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Characterization of the *mus308* Gene in *Drosophila melanogaster*

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ABSTRACT

Among the available mutagen-sensitive mutations in Drosophila, those at the *mus308* locus are unique in conferring hypersensitivity to DNA cross-linking agents but not to monofunctional agents. Those mutations are also associated with an elevated frequency of chromosomal aberrations, altered DNA metabolism and the modification of a deoxyribonuclease. This spectrum of phenotypes is shared with selected mammalian mutations including Fanconi anemia in humans. In anticipation of the molecular characterization of the *mus308* gene, it has been localized cytogenetically to 87C9–87D1,2 on the right arm of chromosome three. Nine new mutant alleles of the gene have been generated by X-ray mutagenesis and one was recovered following hybrid dysgenesis. Characterization of these new alleles has uncovered additional phenotypes of mutations at this locus. Homozygous *mus308* flies that have survived moderate mutagen treatment exhibit an altered wing position that is correlated with reduced flight ability and an altered mitochondrial morphology. In addition, observations of elevated embryo mortality are potentially explained by an aberrant distribution of nuclear material in early embryos which is similar to that seen in the mutant *giant nuclei*.

THIRTY-TWO loci that are required for mutagen resistance have been identified in *Drosophila melanogaster* (LINDSLEY and ZIMM (1992). A molecular analysis of mutations in 24 of these genes has identified 7 that are essential for either excision repair, postreplication repair, or photorepair (BOYD *et al.* 1983). The *phr* gene is absolutely required for photorepair and has a significant influence on excision repair as well (BOYD and HARRIS 1987). The *mus201* and *mei-9* genes are essential for the excision of pyrimidine dimers, whereas *mei-41, mus205, mus302* and *mus310* are required for postreplication repair (BOYD *et al.* 1982; BOYD and SHAW 1982; BOYD, GOLINO and SETLOW 1976; BOYD and SETLOW 1976). With one exception the available mutagen sensitive mutations are hypersensitive either to monofunctional alkylating agents alone or to a combination of monofunctional and bifunctional agents. Mutations in the *mus308* gene are unique in this regard because they exhibit a strong sensitivity to bifunctional DNA cross-linking agents without a concomitant sensitivity to monofunctional alkylating agents (BOYD *et al.* 1981; BOYD, SAKAGUCHI and HARRIS 1990). These mutations do not exhibit a strong defect in either excision or post-replication repair (BOYD *et al.* 1980; BOYD and SHAW 1982).

In the initial report of the *mus308* gene it was genetically mapped to the third chromosome at position 55 ± 5 cM (BOYD *et al.* 1981). Subsequent cytological and biochemical studies have identified a broad spectrum of pleiotropic effects associated with mutations at that locus. Some of these effects are observed after mutagen treatment whereas others occur spontaneously. A deoxyribonuclease activity derived from mitochondria of homozygous *mus308* mutants exhibits an altered charge in the absence of mutagen treatment (BOYD, SAKAGUCHI and HARRIS 1990). An increase in spontaneous chromosomal aberrations has also been observed in larval neuroblasts of *mus308* mutants (GATTI *et al.* 1984). Phenotypes observed after mutagen treatment include a failure to recover normal levels of semi-conservative DNA synthesis in primary cell cultures derived from *mus308* embryos (BROWN and BOYD 1981). In addition, flight muscle mitochondria in *mus308* flies exhibit decreased stability following treatment with nitrogen mustard (SAKAGUCHI *et al.* 1990).

The human disorder Fanconi anemia shares several of these characteristics with the *mus308* mutants. As in the *mus308* mutants, cells derived from Fanconi anemia A patients exhibit a unique hypersensitivity to DNA cross-linking agents (ISHIDA and BUCHWALD 1982), an increase in spontaneous chromosomal aberrations (PINCHEIRA, BRAVO and LOPEZ-SAEZ 1988; ROSENDORFF and BERNSTEIN 1988), a failure to recover semiconservative DNA synthesis after mutagen treatment (MOUSTACCHI and DIATLOFF-ZITO 1985), and an alteration in organellar nuclease activity as judged by isoelectric focusing (SAKAGUCHI *et al.* 1991). Fanconi anemia is characterized by a progressive loss of blood cells, skeletal defects, and stunted growth

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(TIMME and MOSES 1988). Children suffering from this disorder are predisposed to leukemia and generally die from bone marrow failure by late childhood. Four complementation groups (A, B, C and D) have thus far been identified among patients with this disorder (M. BUCHWALD, personal communication). Since the lethal effects of this disorder can be relieved by bone marrow transplantation (EBELL, FRIEDRICH and KOHNE 1989), it is a strong candidate for gene replacement therapy. Several laboratories are therefore currently attempting to clone the normal human genes. The phenotypic similarities between Fanconi anemia A and the *mus308* mutants suggest that Drosophila may provide a genetic model for this disorder as well as an alternative access to the human gene.

In the present study the map position of the *mus308* gene has been refined to 51 ± 1 cM on the third chromosome. With the aid of pre-existing deficiencies in that region of the genome the cytological map position has been identified as 87C9–87D1,2. Ten new mutations at this locus have been generated following X-ray mutagenesis and hybrid dysgenesis. Analysis of those mutants has revealed new *mus308* phenotypes. An elevated embryo mortality documented in *mus308* cultures has been correlated with an aberrant nuclear distribution following staining of embryos with Hoechst 33258. A wing phenotype similar to that observed in the mutant *held up* (LINDSLEY and ZIMM 1992) is also seen in adult survivors of nitrogen mustard (HN2) treated larvae. That defect has been correlated with reduced flight capacity.

MATERIALS AND METHODS

Stocks: The stocks used in the deficiency study are listed in Table 1. All other mutations and chromosomes used are described in LINDSLEY and ZIMM (1992). Cultures were maintained in ½ pint milk bottles or 6 dram vials on E. B. Lewis Standard medium (LEWIS 1960) at 25° unless otherwise specified.

Recombinational mapping: The genetic map position of *mus308* was determined by crossing fifteen *Gl Sb H/Payne* males to thirty *st p^p mus308^{D2}* virgin females in bottles. After seven days the parents were removed and virgin females of genotype *st p^p mus308^{D2}/Gl Sb H* were collected. Thirty of these females were mated to fifteen *st p^p mus308^{D2}* males in each of six mass matings. The flies were transferred at 48-hr intervals and three broods from each mating were initiated. All broods were treated with mutagen 72 hr after the initiation of egg laying. Bottles 1–5 were treated with 1.5 ml of nitrogen mustard solution, and bottle 6 was treated with H_2O as a control. All broods were treated identically. Progeny were scored from day 9 to 17. The same protocol was used to map the *mus308^{D14}* allele.

Cytological mapping: Fifteen males heterozygous for a deficiency and a balancer chromosome carrying *Sb* were crossed to thirty *mus308^{D2}/Tm3 Sb e* virgin females. Two broods were established. Seventy-two hours after the cultures were initiated the first brood was treated with 1.5 ml of nitrogen mustard solution and the second brood with 1.5 ml of H_2O. The progeny were scored through day 17 for the presence of *Df/mus308* progeny (*Sb*$^+$). Each deficiency was also tested for complementation with the recessive lethal loci *l(3)m114-SzD*, *l(3)S5*, and *l(3)S3*, which are balanced with *MKRS*. Progeny were scored for the presence of individuals heterozygous for the deficiency and lethal bearing chromosomes. In addition deficiency heterozygotes were scored for pseudodominance of *kar*.

TABLE 1

Deficiencies used in the localization of *mus308*

Deficiencies	Breakpoints	References
Df(3R)ry^{619}	87D7-9;87E12-F1	HALL and KANKEL (1976)
Df(3R)ry^{1608}	87D4-6;87E1-2	HILLIKER *et al.* (1980)
Df(3R)ry^{1607}	87D3-4;87E2-3	HILLIKER *et al.* (1980)
Df(3R)ry^{614}	87D2-4;87D11-14	HALL and KANKEL (1976)
Df(3R)ry^{1402}	87D2-4;87D14-E2	HILLIKER *et al.* (1980)
Df(3R)ry^{1301}	87D2-4;87E1-2	HILLIKER *et al.* (1980)
Df(3R)ry^{75}	87D2;87D14-E1	LEFEVRE (1971)
Df(3R)ry^{27}	87D1,2;87F1-2	HALL and KANKEL (1976)
Df(3R)ry$^{506\text{-}85C}$	87D1,2; 88E5-6	Indiana Stock Center
Df(3R)karl^{G27}	87B3-5;87D6-12	HALL and KANKEL (1976)
Df(3R)kar^{H5}	87A1-2;87D11	HENIKOFF (1979)
Df(3R)kar^{31}	87B15-C2; 87C9-D2	HALL and KANKEL (1976)
Df(3R)kar$^{SZ\text{-}31}$	87C6-7;87C9-D3,4	GAUSZ *et al.* (1979)
Df(3R)kar$^{SZ\text{-}29}$	87C3-4;87C9-D1,2	GAUSZ *et al.* (1979)
Df(3R)kar$^{SZ\text{-}13}$	86E6-7;87C9-D1	VOELKER *et al.* (1981)
Df(3R)kar$^{SZ\text{-}21}$	87C7;87C8-9	GAUSZ *et al.* (1979)
Df(3R)pb^{win12L} + Antp^{rA74R}	84A1-2; 87C	Indiana Stock Center
Df(3R)ry^{615}	87B12-15;87E8-11	HALL and KANKEL (1976)
Df(3R)ry^{1168}	87B15-C1;87E9-12	HILLIKER *et al.* (1980)

X-ray-induced mutations: A total of 2,638 adult *mwh red e* males were collected and aged 1–4 days. These males were etherized, placed on a Kimwipe in a milk bottle and irradiated with 3000 rad. A Picker X-ray machine equipped with a Machlett OEG 60 tube was used, and the beam was filtered through a 0.95 mm aluminum plate. Groups of 30 irradiated males were immediately mated to *D/TM3, Sb Ser e* females. The F_1 male progeny (4261 *mwh red e/TM3, Sb Ser e*) were crossed individually in vials to *mus308^{D2}/Tm3, Sb Ser e* virgin females. Two broods were established from each mating. The first brood was treated with 0.006% nitrogen mustard and the second brood was left untreated. Absence of the *Sb*$^+$ phenotype in the treated vials indicated a lack of complementation to *mus308^{D2}*.

Dysgenically induced mutation: *Sp/CyO; Δ2–3,Sb/TM6* males were crossed to *Birm 2;st $p^p e^{11}$* females *en masse*. The *Birm 2* chromosome (ROBERTSON *et al.* 1988), contains 17 defective *P* elements which lack the ability to produce transposase. The *Δ2–3* element is a *P* element construct which produces transposase but is stably integrated at 99B (ROBERTSON *et al.* 1988). In the next generation dysgenic *Birm 2/CyO; st p^p e^{11}/Δ2–3, Sb* males were collected and crossed to *D/TM3* virgin females. Individual *+/CyO; st p^p e^{11}/TM3* males (11,433) were collected and mated in vials to *st p^p mus308/TM3* virgin females. Two broods were established; the first brood was treated with 0.006% nitrogen mustard and the second brood was left untreated. The progeny were then scored for the absence of the *Sb*$^+$ phenotype in the treated vial.

Cytological analysis: Polytene chromosome squashes were analyzed for visible aberrations. Fifteen males carrying the X-ray-induced mutations, *mus308$^{D7\text{-}14}$/TM6B* or *Df(3R)mus308D^{16}/TM6B*, were mated to 19 Canton-S females. The females were allowed to lay eggs in bottles for 3 days at 25°. Lyophilized yeast was then added to the bottles

and the cultures were moved to 18°. After 3–6 days permanent orcein-acetate squash preparations were generated from Tb^+ larvae and analyzed for chromosomal aberrations. Such larvae do not carry the TM6B balancer (ASHBURNER 1989).

Lethal complementation: Chromosomes bearing X-ray-induced *mus308* mutations were analyzed for homozygous viability. For those *mus308* mutations that were found to be inviable as homozygotes, complementation with lethal mutations located near the *mus308* locus was performed. Complementation between the chromosomes bearing the X-ray generated *mus308* alleles and the available lethal mutations in the 87C9 to 87D3,4 region was performed by mating *mus308* males balanced with *TM3(Sb)* to virgin females heterozygous for a lethal mutation and a balancer chromosome. The progeny without a balancer chromosome were then scored.

Mutagen sensitivity: To test for mutagen sensitivity 30 *mus308/TM3* females were mated to 15 *mus308/TM3* sibs. The treatment was performed as described under the Cytological Mapping section, with the exception that the larvae were treated with nitrogen mustard concentrations varying between 0.008% and 0.00015%. Two to three matings per dose were established for each allele tested.

Flight capacity: Nitrogen mustard treatment was performed as described above. All flies used in the flight test were collected as virgins from either the mutagen treated or water treated cultures. The flies were then aged for 3–5 days. The flight test was performed at room temperature from 12:00 p.m. to 4:00 p.m., as described by BENZER (1973). The flight distance measurements were taken from the top of the cylinder, which was 31 cm tall, and the flies were dropped from a distance of 19 cm above the cylinder. Males and females of each stock were tested separately.

Viability studies: For each genotype 100 virgin males and 200 virgin females were collected and kept in separate vials. They were mated in bottles for 2 days and then moved to a plexiglass egg collector (BELLEN and KIGER 1988). Eggs collected during the first 2 hr were discarded. Those collected in the subsequent 4-hr period were aligned in a 10 × 10 grid on moist toweling placed on top of fly media in a split bottle. After 24 and 48 hr at 25° the number of unhatched embryos was scored. On the eighth day the number of pupae were scored. By day 12 all adults had eclosed and were scored. In later experiments embryos were placed in a 10 × 10 grid on a petri dish containing 1% agarose. The number of unhatched eggs was scored at 24 and 48 hr.

Fluorescent staining of embryos: Embryos (0–10 hr postfertilization) were stained with 1 μg/ml Hoechst 33258 in Buffer A and mounted as described by BELLEN and KIGER (1988). In an exception to that procedure the embryos were washed three times with fresh buffer for 10 min per wash.

RESULTS

Recombinant mapping: To facilitate cytological mapping of *mus308* the genetic map position has been refined beyond the original localization to 55 ± 5 cM (BOYD *et al.* 1981). In the previous mapping analysis *mus308* had been mapped distal to *cu* (BOYD *et al.* 1981) and in a separate recombination experiment the locus was mapped proximal to *red* (E. A. LEONHARDT, unpublished). The mapping scheme outlined in Figure 1 positions *mus308* relative to the dominant markers *Gl*, *Sb* and *H* and the recessive markers *st* and

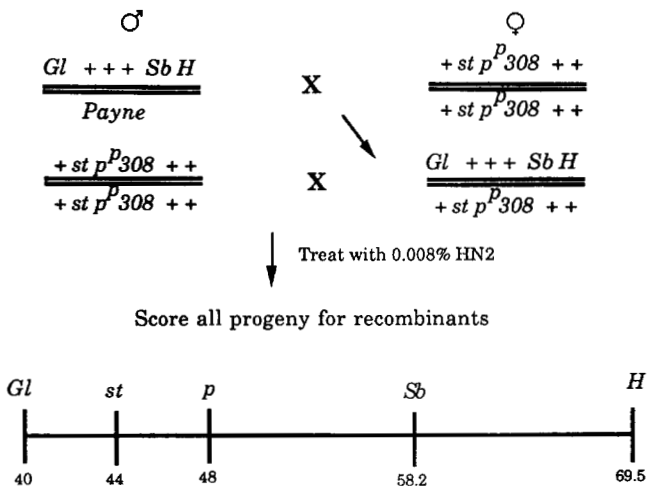


FIGURE 1.—Recombination mapping of the *mus308* locus. The genetic scheme used to map *mus308* employed the dominant markers *Gl*, *Sb*, *H* and the recessive markers *st* and p^p. The progeny of the second cross were treated with 0.008% HN2 and the survivors were scored for the presence of the five markers. The published map positions of all five markers are shown below the cross. The *Gl Sb H* stock was maintained with the Payne balancer chromosome.

p^p. By treating the F_1 progeny with HN2 the *mus308* mutation could be identified since homozygous *mus308* flies do not survive treatment. Because reciprocal recombinant classes are recovered from the interval between p^p and *Sb*, as seen in Table 2, that interval contains the *mus308* gene. Recombinant classes used to calculate the map position for *mus308* are marked with an (*a*). There were 8 single crossover events in the interval between p^p and *mus308*, and 31 single crossover events occurred between *mus308* and *Sb*. When the double crossover events seen in Table 2 were included, the map position was calculated as 51 ± 1 cM (O'BRIEN and MACINTYRE 1978) between the genetic markers p^p and *Sb*. Taken together, these data establish the genetic position of *mus308* in the interval between *cu* (50.0) and *red* (53.6) on the right arm of chromosome three, at 51 ± 1 cM.

Cytological mapping: Nineteen chromosomes bearing deficiencies in the region flanked by *cu* and *red* were employed in a cytogenetic localization of the *mus308* gene. Previous cytological characterization of those deficiencies is summarized schematically in Figure 2 together with an analysis of their complementation to 6 genes in that region. Previously identified lethals in this region (GAUSZ *et al.* 1979; HILLIKER, CLARK and CHOVNICK 1980) were sent to us by A. CHOVNICK who also provided the complementation data for those mutants. Those results have been independently confirmed in the current study. The malic enzyme (*Men*) locus had previously been mapped to the region 87C9 to D1,2 by VOELKER *et al.* (1981) with the aid of several of these deficiencies. Their complementation results involving that gene are also summarized in Figure 2. Those data together with our own analysis of complementation of the

TABLE 2

Recombination mapping of *mus308*

Genotypes of recombinant chromosomes	Recombinant intervals	No. of individuals recovered from treated bottles	No. of individuals recovered from H_2O bottles
Gl + + Sb H	Parental chromosome	557	330
+ st p^p + +	Parental chromosome	0	315
+++ Sb H	*Gl–st*	13	7
Gl st p^p + +	*Gl–st*	0	8
+ st + Sb H	*st–p^p*	12	13
Gl + p^p + +	*st–p^p*	0	13
+ st p^p Sb H[a]	*p^p–Sb*	8	37
Gl + + + +[a]	*p^p–Sb*	31	27
+ st p^p + H	*Sb–H*	0	24
Gl + + Sb +	*Sb–H*	74	34
+ + + + +[a]	Gl–st; p^p–Sb	4	1
+ + + Sb +	*Gl–st; Sb–H*	1	0
Gl + p^p Sb H[a]	*st–p^p; p^p–Sb*	1	0
+ st + + +[a]	*st–p^p; p^p–Sb*	1	2
Gl + p^p +H	*st–p^p; Sb–H*	2	0
+ st + Sb +	*st–p^p; Sb–H*	0	1
Gl + + + H[a]	*p^p–Sb; Sb–H*	3	1
+ st p^p Sb +[a]	*p^p–Sb; Sb–H*	0	2
Total		707	815

Data from the mating scheme outlined in Figure 1.

[a] Recombinant classes used in the calculation of the map position. The recombinant classes that were not represented in either the treated or control bottles are omitted.

mus308 mutations with these deficiencies provides a unique order for five of these genes. The deficiencies *Df(3R)kar^{SZ-31}*, *Df(3R)kar^{SZ29}*, *Df(3R)kar^{SZ-13}* and *Df(3R)kar^{SZ-21}* were particularly informative in establishing the proximal to distal order of: *kar, l(3)m114-SzD, mus308, Men* and *l(3)S5; l(3)S3]*. The two most distal loci, *l(3)S5* and *l(3)S3*, have not been ordered relative to each other, and only one deficiency establishes the relative positions of *mus308* and *Men*. This analysis therefore establishes the cytological position of the *mus308* gene at 87C9 to 87D1,2 on the right arm of chromosome three.

Generation of *mus308* mutations: New *mus308* alleles were induced by X-rays and recovered as shown in Figure 3A. The mutagenized stock was isogenic for a single third chromosome bearing the markers *mwh, red* and *e*. Males of that genotype were irradiated and mated *en masse* to *D/TM3* virgin females. Among 4,261 male progeny retrieved in the F_1 generation, 26% were infertile, and nine carried 3rd chromosomes that failed to complement *mus308^{D2}* for HN2 resistance. The nine new lines are designated *mus308^{D7-14}* and *Df(3R)mus308D^{16}*. The four lines *mus308^{D10-13}* are potentially derived from a single mutational event, because the males bearing those mutations were obtained from a single initial culture. Southern analysis which supports this conclusion has revealed a common RFLP in all four strains (J. B. BOYD, unpublished results).

Polytene chromosomes heterozygous for the X-ray induced mutations were surveyed for chromosomal aberrations as summarized in Table 3. Cytologically visible mutations were detected in three of the eleven mutant stocks. The deficiency *Df(3R)mus308D^{16}* exhibits breakpoints at 87C1–2 and 87D1,2–D3,4; *mus308^{D8}* contains an inversion that does not involve the 87 region of the third chromosome; and the *mus308^{D7}* stock carries a translocation between 3R and 2R (T(2;3)43;93). In addition, the homozygous inviable chromosomes *mus308^{D7-9}*, *mus308^{D11}* and *Df(3R)mus308D^{16}* were tested for complementation with lethal mutations that had previously been localized to this region of the genome. As expected the deficiency *Df(3R)mus308D^{16}* failed to complement the lethal mutation *l(3)lm114-SZD* as well as *kar* and *mus308*.

The dysgenic scheme outlined in Figure 3B was also employed in an effort to recover a transposon insertion in the *mus308* gene. Among 11,433 third chromosomes analyzed in that screen, one carries a new *mus308* allele that is designated *mus308^{D15}*. That mutation is not, however, associated with a *P* element insertion as judged by Southern analysis, since no new *P* insertions were visualized in the strain (data not shown).

Analysis of mutagen sensitivity: The level of HN2 sensitivity of each new homozygous viable allele of *mus308* was compared with that of *mus308^{D2}*, an EMS induced mutation (Figure 4). Matings were established between *mus308* virgins, heterozygous for the balancer chromosome *TM3*, and their sibs. The progeny were treated as larvae with various doses of HN2, and the ratio of homozygous *mus308* survivors to heterozygous survivors was calculated. The data presented in Figure 4 reveal a comparable sensitivity among all tested mutants under conditions that have little influence on the control strain. Above 0.002% HN2, negligible survival is observed in any of the *mus308* mutants and all stocks exhibit wildtype survival levels at 0.00025% HN2. Since *mus308^{D10}*, *mus308^{D12}* and *mus308^{D13}* are potentially derived from a single mutational event, only the data for *mus308^{D10}* are shown. The other two mutants were tested and found to have similar sensitivities.

In the above experiment a large percentage of the surviving homozygous flies were observed to exhibit a phenotype similar to that of the mutant *wings held up* (LINDSLEY and ZIMM 1992) which is characterized by an erect resting wing position. Figure 5 depicts homozygous *mus308^{D14}* survivors after HN2 treatment. As can be seen the trait is not fully penetrant,

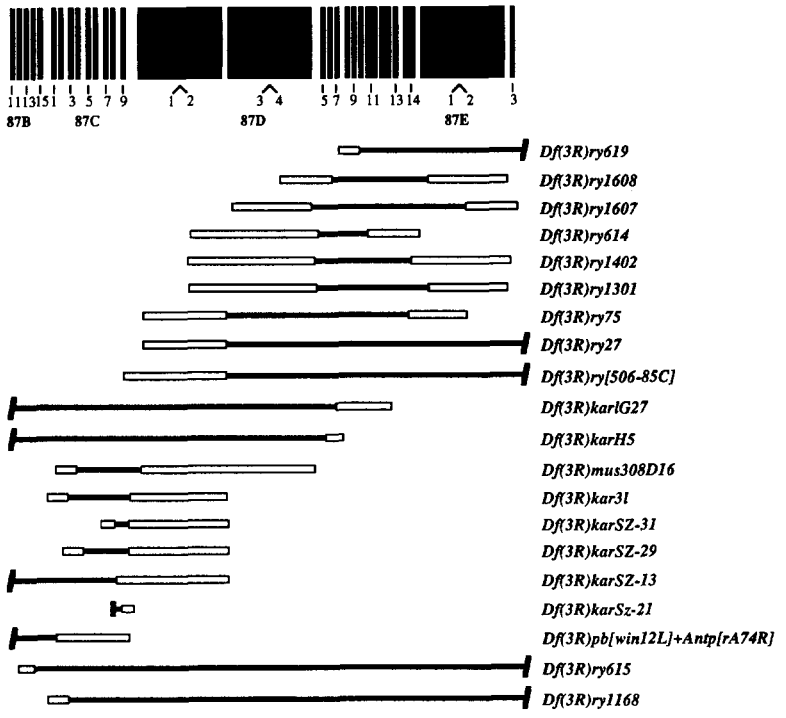


FIGURE 2.—Cytogenetic localization of the *mus308* locus. The bold black lines show the extent of the deficiencies and the open boxes indicate uncertainties in the cytological breakpoints. Complementation results obtained between the deficiencies and mutations in the following genes; *mus308*, *Men*, *l(3)m114*, *l(3)S5* and *l(3)S3* is also indicated. (+) indicates complementation; (−) a lack of complementation. The deficiencies are listed in Table 1. (*) Complementation results obtained with the lethal mutations *kar*, *l(3)m114*, *l(3)S5* and *l(3)S3* are from A. CHOVNICK (personal communication). (¶)Data from (VOELKER *et al.* 1981). (*)Data from (HILLIKER *et al.* 1980).

although it was seen in all *mus308* alleles tested. This phenotype is difficult to quantify because the flies become stuck in the medium shortly after eclosion. Since the *mus308* alleles used in this study were generated in three different genetic backgrounds, the erect wing trait is due to the *mus308* mutations and not to a secondary mutation. The homozygous *mwh red e* survivors, which carry the parental chromosome, did not display this trait nor did any of the water treated cultures of *mus308*.

Flight capacity: The aberrant wing phenotype described above suggested that *mus308* flies surviving HN2 treatment as larvae may have difficulty flying. Accordingly, the effect of HN2 treatment on the flight capacity of *mus308* flies was analyzed as described in Table 4. A mutagen insensitive stock carrying the white (*w*) marker was employed as a control in these experiments, because the *mwh red e* chromosome carries markers that adversely effect flight. Treatment with either 0.002% or 0.004% HN2 had no effect on either the survival or flight capacity of that stock. In contrast, the flight capacity of $mus308^{D15}$ males and females was reduced by 80 and 56%, respectively, after treatment with a dose of 0.001%. Treatment of an alternate allele ($mus308^{D12}$) with a still lower dose of HN2 (0.0005%) resulted in a 20% reduction in flight capacity; confirming an association of this phenotype with the *mus308* defect. This result is particularly remarkable because the dose of 0.0005% HN2 employed in that experiment had no effect on survival. A cross-link sensitive mutation in an alternate gene ($mei\text{-}41^{D5}$) was employed to establish that flight defects are not generally observed following treatment of HN2 sensitive mutants. The $mei\text{-}41^{D5}$ cultures were treated with an HN2 dose which kills 69% of the mutant progeny. The ability of the *mei-41* survivors to fly was not affected to the extent seen in the $mus308^{D15}$ trials, even though a larger percentage of the $mus308^{D15}$ flies survived the mutagen treatment. Treated *mei-41* males exhibit a reduction in flight capacity of only 11% relative to that of the untreated stock and that value for females is 14%.

Viability studies: Because the *mus308* stocks were perceived to exhibit low fertility during culture, the viability of homozygous *mus308* individuals was analyzed at each stage of development. With the exception of embryos, survival was normal at all tested stages of development (our unpublished observations). Accordingly, the hatching frequency of homozygous *mus308* embryos was quantified as documented in Table 5. The parental control stock *mwh red e* is seen to exhibit an embryo mortality frequency of 9% (Table 5). In contrast, homozygous $mus308^{D2}$ and $mus308^{D14}$ mutants have mortality rates of 21 and 29%, respectively. Whether this mortality is due to a maternal or early zygotic effect is the subject of future study.

Fluorescent staining of embryos: In an effort to identify the origin of the embryo lethality documented above, the distribution of chromatin was monitored in developing embryos. Zero- to 10-hr embryos from homozygous *mus308* parents were stained with the nuclear staining dye Hoechst 33258. In that analysis *mus308* embryos exhibit a variety of abnormal phenotypes that have been catalogued into five distinct classes (Figure 6). Class 0 embryos are phenotyp-

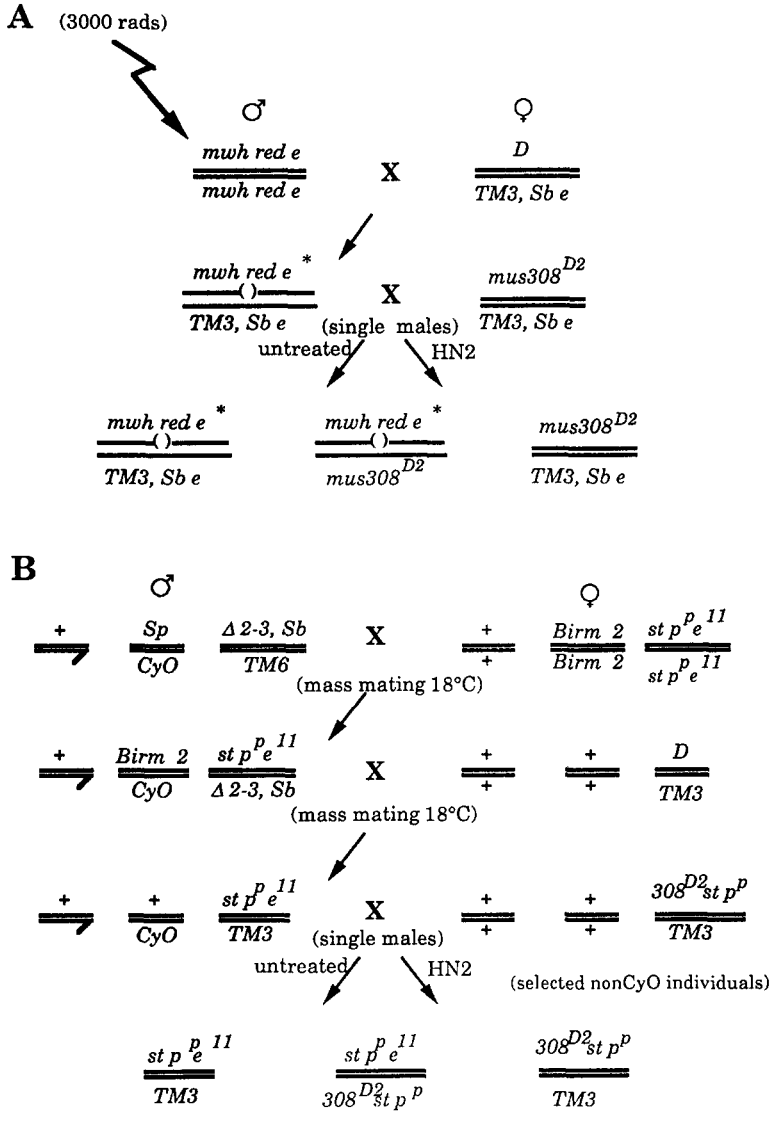


FIGURE 3.—Crosses used to retrieve new *mus308* mutations and deficiencies. (A) Genetic scheme used to generate $mus308^{D7}$ $mus308^{D14}$ and $Df(3R)mus308D^{16}$ following X-ray mutagenesis. (B) Dysgenic crosses employed to generate $mus308^{D15}$. See MATERIALS AND METHODS for a detailed description of the crosses.

TABLE 3

Cytogenetic analysis of X-ray-generated chromosomes

Mutation	Homozygous viability[a]	Cytological aberration[b]	Complementation[c]				
			kar	*lm114-SZD*	$mus308^{D2}$	*lS3*	*lS5*
$mus308^{D7}$	−	T(2;3)43;93	+	+	−	+	+
$mus308^{D8}$	−	In(3L) 79DE-80C| 61D-79CD	+	+	−	+	+
$mus308^{D9}$	−	None	+	+	−	+	+
$mus308^{D10}$	+	None	///	///////	−	///	///
$mus308^{D11}$	−	None	+	+	−	+	+
$mus308^{D12}$	+	None	///	///////	−	///	///
$mus308^{D13}$	+	None	///	///////	−	///	///
$mus308^{D14}$	+	None	///	///////	−	///	///
$Df(3R)mus308^{D16}$	−	Df(3R) 87C1-2; 87D1,2-3,4	−	−	−	+	+

[a] (+) Individuals homozygous for the *mus*-bearing chromosome are viable, (−) homozygous individuals are not viable.
[b] None indicates no visible aberrations.
[c] (−) indicates a failure to complement, (+) indicates complementation between *mus308* and three lethals that map close to *mus308*: *lm114-SZD*, *lS3* and *lS5*. Barring (////) indicates that the *mus308*-bearing chromosome was not tested for complementation with a lethal mutation.

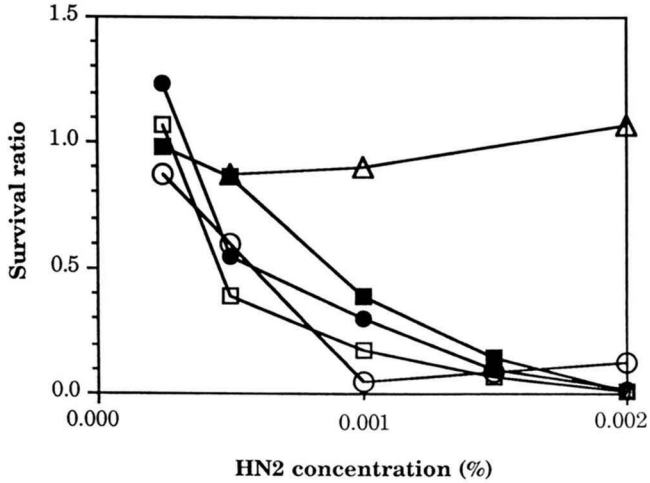


FIGURE 4.—HN2 sensitivity of homozygous *mus308*^{D10}, *mus308*^{D14} and *mus308*^{D15} individuals. The ratios of homozygous survivors to survivors heterozygous for the balancer chromosome are shown after HN2 treatment. These values have been normalized with respect to the water treated samples. Only the results for doses below 0.002% are shown. Above that dose survival of the mutants ie negligible. The number of survivors at each dose ranged from 56 to 1800. (□——□) *mus308*^{D10}; (■——■) *mus308*^{D14}; (●——●) *mus308*^{D15}; (○——○) *mus308*^{D2}; (△——△) *mwh red e*.

ically normal; class 1 embryos have disorganized peripheral nuclei; class 2 embryos exhibit a large mass of chromatin with peripheral nuclei still present; class 3 embryos possess two large chromatin masses; and class 4 embryos have only one large mass (Figure 6, A–F). Figure 6A depicts a normal preblastoderm embryo and 6F an unfertilized egg (center), whereas Figure 6, B–E, illustrates representatives from each of the abnormal classes, 1 through 4, respectively. Classification of both homozygous *mus308* and *mwh red e* embryos into these classes is presented in Figure 6G. Over 99% of the control embryos (*mwh red e*) exhibit a normal chromatin distribution. In *mus308*^{D2}, however, 13.1% of the embryos are abnormal with class 1 being the predominant class. Among 221 *mus308*^{D2} embryos no class 3 embryos were seen, although this class was most frequent in the *mus308*^{D14} stock. In *mus308*^{14} 30.2% of the embryos in the 0–10-hr collection were abnormal. The larger proportion of aberrant *mus308*^{D14} embryos relative to *mus308*^{D2} embryos correlates with the lethality of those embryos which is 29 and 21%, respectively (Table 5). Confirmation that this phenotype is associated with the *mus308* mutations was provided by analysis of *mus308*^{D14}/*mus308*^{D2} heteroallelic embryos which exhibit all four abnormal classes (data not shown).

DISCUSSION

In this study the *mus308* locus has been cytogenetically mapped to position 87C9-D1,2 on the right arm of chromosome three. The proximal to distal gene

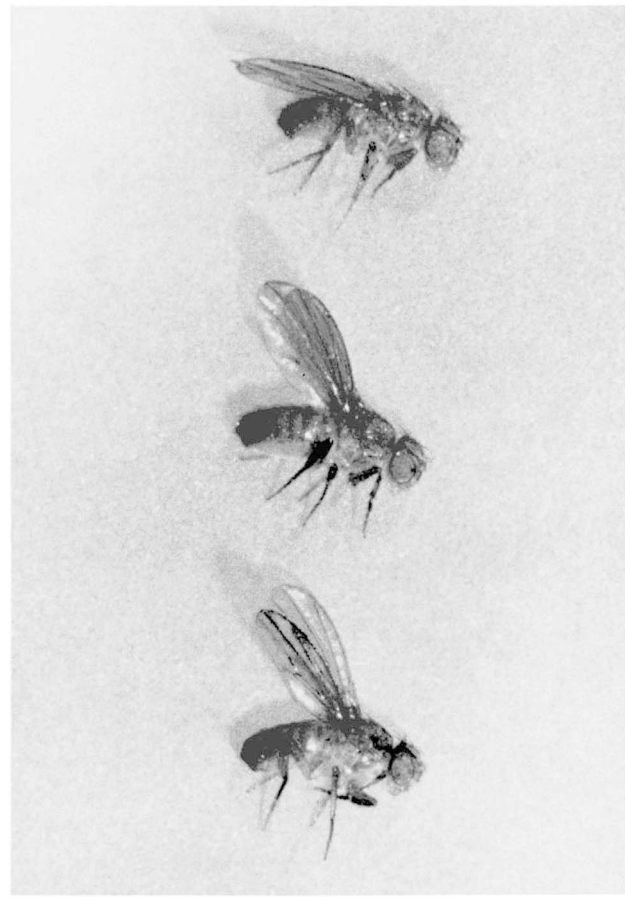


FIGURE 5.—Wing phenotype of *mus308*^{D14} flies after treatment with HN2. A culture of homozygous *mus308*^{D14} larvae was treated with 0.0005% HN2. Shown in the picture are three homozygous *mus308* individuals that survived the treatment and eclosed. The wings of two of the three *mus308* survivors are held in an upright position. The third exhibits the normal wing position.

order in that genomic region has been established as *kar, l(3)m114, mus308, Men* and (*l(3)S3* and *l(3)S5*). In addition, ten new alleles of the *mus308* gene have been retrieved from two separate mutagenesis schemes. Nine were generated with X-ray mutagenesis and one was obtained following genetic mobilization of *P* elements (see Figure 2). The latter mutation, *mus308*^{D15} does not contain a new *P* insertion as shown by Southern analysis. Since that mutation was generated during *P* mobilization, however, it may carry a molecularly detectable lesion which was generated by the insertion and subsequent excision of a transposable element.

Cytological analysis of the new mutants has revealed that six have no visible chromosomal aberrations, the chromosome carrying *mus308*^{D8} has an inversion involving most of the left arm of chromosome three, the chromosome bearing *mus308*^{D9} carries a translocation involving the right arms of chromosome two and three, and *Df(3R)mus308D*^{16} is a deficiency between 87C1,2 and 87D3,4 (see Table 3 for details). The viable mutants retrieved in this study are shown

TABLE 4

Flight capacity of HN2-sensitive mutants

Genotype	Treatment	Percent survival[a]	Males		Females	
			Mean flight in cm[b]	Percent of control[c]	Mean flight in cm[b]	Percent of control[c]
w	H_2O		20.9 (497)		18.1 (474)	
w	0.002% HN2	100	20.8 (485)	99.5	19.5 (561)	>100
w	0.004% HN2	100	20.2 (497)	96.7	19.0 (438)	>100
$mus308^{D15}$	H_2O		19.8 (236)		16.7 (256)	
$mus308^{D15}$	0.001% HN2	40	4.0 (262)	20.2	7.4 (196)	44.3
$mus308^{D2}$	H_2O		20.8 (551)		19.3 (826)	
$mus308^{D2}$	0.0005% HN2	100	15.1 (574)	72.6	14.9 (633)	77.2
$mei\text{-}41^{D5}$	H_2O		20.7 (478)		20.0 (457)	
$mei\text{-}41^{D5}$	0.002% HN2	31	18.4 (253)	88.9	17.2 (231)	86

Effect of HN2 treatment of larvae on the flight capacity of homozygous *mus308*, *mei-41* and *w* flies.

[a] Ratio of HN2-treated progeny to H_2O-treated progeny.

[b] The average distance that flies come out of the free fall and stick to the oiled cylinder as measured from the bottom of the cylinder.

[c] The ratio of flight measurements of treated flies/H_2O treated flies.

TABLE 5

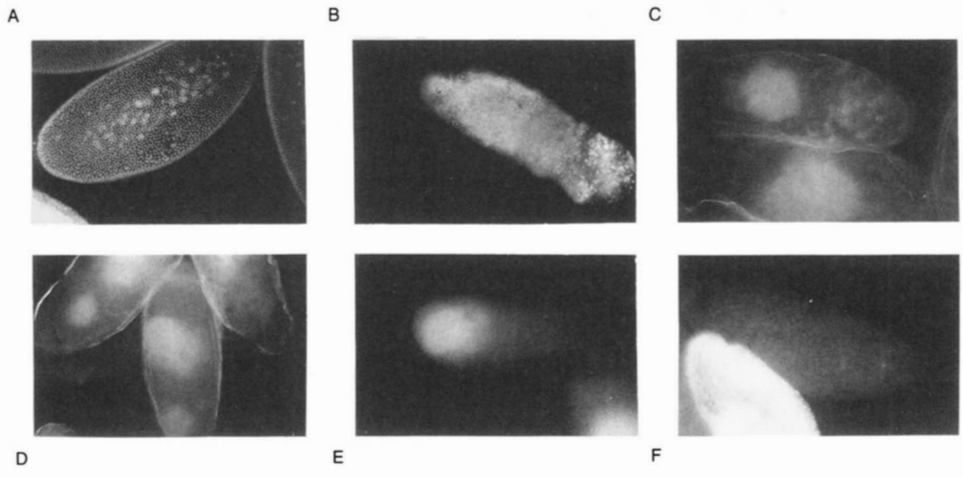
The analysis of *mus308* embryonic mortality

Homozygous genotype	No. of eggs tested	Percent mortality
mwh red e	400	9
$mus308^{D2}$	797	21
$mus308^{D14}$	700	29

to exhibit HN2-sensitivities comparable to that of the EMS-induced allele $mus308^{D2}$.

During the analysis of mutagen sensitivity it was observed that a large percentage of homozygous *mus308* survivors hold their wings erect. This trait is difficult to quantify because the flies become mired in the media immediately after eclosion. The phenotype was only observed after HN2 treatment of the mutants; treated control flies and untreated mutant flies assume a normal wing position. Since other mutants with this phenotype have been demonstrated to affect flight (HOMYK and EMERSON 1988), two of the *mus308* alleles were tested for flying ability. Without treatment the *mus308* flies do not differ from control flies in their capacity to fly. However, flies recovered from treated mutant larvae exhibit a significant decrease in flight capacity. The abnormal wing position therefore correlates well with reduced flight capacity. As mentioned, mutations in several other genes exhibit a wings-held-up phenotype in the absence of mutagen treatment (HALL 1982). Several of those that have been cloned and characterized have been shown to encode muscle specific products (KARLIK, COUTU and FYRBERG 1984; KARLIK and FRYBERG 1985; MAHAFFEY *et al.* 1985). In other cases, however, the role of the gene product in muscle differentiation is not apparent; *dpp* encodes a protein with homology to transforming growth factor-β (ST. JOHNSTON *et al.* 1990) and the *ewg* peptide is thought to be involved in neurogenic development (FLEMING, DESIMONE and WHITE 1989). A third gene, *mit11*, is involved in mitosis (SMITH, BAKER and GATTI 1985). Alteration in flight capacity is therefore not an accurate predictor of function for the *mus308* gene.

Although each major stage of the life cycle was analyzed for mortality of homozygous *mus308* individuals, an increase was observed only during the embryonic stage. When embryonic nuclei were visualized with Hoechst staining, a substantial percentage of the mutant embryos exhibited an aberrant chromatin distribution. In that analysis 13–30% of the embryos exhibit disoriented nuclei and/or large clumps of chromatin. It should also be noted that the *mus308* embryos are extremely fragile compared to the control embryos in that the mutant embryos disintegrate under standard conditions employed to remove the vitellin membrane. This fragility implies a possible aberration in the cytoskeleton. There are several possible explanations for the aberrant chromatin segregation observed in *mus308* embryos. First, the clumping of nucleic matter seen in the embryos could be due to premature termination of DNA synthesis which would render the homologs inseparable at mitosis. Second, a defect in spindle formation could interfere with normal chromosome segregation. A third explanation is that the spatial distribution of nuclei is perturbed due to improper nuclear migration. Further study is therefore required to establish the primary cause of this mutant phenotype. Large chromatin masses have also been seen in mutants of genes that have been implicated in Drosophila mitosis such as *gnu* (FREEMAN, NUSSLEIN-VOLHARD and GLOVER 1986; FREEMAN and GLOVER 1987), *quartet* (ZAHNER and CHENEY 1990), and *polo* (GLOVER *et al.* 1989). Other mutants that perturb mitosis are also mutagen-sensitive. The *mus101* gene in Drosophila, which was



G **Analysis of Nuclei in *mus308* Embryos**

Genotype	class 0	class 1	class 2	class 3	class 4	unfertilized eggs
mus308^{D2} (221)	82.4%	9.5%	.9%	0%	2.7%	4.5%
mus308^{D14} (149)	59.7%	6.7%	8.7%	10.1%	4.7%	10.1%
mwh red e (118)	95.8%	.9%	0%	0%	0%	3.4%

FIGURE 6.—Chromatin distribution in *mus308* embryos. Representives of each of the six morphological classes (A–F) seen when 0–10-hr embryos were stained with Hoechst and visualized with fluorescence microscopy. A, Normal embryo; B, class 1 embryo; C, class 2 embryo; D, class 3 embryo; E, class 4 embryo; F, unfertilized egg. The 0–10-hr post fertilization embryos were scored for the number present in each of these classes. The table (G) lists the percentages of each class of embryos found in the wildtype control (*mwh red e*) and in the two *mus308* mutants. The numbers in brackets are the total number of embryos scored. For a complete description of the classes refer to RESULTS.

initially identified through an analysis of mutagen sensitive mutations also exhibits mitotic defects (GATTI, SMITH and BAKER 1983). In yeast the *cdc7* gene, which encodes a protein kinase, is not only necessary for the initiation of DNA synthesis but it is also thought to be involved in error prone repair (BUCK, WHITE and ROSAMOND 1991; NJAGI and KILBEY 1982). The yeast gene *hrr25*, which encodes another protein kinase, also has a dual role in repair and mitosis (HOEKSTRA *et al.* 1991).

Neither the embryonic phenotype seen in untreated cultures nor the wing phenotype seen in HN2 treated cultures is fully penetrant. This may be due to a threshold effect. During times of replicative stress, which may occur during the repair of DNA cross-links after HN2 treatment or during early embryogenesis, the mutant protein may not be able to meet these demands in all individuals.

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