°.

The genetic screen described in Figure 1 generated a total of 1056 sublines containing newly transposed *P(ry*$^+$*)* insertions. Among them 61 showed *Y* linkage; of the remaining 995 autosomal insertions, 101 behaved as autosomal heterochromatin insertions, since rosy$^+$ eye color expression in these sublines was greatly enhanced by the presence of an extra *Y* chromosome. All previously characterized *P(ry*$^+$*)* insertions responding to the *Y* chromosome are located in heterochromatin (Zhang and Spradling 1994). The *Y* chromosomes with the newly transposed elements were tested for male fertility (see materials and methods). We found that seven independently derived *Y* chromosome sublines contain *Y*-linked male sterile mutations, *ms(Y)s*. *XY* males carrying *ms(Y)16*, *ms(Y)28*, *ms(Y)61*, *ms(Y)69*, and *ms(Y)77* are completely sterile. The *ms(Y)15* and *ms(Y)23* mutations are leaky, producing occasional progeny in the fertility test (see below).

Male fertile reversions in germ line cells of the *X/ms(Y)* males: To investigate whether the *Y*-linked male sterile phenotype is caused by a *P*-element insertion, each of the male sterile *Y* chromosomes was tested for its ability to revert to the wild type. Since the functions of the *Y* chromosome are cell autonomous and required only in the male germ line (Marsh and Wieschaus 1978), we asked whether fertile sperm could be produced, by excising the *P* element, from germ line cells of the *X/ms(Y)* mutant males. For each of the *ms(Y)* mutations, mutant *X/ms(Y); Sb Δ2-3 / ry*^{506} males were produced from a cross between *C(1;Y)1, y / ms(Y); ry*^{506} */ ry*^{506} males and *y / y; Sb Δ2-3 / TM6, Ubx* females. To test male fertility the *X/ms(Y); Sb Δ2-3 / ry*^{506} males were individually crossed to five females of genotype *y / y; ry*^{506} */ ry*^{506} in vials. The results showed that males carrying six *ms(Y)* mutations, *ms(Y)15*, *16*, *23*, *28*, *61*, and *69*, produced revertants in the presence of a transposase source (Table 1). The remaining *ms(Y)* chromosome, *ms(Y)77*, failed to produce any revertants, suggesting that this mutation has an origin other than the *P*-element insertion.

As shown in Table 1, *X/ms(Y)16*, *X/ms(Y)28*, *X/ms(Y)61*, and *X/ms(Y)69* males produced no progeny. When the transposase source was present, these males produced progeny ranging from 0.8–11.4 per male, indicating that the *P(ry*$^+$*)* insertions in these *Y* chromosomes are the causes of the male sterile mutations. To

TABLE 1

Reversion of the *ms(Y); SbΔ2-3/ry*^{506} males

Genotype[a]	Total males	% with progeny	Progeny/male[b]
X/ms(Y)15; Sb Δ2-3/ry^{506}	15	100	17.6 ± 12.9
X/ms(Y)15; TM6/ry^{506}	16	50	1.1 ± 2.7
X/ms(Y)16; Sb Δ2-3/ry^{506}	40	90	11.4 ± 14.6
X/ms(Y)16; TM6/ry^{506}	50	0	0
X/ms(Y)23; Sb Δ2-3/ry^{506}	13	85	22.2 ± 20.4
X/ms(Y)23; MT6/ry^{506}	16	12	0.4 ± 1.65
X/ms(Y)28; Sb Δ2-3/ry^{506}	36	94	10.6 ± 15.2
X/ms(Y)28; TM6/ry^{506}	40	0	0
X/ms(Y)61; Sb Δ2-3/ry^{506}	26	23	2.4 ± 9.4
X/ms(Y)61; TM6/ry^{506}	50	0	0
X/ms(Y)69; Sb Δ2-3/ry^{506}	20	20	0.8 ± 3.1
X/ms(Y)69; TM6/ry^{506}	50	0	0
X/ms(Y)77; Sb Δ2-3/ry^{506}	38	0	0
X/ms(Y)77; TM6/ry^{506}	50	0	0
X/ms(Y)104b; Sb Δ2-3/ry^{506}	22	100	6.9 ± 7.9
X/ms(Y)104b; TM6/ry^{506}	40	10	0.1 ± 0.5

[a] For all of the *ms(Y)* mutations, the *X/ms(Y); Sb Δ2-3/ry*^{506} and *X/ms(Y); TM6/ry*^{506} males are siblings, produced from a cross between *y/y; Sb Δ2-3/TM6* females and *C(1;Y)1, y/ms(Y); ry*^{506}*/ry*^{506} males.

[b] Values are means ± SE. The ratio of progeny/male was calculated from total number of the tested males.

confirm that the male progeny were actual revertants, we further examined fertility of 5–10 individual males that were produced in each of the six reversion tests. These males remained fertile in the absence of the transposase source, and thus were true revertants. The individual *ms(Y)* mutations were reverted at different frequencies ranging from 0.8 to 22.2 progeny/male, and the number of progeny produced from the individual *X/ms(Y); Sb Δ2-3/ry*^{506} males varied greatly, which is reflected by the large standard deviations for the reversion rates (Table 1).

Two of the *ms(Y)* mutations, *ms(Y)15* and *ms(Y)23*, are leaky, as shown in the control matings without a transposase source (Table 1). While most of the *X/ms(Y)15* and *X/ms(Y)23* males were sterile, some produced a small number of progeny [1.1 progeny/male for *ms(Y)15* and 0.4 progeny/male for *ms(Y)23*]. In the presence of a transposase source, the *X/ms(Y)15* males produced 17.6 progeny/male, and the *X/ms(Y)23* males produced 22.2 progeny/male (Table 1).

In addition to the *ms(Y)* mutations described above, another *ms(Y)* mutation, *ms(Y)104b*, was isolated in an earlier testing screen similar to that in Figure 1. The *ms(Y)104b* mutation also reverted in a reversion experiment as described in Table 1. A fraction of the *X/ms(Y)104b* males were leaky, producing 1–5 progeny each (0.1 progeny/male, Table 1). Further tests indicated that young mutant males were more likely to produce a small number of progeny, while older males (>4 days) were sterile. Similar results were obtained

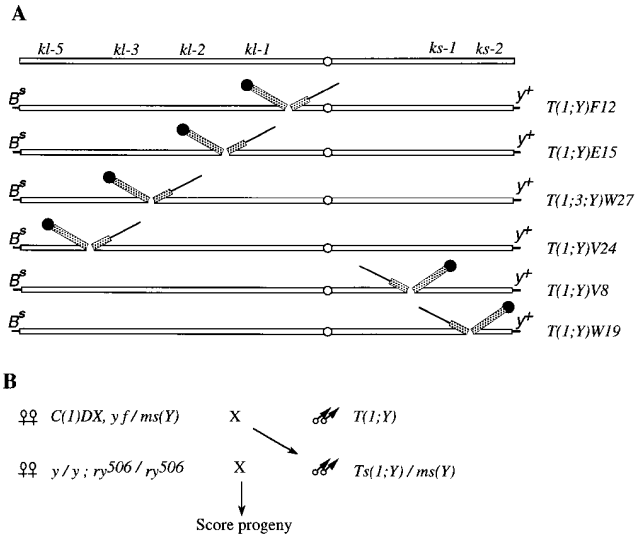

Figure 2.—*Ts(1;Y)* chromosomes used in genetic complementation to map the *ms(Y)* mutations. (A) Schematic drawings of the reciprocal *X-Y* translocations (adapted from Hardy *et al.* 1981). The uppermost open box represents a normal *Y* chromosome. Above the *Y* chromosome are six male fertility genes (*kl-5*, *kl-3*, *kl-2*, *kl-1* on the long arm; *ks-2*, *ks-1* on the short arm). (B) Genetic crosses to construct *Ts(1;Y)/ms(Y)* males and to test male fertility. Open boxes, *Y*-chromosomal; stippled boxes, *X* heterochromatin; thin lines, *X* euchromatin; open circles, *Y* centromeres; solid circles: *X* centromeres. The *T(1;3;Y)W27* chromosome also carries a reciprocal translocation between the *X*, *Y*, and third chromosomes but acts like the other *T(1;Y)* chromosomes in the genetic complementation experiments.

for young males carrying EMS-induced *ms(Y)* mutations (Kennison 1981).

Genetic mapping of the *ms(Y)* mutations by complementation: The above reversion tests showed that seven of the *ms(Y)* mutations are induced by the *P(ry*$^{+}$*)* insertions. Southern hybridization, using *P(ry*$^{+}$*)* sequences as probes, showed that each of the *ms(Y)* mutations is associated with a single *P(ry*$^{+}$*)* element (data not shown). Therefore, each of these *ms(Y)* chromosomes contains a single mutation. To map the *ms(Y)* mutations in the *Y* chromosome, a series of *X-Y* translocation chromosomes were employed in genetic complementation analyses. In Figure 2, we used six *Ts(1;Y)* chromosomes which are individually marked with either *y*$^{+}$ (*V24*, *W27*, *E15*, *F*$_{1}$*2*) or *B*^{S} (*W19*, *V8*). The *Ts(1;Y)* chromosomes carry the entire euchromatic portion of the *X* chromosome, but vary in the amount of *Y* chromosome material. This set of *Ts(1;Y)* chromosomes allowed us to map the *ms(Y)* mutations to four loci on the *Y* long arm (*kl-5*, *kl-3*, *kl-2*, *kl-1*) and two loci on the *Y* short arm (*ks-1*, *ks-2*).

For each *P*-induced *ms(Y)* mutation, we set up a series of genetic crosses between *C(1)DX, y f/ms(Y)* females and each of the six *T(1;Y)* males. From these crosses *Ts(1;Y)y*$^{+}$ or *B*^{S}*/ms(Y)* males were produced and their fertility was tested (Figure 2). The results from the male

fertility tests are used to map the *ms(Y)* mutations (Table 2). The *ms(Y)16* mutation is mapped to the *kl-5* region on the long arm because it was unable to complement *Ts(1Lt;YSt)V24*, which is $kl\text{-}5^{-}kl\text{-}3^{+}kl\text{-}2^{+}kl\text{-}1^{+} \cdot ks\text{-}1^{+}ks\text{-}2^{+}$. Three of the *ms(Y)* mutations, *28*, *61*, and *104b*, are mapped to the *kl-3* region on the long arm, since they were complemented by *Ts(1Lt;YSt)V24*, but not *Ts (1Lt;YSt;3)W27*, which is $kl\text{-}5^{-}kl\text{-}3^{-}kl^{-}\text{-}2^{+}kl\text{-}1^{+} \cdot ks\text{-}1^{+}ks\text{-}2^{+}$. The other three *ms(Y)* mutations, *15*, *23*, and *69*, were complemented by all *Ts(1;Y)s* except *Ts(1Lt:YLt)V8*, which is $kl\text{-}5^{+}kl\text{-}3^{+}kl\text{-}2^{+}kl\text{-}1^{+} \cdot ks\text{-}1^{+}ks\text{-}2^{-}$. Therefore, they are mapped to the *ks-2* region on the short arm. Finally, the *ms(Y)77* chromosome, which failed to revert as described above, was complemented only by the *Ts (1Lt;YSt)W19* and *V8* chromosomes that have deletions on the short arm, indicating that it has a mutation on the long arm. Furthermore, since *Ts(1Lt;YSt)V24 / ms(Y)77* males were sterile (Table 2), the *ms(Y)77* chromosome contains at least a mutation in the *kl-5* region.

Physical mapping of the *P(ry⁺)* elements in the *ms(Y)* mutations by *in situ* hybridization: Because the *Y* chromosome is not amplified in polytene nuclei of the larval salivary glands, physical locations of the *P(ry⁺)* elements that induced the *ms(Y)* mutations cannot be mapped using the polytene chromosomes. As a result, we determined their locations in the *Y* chromosome by using fluorescence *in situ* hybridization to the mitotic chromosomes prepared from larval neuroblasts (Zhang and Spradling 1994). Shown in Figure 3, the *P(ry⁺)* elements in the *ms(Y)* mutations were seen *in situ* within regions of the *ms(Y)* chromosomes where the male sterile mutations have been mapped by the genetic complementation analyses described above. As shown in Table 2, three *ms(Y)* mutations (*28*, *61*, and *104b*) are mapped by genetic complementation to the *Y* chromosomal *kl-3* region. Fluorescence *in situ* hybridization showed that the *P(ry⁺)* elements that induced these mutations are located in the same region between h7-9 (Figure 3). Each of the other three *ms(Y)* mutations (*15*, *23*, and *69*), which have been mapped to the *ks-2* region on the short arm by genetic complementation, is associated with a single *P(ry⁺)* element within the *Y* chromosomal h24-25 region on the short arm (Figure 3). The *ms(Y)16* mutation is associated with a *P(ry⁺)* element in the *kl-5* region, which is also consistent with our genetic complementation results (Table 2).

By staining with DAPI, we found that the *ms(Y)77* chromosome contains a large deletion on its long arm, with a distal breakpoint in h1-3 and a proximal breakpoint in h11-13 where the starting element in *Y95-2* was located (Zhang and Spradling 1994; Figure 3). The deletion associated with the *ms(Y)77* chromosome is consistent with results obtained from the above reversion experiments, indicating that the insertion in the *ms(Y)77* chromosome alone is not the cause of the male sterile phenotype.

Defects in spermatogenesis: We examined *ms(Y)* mu-

TABLE 2

Genetic complementation between *Ts(1;Y)* chromosomes and the *ms(Y)* mutations

	Ts(1Lt;YSt) V24	*Ts(1Lt;YSt; 3) W27*	*Ts(1Lt; YSt) E15*	*Ts(1Lt;YSt) F12*	*Ts(1Lt;TLt) V8*	*Ts(1Lt;YLt) W19*	*ms(Y)* locus
	$kl\text{-}5^{-}\ kl\text{-}3^{+}kl\text{-}2^{+}kl\text{-}1^{+}$ $ks\text{-}1^{+}ks\text{-}2^{+}$	$kl\text{-}5^{-}\ kl\text{-}3^{-}\ kl\text{-}2^{+}kl\text{-}1^{+}$ $ks\text{-}1^{+}ks\text{-}2^{+}$	$kl\text{-}5^{-}\ kl\text{-}3^{-}\ kl\text{-}2^{-}\ kl\text{-}1^{+}$ $ks\text{-}1^{+}ks\text{-}2^{+}$	$kl\text{-}5^{-}\ kl\text{-}3^{-}\ kl\text{-}2^{-}\ kl\text{-}1^{-}$ $ks\text{-}1^{+}ks\text{-}2^{+}$	$kl\text{-}5^{+}kl\text{-}3^{+}kl\text{-}2^{+}kl\text{-}1^{+}$ $ks\text{-}1^{-}\ ks\text{-}2^{-}$	$kl\text{-}5^{+}kl\text{-}3^{+}kl\text{-}2^{+}kl\text{-}1^{+}$ $ks\text{-}1^{+}ks\text{-}2^{-}$	
ms(Y)16	S	S	S	S	F	F	*kl-5*
ms(Y)28	F	S	S	S	F	F	*kl-3*
ms(Y)61	F	S	S	S	F	F	*kl-3*
ms(Y)104b	F	S	S	S	ND	ND	*kl-3*
ms(Y)15	F	F	F	F	S	S	*ks-2*
ms(Y)23	F	F	F	F	S	S	*ks-2*
ms(Y)69	F	F	F	F	S	S	*ks-2*
ms(Y)77	S	S	S	S	F	F	Deficiency[a]

F, male fertile; S, male sterile; ND, not determined.

[a] The *ms(Y)77* chromosome carries a large deletion on the long arm. See text for details.

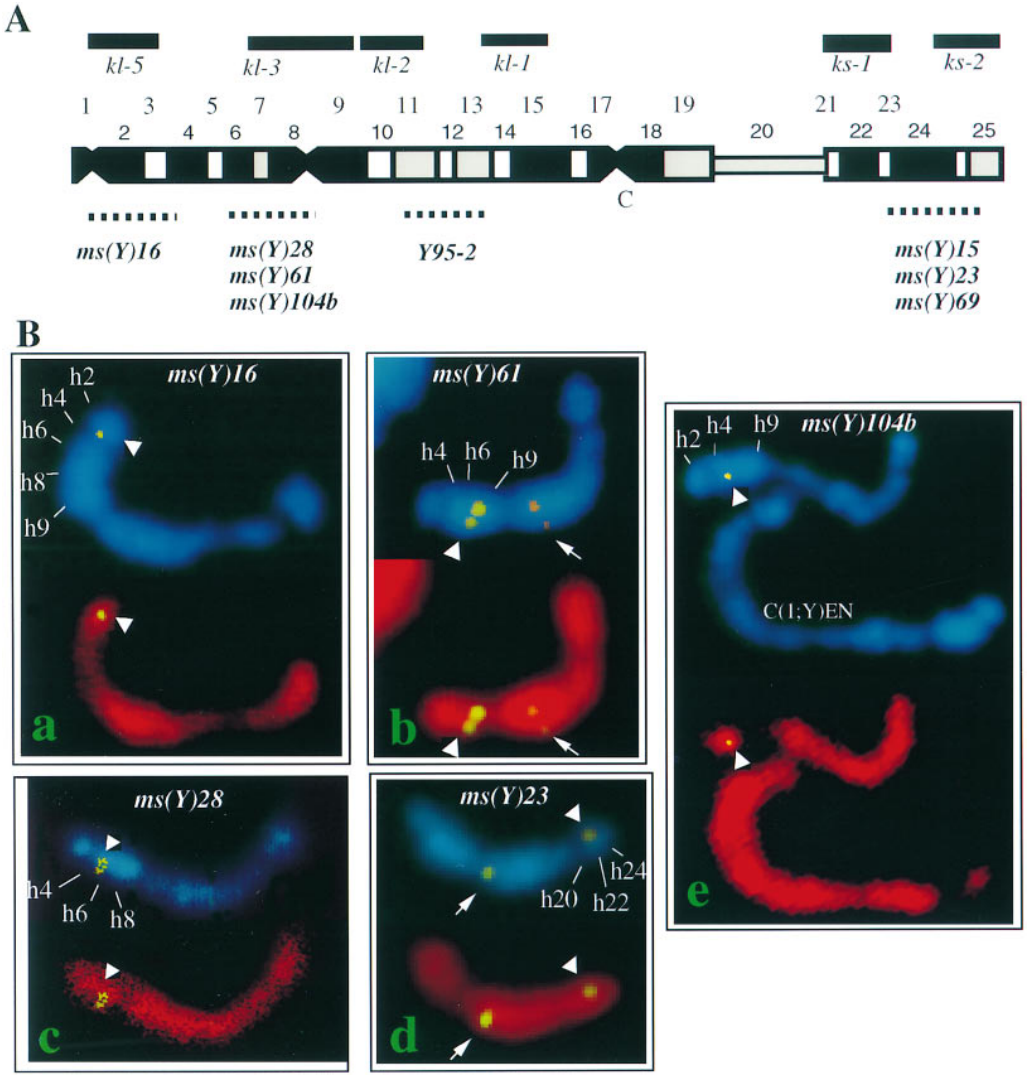

Figure 3.—Mapping *P(ry$^+$)* elements in the *ms(Y)* mutations into the *Y* chromosome by fluorescence *in situ* hybridization. (A) Schematic drawings of the *Drosophila melanogaster Y* chromosome with labeled banding patterns (1–25, above the diagram) (Gatti and Pimpinelli 1983). The strength of DAPI staining is represented by the degree of shading in the diagram. Six male fertility genes (*kl-5*, *kl-3*, *kl-2*, *kl-1* on the long arm; *ks-2*, *ks-1* on the short arm) and their relative locations (thick bars) are shown above the *Y* chromosome. Below the *Y* chromosome are dashed lines indicating approximate regions where the *P* elements were mapped. (B) Images of fluorescence *in situ* hybridization to map the *P* elements onto the mitotic *Y* chromosome. In each panel the *Y* chromosome was stained with either DAPI (blue) or propidium iodide (red). *In situ* signals for the *P* elements (yellow, indicated by arrowheads) were captured along with the *Y* chromosomes stained with propidium iodide. The signals were superimposed onto the DAPI-stained *Y* chromosome by using Photoshop software. The starting *P* element in *Y95-2* (arrows in panels b and d) was often retained during transposition, which was determined by restriction analyses (data not shown). The *Y* chromosomes shown here carry the following *P*-induced *ms(Y)* mutations: *ms(Y)16* (a), *ms(Y)61* (b), *ms(Y)28* (c), *ms(Y)23* (d), and *ms(Y)104b* (e).

tant phenotypes in the adult testes by using light and electron microscopes. In the mutant testes carrying each of the seven *ms(Y)* alleles, the most discernible phenotype is the appearance of degrading materials in the basal testicular region. The absence of sperm in the seminal vesicles is common to the mutant testes. In spite of the degenerating phenotypes during late spermatogenesis, no significant deviations from the wild type were seen by phase-contrast microscopy in the mutant germ-line cells before, during, and shortly after the meiotic divisions.

When the postmeiotic nuclei were examined by staining the testes with DAPI, the *ms(Y)* mutations in the *kl-5* region, *ms(Y)16*, and in the *kl-3* region, *ms(Y)28*, *61*, and *104b*, displayed nearly normal nuclear condensation, except for the appearance of occasional singular needle-shaped nuclear heads. The organized bundles of elongated sperm heads were eventually disintegrated in the basal region of the testes before the spermatids reached maturity. When phenotypes of the *ms(Y)28*, *61*, and *104b* mutations were examined using an electron microscope, noticeable defects were found in the mutant sperm flagellar axoneme. The *ms(Y)28* and *61* mutations in the *kl-3* region resulted in loss or great reduction of the axonemal outer arm dyneins (Figure 4), similar to the deletion of the *kl-3* region (Hardy *et al.* 1981). The *ms(Y)104b* mutation displayed similar axonemal phenotypes, though partially formed axonemal outer arms were sometimes seen, which may reflect its leaky fertility (Table 1). Interestingly, electron microscopic analyses often display abnormal cellular membrane structure in the spermatid tails of the mutants, seen as discontinuous at early postelongation stage (Figure 4). However, further examination is needed to reveal whether the abnormal membrane appearance is specifically associated with the mutant testes. Shortly after

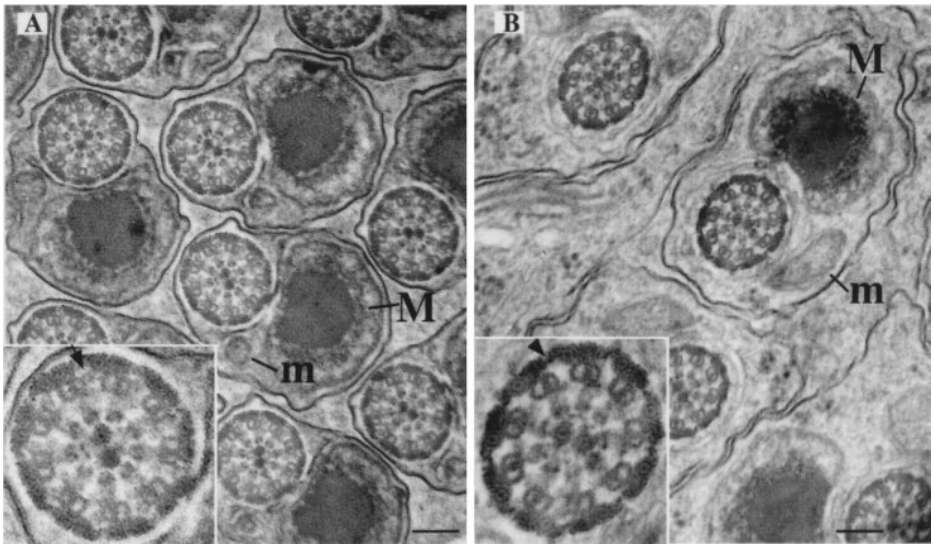

Figure 4.—Electron micrographs of postelongation spermatid tails. (A) Wild-type spermatid tails. Outer dynein arms (arrow) protrude from the A tubules of the peripheral doublets. (B) Abnormal axonemal structure from a mutant male, *X/ms(Y)28*, at slightly younger stage, in which the outer arms are missing, or greatly reduced (arrowhead). The sections of spermatid tails are viewed in the head-tail direction. *M*, major mitochondrial derivative; *m*, minor mitochondrial derivative. Bars, 0.1 μm.

the developmental stage at which the above defects were shown, the axonemes of 64 spermatids along the entire length of the bundle were degenerated rapidly, which was followed by complete disintegration of the mitochondrial derivatives and the spermatid membrane.

In contrast to the above late phenotypes associated with the *kl-5* and *kl-3* mutations during spermatogenesis, the *ms(Y)* mutations in the *ks-2* region, *15*, *23*, and *69*, showed early postmeiotic defects. Staining with DAPI revealed that the 64 elongated spermatid nuclei in a cyst were scattered throughout the basal third of the testes. Hardy *et al.* (1981) showed that *ks-2* deletion displays abnormal alignment between axoneme and onion nebenkern in early postmeiotic spermatids. We have labeled the male germ-line cells with *lacZ* enhancer-trap lines that express β-galactosidase activity in the adult testes (see materials and methods). When the germline cells were labeled with X-gal staining, the *ms(Y)15*, *23*, and *69* mutations showed identical defects in early postmeiotic cells. In the wild-type testes X-gal staining is localized in the nuclei of the germ-line cells, resulting from a nuclear targeting signal upstream of the *lacZ* reporter gene (Bier *et al.* 1989). In the testes of the *X/ms(Y)* mutants, however, the staining lost nuclear localization in the postmeiotic spermatids, while the *lacZ* staining pattern was normal in the nuclei of the primary and secondary spermatocytes. Instead, X-gal staining was dispersed throughout the spermatid bundles, indicating a defect in the spermatid nuclear membrane structure. Though it is unclear at which postmeiotic stage this defect first appeared, it may take place soon after the initiation of spermatid nuclear elongation.

Suppressors of PEV and ry^+ gene expression: When the ry^+ gene is relocated in the heterochromatin, it is subject to PEV and its activity is enhanced by the presence of an extra *Y* chromosome (Karpen and Spradling 1992; Zhang and Spradling 1994). The *P*(ry^+) elements in the *ms(Y)* mutations apparently inserted into *Y* chromosome regions where PEV is strong, because expression of the ry^+ gene is greatly affected by two PEV modifiers, *i.e.*, the amount of heterochromatin and temperature (Spofford 1976). For the seven *ms(Y)* mutations described in Table 1, all regular *X/ms(Y)* males remained phenotypically rosy when the flies were reared at temperatures ranging from 18° to 25°.

In the presence of an extra *Y* chromosome to suppress PEV, the vast majority of *C(1)RM/ms(Y)* females or *C(1;Y)/ms(Y)* males failed to express the ry^+ gene when the flies were reared at 18°. At 22°, more females and males with the extra *Y* chromosome showed $rosy^+$ eye color, though the level of ry^+ expression varied among individuals of the same genotype. An exception is the ry^+ gene in *ms(Y)61*, which is affected little if at all by the presence of the extra *Y* chromosome. The ry^+ gene in *ms(Y)61* remained completely silent in females or males carrying an extra *Y* chromosome when the flies were reared at 22°.

As the temperature was increased to 25°, all but one *ms(Y)* insertions expressed $rosy^+$ eye color in approximately 80–100% of *C(1)RM/ms(Y)* females or *C(1;Y)/ms(Y)* males. For the *ms(Y)61* insertion, expression of weak $rosy^+$ eye color was observed in approximately 50% of *C(1)RM/ms(Y)61* females and 30% of *C(1;Y)/ms(Y) 61* males. Therefore, expression of $rosy^+$ eye color in *ms(Y)61* requires a combination of PEV suppression, *i.e.*, an extra *Y* chromosome and a temperature of 25°.

Expression of the *P*-carrying ry^+ gene is also modified by the amount of *X*-chromosomal heterochromatin. The *C(1)DX* chromosome contains less heterochromatin than *C(1)RM*. Because *C(1)DX* is deficient for the *bb* locus, females carrying this compound *X* chromosome are lethal, unless compensated with a *Y* chromosome (Lindsley and Zimm 1992). We observed unusually strong PEV on the ry^+ gene expression in females of *C(1)DX* carrying any one of the *ms(Y)* chromosomes; at 18° or 22°, the ry^+ gene in all seven *ms(Y)* mutations

described in Table 1 was silent in *C(1)DX / ms(Y)* females. When the temperature was increased to 25°, two of these *P(ry$^+$)* elements, *ms(Y)15* and *16*, expressed the *ry$^+$* genes in a small fraction (<20%) of the *C(1)DX / ms(Y)* females, but the *P(ry$^+$)* elements in the remaining five *ms(Y)* mutations remained silent.

Enhancer-trap expression in the *Y* chromosome: In addition to the *ry$^+$* gene, the *P(ry$^+$)* element carries a *lacZ* construct with a *P* promoter (Mlodzik and Hiromi 1992), functioning as an efficient enhancer trap. We examined *lacZ* activity in the adult testes for all 61 *P* insertions in the *Y* chromosome by staining for X-gal activity (see materials and methods). All seven *P* elements in the *ms(Y)* mutations failed to produce *lacZ* staining, while three other insertions expressed weak *lacZ* activity in the adult testes.

DISCUSSION

Isolation of *P* elements in the *Y* chromosome and modifiers of position effect variegation: When transposed into the *Y* chromosome, *P* elements genetically marked with the *ry$^+$* gene are subject to heterochromatic position effect (Berg and Spradling 1991; Karpen and Spradling 1992). The *Y95-2* chromosome, which contains a *P(ry$^+$)* element on the long arm, was isolated serendipitously in a screen to identify newly transposed *P* elements through Southern hybridization (Zhang and Spradling 1993). The *Y95-2* insertion is genetically silent, even in the presence of an extra *Y* chromosome to suppress PEV. When the *P* element in *Y95-2* was used as a starting element and PEV was suppressed by an extra *Y* chromosome, a genetic screen recovered approximately 5% of total new insertions in the *Y* chromosome (Zhang and Spradling 1994). However, the screen failed to isolate any *ms(Y)* mutations and all fourteen of the isolated *P(ry$^+$)* elements are located on the long arm of the *Y* chromosome.

In this study, we added an additional PEV modifier, *i.e.*, temperature increase, in the genetic screen to isolate the *Y*-linked insertions. In addition to a relatively small increase in frequency of recovering *P* elements in the *Y* chromosome (5.8 *vs.* 5%), the insertions isolated in this screen have new characteristics. Some of these *P* elements are subject to strong PEV so that the *ry$^+$* expression in adult eyes is detectable only when a combination of two PEV modifiers is used. In particular, the *ry$^+$* gene in the *ms(Y)61* mutation, an extreme example, failed to express without the simultaneous presence of the modifiers. Therefore, some of the *ms(Y)* mutations isolated in this study would have escaped detection had our screen been performed at 22°. Our results also showed that the insertion sites of these *P* elements are distributed into different regions along the *Y* chromosome (Figure 3). Three *ms(Y)* insertions, *ms(Y)15*, *23*, and *69*, are located within the *ks-2* gene on the short arm, where no *P* element was recovered previously. In addition to these insertions in the *Y* chromosome, by using the combination of the PEV modifiers in the screen, we observed a threefold increase in recovering *P* elements in autosomal heterochromatin (9.5 *vs.* 3.2%).

***LacZ* expression from the *Y*-linked *P* elements:** Studies with X-gal staining in Drosophila germ lines revealed that large numbers of enhancer-trap lines expressed the *lacZ* gene. For example, *lacZ* staining was observed in the ovaries of 30–50% examined euchromatic enhancer-trap insertions (Fasano and Kerridge 1988; Grossniklaus *et al.* 1989; A. Spradling, personal communication). In the germ line of adult testes, *lacZ* staining was seen in 68% euchromatic insertions (Gönczy *et al.* 1992). In contrast, among the 61 *Y*-linked *P(ry$^+$)* elements with an enhancer-trap construct reported here, only three (5%) express weak *lacZ* activity in the adult testes. Interestingly, none of the *ms(Y)* mutations expresses the *lacZ* activity in adult testes. The lack of male germ-line *lacZ* expression from the *Y*-linked *P* insertions could be explained if heterochromatic regions (at least those targeted by the *P* elements) were devoid of conventional enhancer elements (Zhang and Spradling 1994). Alternatively, special *cis*-regulatory elements, such as heterochromatic promoters, are required for expressing genes in heterochromatin (see Weiler and Wakimoto 1995).

Hypersensitivity of the *Y* chromosome to *P*-element mutagenesis: The *Y* chromosome has a large physical size in *D. melanogaster* and contains approximately 4×10^7 bp DNA sequences. Three of the six male fertility genes in the *Y* chromosome, *kl-5*, *kl-3*, and *ks-1*, occupy a significant proportion of the *Y* chromosome (Gatti and Pimpinelli 1983; Bonaccorsi *et al.* 1988). Each of these genes contains about 10% of the *Y* chromosome, or 4×10^6 bp, which is at least two orders of magnitude larger than most euchromatic genes. The large size may be responsible for hypersensitivity of the *Y*-linked genes to EMS or X-ray treatment (see Lindsley and Tokuyasu 1980; Fuller 1993). Because the *Y*-linked genes contain largely abundant repetitive sequences (Bonaccorsi and Lohe 1991), the hypersensitivity of the male fertility genes in the *Y* chromosome could be explained if both EMS and X-ray treatments induced large chromosomal rearrangements at high frequency.

Results reported here suggest that the *ms(Y)* mutations are also induced at high frequency by *P*-element insertional mutagenesis. Among the 61 *Y*-linked *P*-element insertions isolated in our genetic screen, 6 (or 10%) induced male sterile mutations in three of the six male fertility genes (Table 1). High frequency recovery of *ms(Y)* mutations among randomly selected *P*-element insertions indicates that either the genetic screen has a selection advantage for *ms(Y)* mutations, or the fertility genes are hypersensitive to *P*-element insertions. Studies using enhancer trap showed that *P*-element transposes preferentially into genomic sites where transcription is active (Bownes 1990). In our experiments, however,

transposition of the *P(ry*$^+$*)* element into the *Y* chromosome took place in female germ lines where the *Y* chromosome has no essential function (Figure 1). The use of PEV modifiers in our screen may have preferentially allowed the recovery of *P* elements in the *Y*-linked male fertility genes, if PEV within these *Y*-chromosomal regions is particularly sensitive to the modifiers.

Our results may otherwise suggest an alternative explanation for the high frequency of the *ms(Y)* mutations. No apparent large deletions or other chromosomal rearrangements were seen in the *ms(Y)* chromosomes, except *ms(Y)77*, which contains a large deletion. The male sterile phenotypes were reverted after the *P* elements were exposed to a transposase source. To explain why the *Y* chromosome is hypersensitive to *P*-element mutagenesis, which caused no large chromosomal rearrangement, we propose that some of the *ms(Y)* mutations could have been induced by insertions of the genetically engineered *P(ry*$^+$*)* element into abundant noncoding repetitive regions within the *Y*-linked male fertility genes. Horowitz and Berg (1995) reported a mechanism by which essential gene expression is disrupted by the *P(ry*$^+$*)* element, which inserted into a large intron of the *psq* gene. The induced psq mutations resulted from aberrant transcriptional termination within the *P(ry*$^+$*)* element. It is conceivable that *Y* chromosome hypersensitivity to EMS and X-ray mutagenesis could have arisen from small DNA sequence changes within the abundant repetitive sequences of the male fertility genes, disrupting gene expression by mechanisms such as premature transcriptional termination.

Male sterile phenotypes and the *Y*-linked male fertility genes: The *kl-3* and *kl-5* genes are proposed to encode large subunits of the sperm flagellar dynein complex (Hardy *et al.* 1981; Goldstein *et al.* 1982; Gepner and Hays 1993). We showed that all three *P*-induced *kl-3* mutant males, *X/ms(Y)28*, *ms(Y)61*, and *ms(Y)104b*, are able to initiate normal germ-line development and form differentiated spermatids, but late spermatogenesis produces defective sperm. Consistent with the proposed function of the *kl-3* gene, our ultrastructural analysis revealed that the *kl-3* alleles are associated with defects within the axonemal outer dynein arms extending from the A tubules of the peripheral doublets (Figure 4), similar to that seen in the *kl-3* deletions (Hardy *et al.* 1981). In addition to the lost or greatly reduced outer dynein arms, electron micrographs of postelongation spermatid tails from the mutants showed cellular membrane defects (Figure 4) before degradation of the axonemes in the entire spermatid bundle. It is unknown if the membrane phenotype at spermatid elongation stage resulted from a primary defect in the outer arms of the axonemes.

Hardy *et al.* (1981) showed that the earliest defect associated with the *ks-2* deficiency occurs during the onion nebenkern stage of the round spermatids, manifested as misalignment of developing axonemes with the mitochondrial derivatives. Our results also showed that three *ks-2* alleles induced by single *P*-element insertions, *ms(Y)23*, *15*, and *69*, displayed early postmeiotic defects. Under the light microscope, all three mutants displayed defects during nuclear condensation stage at which the 64 spermatid nuclei in a cyst are scattered. Studies using X-gal staining of the mutant germ-line nuclei suggest that the *ks-2* mutations are associated with a nuclear membrane defect, because the *lacZ* activity lost nuclear localization.

In addition to the above proposed functions for the *Y*-linked genes, such as encoding for dynein subunits, there have been continuous discussions whether the *Y* chromosome contains nonconventional genes without coding capacity for proteins (see Hennig 1993). "RNA-product" theories propose that the *Y*-linked genes are transcribed into large RNA molecules which do not code for proteins. The newly synthesized transcripts remain with the *Y* chromosomal regions where they are transcribed from to form the giant lampbrush-like structures in the primary spermatocytes (Hennig 1985; Bonaccorsi *et al.* 1988; Gatti and Pimpinelli 1992). These nuclear scaffolds are proposed to play essential roles during spermatogenesis. The *P*-induced alleles of the *Y*-linked *kl-5*, *kl-3*, and *ks-2* genes described in this paper will facilitate molecular characterization of these heterochromatic genes with unusual properties.

We thank the Bloomington Drosophila Stock Center for providing stocks, Dr. Marie Cantino and the University of Connecticut EM Lab for help with electron microscopy, and Douglas Malton for technical assistance. Our many thanks to Dr. Allan Spradling for advice. The ms(Y)104b mutation was isolated in Dr. Spradling's lab. We also thank Dr. Dan Lindsley for critical comments. This work was supported by a startup grant from the University of Connecticut and a grant from the American Cancer Society (ACSIN52L-13).

LITERATURE CITED

Berg, C., and A. Spradling, 1991 Studies on the rate and site-specificity of *P* element transposition. Genetics **127:** 515–524.

Bier, E., H. Vaessin, S. Shepherd, K. Lee, K. McCall *et al.*, 1989 Searching for pattern and mutation in the Drosophila genome with a *P-lacZ* vector. Genes Dev. **3:** 1273–1287.

Bonaccorsi, S., and A. Lohe, 1991 Fine mapping of satellite DNA sequences along the *Y* chromosome of *Drosophila melanogaster*: relationship between satellite sequences and fertility factors. Genetics **129:** 177–189.

Bonaccorsi, S., C. Pisano, F. Puoti and M. Gatti, 1988 *Y* chromosome loops in *Drosophila melanogaster*. Genetics **120:** 1015–1034.

Bownes, M., 1990 Preferential insertion of *P* elements into genes expressed in the germ-line of *Drosophila melanogaster*. Mol. Gen. Genet. **222:** 457–460.

Bridges, C. B., 1916a Non-disjunction as proof of the chromosome theory of heredity. Genetics **1:** 1–52.

Bridges, C. B., 1916b Non-disjunction as proof of the chromosome theory of heredity. Genetics **1:** 107–163.

Casadaban, M., A. Martinez-Arias, S. Shapira and J. Chou, 1983 Beta-galactosidase gene fusions for analyzing gene expression in *Escherichia coli* and yeast. Methods Enzymol. **100:** 293–308.

Castrillon, D., P. Gonczy, S. Alexander, R. Rawson, C. Eberhart *et al.*, 1993 Towards a molecular genetic analysis of spermatogenesis in *Drosophila melanogaster*: characterization of male-sterile

mutants generated by single *P* element mutagenesis. Genetics **135:** 489–505.
Cooley, L., R. Kelley and A. Spradling, 1988 Insertional mutagenesis of the Drosophila genome with single *P* elements. Science **239:** 1121–1128.
Fasano, L., and S. Kerridge, 1988 Monitoring positional information during oogenesis in adult Drosophila. Development **104:** 245–253.
Fuller, M., 1993 Spermatogenesis, pp. 71–147 in *The Development of Drosophila melanogaster*, edited by M. Bate and A. Martinez. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Gatti, M., and S. Pimpinelli, 1983 Cytological and genetic analysis of the *Y* chromosome of *Drosophila melanogaster*: I. Organization of the fertility factors. Chromosoma **88:** 349–373.
Gatti, M., and S. Pimpinelli, 1992 Functional elements in *Drosophila melanogaster* heterochromatin. *Annu. Rev. Genet.* **26:** 239–275.
Gepner, J., and T. Hays, 1993 A fertility region on the *Y* chromosome of *D. melanogaster* encodes a dynein microtubule motor. Proc. Natl. Acad. Sci. USA **90:** 11132–11136.
Goldstein, L., R. Hardy and D. L. Lindsley, 1982 Structural genes on the *Y* chromosome of *D. melanogaster*. Proc. Natl. Acad. Sci. USA **79:** 7405–7409.
Gönczy, P., S. Viswanathan and S. DiNardo, 1992 Probing spermatogenesis in Drosophila with P-element enhancer detectors. Development **114:** 89–98.
Grossniklaus, U., H. Bellen, C. Wilson and W. Gehring, 1989 *P*-element-mediated enhancer detection applied to the study of oogenesis in Drosophila. Development **107:** 189–200.
Hardy, R. W., K. T. Tokuyasu and D. L. Lindsley, 1981 Analysis of spermatogenesis in *Drosophila melanogaster* bearing deletions for *Y*-chromosome fertility genes. Chromosoma **88:** 593–617.
Hazelrigg, T., P. Fornili and T. Kaufman, 1982 A cytogenetic analysis of X-ray induced male sterile on the *Y* chromosome of *Drosophila melanogaster*. Chromosoma **87:** 535–559.
Henikoff, S., 1992 Position effect and related phenomena. Curr. Opin. Genet. Dev. **2:** 907–912.
Hennig, W., 1985 *Y* chromosome function and spermatogenesis in *Drosophila hydei*. Adv. Genet. **23:** 179–234.
Hennig, W., 1993 Conventional protein coding genes in the Drosophila *Y* chromosome: is the puzzle of the fertility gene function solved? Proc. Natl. Acad. Sci. USA **90:** 10904–10906.
Horowitz, H., and C. Berg, 1995 Aberrant splicing and transcription termination caused by *P* element insertion into the intron of a Drosophila gene. Genetics **139:** 327–335.
Karpen, G. H., and A. C. Spradling, 1992 Analysis of subtelomeric heterochromatin in a Drosophila minichromosome by single *P* element insertional mutagenesis. Genetics **132:** 737–753.
Kemphues, K., E. Raff, R. Raff and T. Kaufman, 1980 Mutation in a testis-specific β-tubulin in Drosophila: analysis of its effects on meiosis and map location of the gene. Cell **21:** 445–451.
Kennison, J. A., 1981 The genetic and cytological organization of the *Y* chromosome of *Drosophila melanogaster*. Genetics **98:** 529–548.
Lindsley, D., and K. T. Tokuyasu, 1980 Spermatogenesis, pp. 225–294 in *Genetics and Biology in Drosophila*, Ed. 2, edited by M. Ashburner and T. Wright. Academic Press, New York.
Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, New York.
Livak, K., 1990 Detailed structure of the Drosophila melanogaster Stellate genes and their transcripts. Genetics **124:** 303–316.
Lloyd, V., D. Sinclair and T. Grigliatti, 1997 Competition between different variegating rearrangements for limited heterochromatic factors in Drosophila melanogaster. Genetics **145:** 945–959.
Marsh, J. L., and E. Wieschaus, 1978 Is sex determination in germ line and soma controlled by separate genetic mechanisms? Nature **272:** 249–251.
Mlodzik, M., and Y. Hiromi, 1992 The enhancer trap method in Drosophila: its application to neurobiology, pp. 397–414 in *Gene Expression in Neural Tissues (Methods in Neuroscience 9)*, edited by P.M. Cann. Academic Press, Orlando, Florida.
Montell, D. J., P. Rorth and A. C. Spradling, 1992 *slow border cells*, a locus required for a developmentally regulated cell migration during oogenesis, encodes a Drosophila C/EBP homolog. Cell **71:** 51–62.
Ritossa, F., 1976 The *bobbed* locus, pp. 801–846 in *The Genetics and Biology of Drosophila*, Vol. 1b, edited by M. Ashburner and E. Novitiski. Academic Press, New York.
Roseman, R., E. Johnson, C. Rodesch, M. Bjerke, R. Nagoshi *et al.*, 1995 A *P* element containing suppressor of Hairy-wing binding regions has novel properties for mutagenesis in *Drosophila melanogaster*. Genetics **141:** 1061–1074.
Rubin, G. M., and A. C. Spradling, 1983 Vector for *P* element-mediated gene transfer in Drosophila. Nucleic Acids Res. **11:** 6341–6351.
Rushlow, C., and A. Chovnick, 1984 Heterochromatic position effect on the *rosy* locus of *Drosophila melanogaster*: cytological, genetic and biochemical characterization. Genetics **108:** 589–602.
Rushlow, C., W. Bender and A. Chovnick, 1984 Studies on the mechanism of heterochromatic position effect at the *rosy* locus of *Drosophila melanogaster*. Genetics **108:** 603–615.
Spofford, J. B., 1976 Position-effect variegation in Drosophila, pp. 955–1018 in *The Genetics and Biology of Drosophila*, Vol. 1c, edited by M. Ashburner and E. Novitiski. Academic Press, New York.
Tokuyasu, K. T., W. J. Peacock and R. W. Hardy, 1972 Dynamics of spermatogenesis in Drosophila melanogaster: I. Individualization process. Z. Zellforsch. **124:** 479–506.
Wallrath, L., and S. Elgin, 1995 Position effect variegation in Drosophila is associated with an altered chromatin structure. Genes Dev. **9:** 1263–1277.
Weiler, K., and B. Wakimoto, 1995 Heterochromatin and gene expression in Drosophila. Annu. Rev. Genet. **29:** 577–605.
Zhang, P., and A. Spradling, 1993 Efficient and dispersed local *P* element transposition from Drosophila females. Genetics **133:** 361–373.
Zhang, P., and A. Spradling, 1994 Insertional mutagenesis of Drosophila heterochromatin with single *P* elements. Proc. Natl. Acad. Sci. USA **91:** 3539–3542.

Communicating editor: S. Henikoff