Genetic Analysis of Chromosome Region 63 of *Drosophila melanogaster*

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ABSTRACT

The salivary chromosome region including cytological division 63 of *Drosophila melanogaster* was genetically analyzed in order to (1) characterize this previously unstudied region and (2) attempt to isolate mutations in the *hsp82* gene. Seven deletions which span this region were isolated, including four which remove the *hsp82* gene. A *Minute* mutation was mapped to this region and this *Minute* was used to isolate duplications in the 63 region. These duplications map the *Minute* to 63B8–C1. F_2 screens were initiated using deletions which remove the *hsp82* gene. Over 15,000 chromosomes were screened, yielding 40 lethal mutations which comprise 14 complementation groups. Several of these mutations map outside the 63 region and appear to give second site interaction with the *Minute* locus. Four loci, including the *Minute* gene, are candidates for hsp82 mutations by cytogenetic mapping. These loci were tested for complementation with a *P* element carrying the *hsp82* gene. However, none of the mutations was rescued.

CURRENTLY, genetic and molecular analysis of the *Drosophila melanogaster* genome has proceeded in two directions. In the first approach, one starts with a visible phenotype of interest and uses this phenotype to aid in identifying chromosomal rearrangements in the region of this gene. Such rearrangements are then useful in molecular analysis of the gene and the surrounding area: they provide convenient landmarks that are useful in molecular mapping as well as providing entry points for chromosomal walks. In the second approach, one maps a cloned gene by *in situ* hybridization and then proceeds to use available rearrangements to isolate mutations in that gene. Frequently, such genes fall into areas that have not been well characterized genetically. In these cases, genetic analysis of the region is useful not only in studying the functions of these genes of interest, but also in identifying and mapping new loci and previously identified loci that had not been precisely mapped.

One region of the Drosophila genome which has not been well studied is the 63 interval of the third chromosome. A small number of genes that have been identified by molecular techniques have been mapped to this region. These genes include a major heat-shock gene, *hsp82*, and two flanking transcripts at 63B (O'CONNOR and LIS 1981; BLACKMAN and MESLESON 1986); an ecdysone-inducible membrane protein at 63E (NATZLE, HAMMONDS and FRISTROM 1986), a gene with homology to spectrin and dystrophin gene at 63CD (DUBREUIL *et al.* 1990), and a ubiquitin gene has been mapped to 63 (ARRIBAS, SAMPEDRO and

IZQUIERDO 1986). One mutation, *M(3)LS3* (LINDLSEY *et al.* 1972), has been mapped using segmental aneuploids to this region; however, no other genes defined by mutations have been localized in the 63 region. Finally, several puffs in addition to the heat shock puff produced by the *hsp82* gene have been identified in this region. These include a late ecdysone puff at 63E (ASHBURNER *et al.* 1974) and a minor heat shock puff at 63F (BONNER and KERBY 1982). The gene products of these puffs have not been identified.

This report discusses the identification of deletions and duplications in the 63 interval and their use in isolating and mapping mutations. We have specifically focused on the 63B region because we had hoped to identify mutations in the *hsp82* gene. Because the hsp82 protein is highly conserved evolutionarily, because it appears to be present in only one detectable copy in Drosophila, and because deletion of the hsp82-coding loci in yeast is lethal (BORKAVITCH *et al.* 1989), we expected that a lethal or visible phenotype would be produced. Although we have identified many lethal loci in this region, we show that none of these mutations represents the hsp82 locus.

MATERIALS AND METHODS

Drosophila culture and strains: Flies were grown on a standard cornmeal-molasses medium. Most mutations, balancers and other rearrangements are described in LINDSLEY and GRELL (1968) or LINDLSEY and ZIMM (1985, 1986, 1987). Strains used in this study that are not described in the above references include: (1) *28AC* which carries a *P* element insert at 63D containing a truncated *hsp28* gene and a *ry* marker gene (HOFFMANN and CORCES 1984); (2) lines containing *P* element inserts of the *Drosophila pseudoobscura hsp82* gene at cytological locations 21, 41, 45 and/

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or 64 (C. LAIRD and E. JAFFE, personal communication); (3) *P* elements marked with a neomycin resistance gene: *neo9* and *neo10* both of which map to 63C (COOLEY, KELLY and SPRADLING 1988); (4) *BK116*, a direct tandem duplication from 63A–64C (LEICHT and BONNER 1988); (5) *In(3LR)T33*, an inversion between 64C and 97–98 (J. J. BONNER, unpublished), and (6) *IN(3LR)f19*, an inversion between 62A and 98A (CRAYMER 1984).

Isolation of deficiencies: *28AC* (*ry$^+$*) males were aged 3–5 days, irradiated with 3500–4000R of X-rays and crossed (two males to six to eight females per vial) to *ve st ry* virgin females. The flies were transferred every 1–2 days and males were removed after 3 days. *ry$^-$* progeny were mated to *TM3Sb/TM6B* flies and several single progeny from each line were then mated to *ve st ry/TM3Sb* (if progeny carried *TM6B* chromosome) or *ve st ry/TM6B* (if progeny carried *TM3Sb* chromosome). If a cross gave any *ve* or *ve st* progeny, then this set was discarded, since this result indicates that the male parent carried the unmutagenized maternal *ve st ry* chromosome. If this cross gave *ve$^+$* progeny, then flies of the genotype *ve$^+$/TM6 or ve$^+$/TM3 Sb* were used to set up a stock which was then used for cytology. Note that if the original *ry* fly was female, *ve* could recombine onto the mutagenized chromosome (there is roughly 5% recombination in the cytological interval between *ve* and 63). At least ten lines from each original *ry$^-$* fly were set up when possible, and several *ve$^+$* lines were examined cytologically when the original fly was female.

Cytological analysis: Third instar larvae were dissected in TB1 (15 mM potassium phosphate, pH 7.0, 80 mM KCl, 16 mM NaCl, 5 mM MgCl$_2$, 1% PEG 6000 (Carbowax) and stained for several minutes in lactic-acetic orcein. Squashes were viewed by phase contrast microscopy using a Zeiss photomicroscope. For examination of heat shock puffs, larvae were heat shocked in 1.5-ml Eppendorf tubes in a 37° water bath for 25 min before dissection.

Isolation of duplications: In the *Pdp* duplication series, males of the genotype *In(3L)P*, *ry* were crossed to *gt w^a* females. In the next generation virgin females of the genotype *gt^1 w^a/+; In(3LP)/+* were irradiated with 3000 R using a cesium source. Females were crossed to males that carried the point *Minute (l(3)63bf^1 or l(3)63Bf^2* balanced over *TM3Sb* or *TM6B*. Each of 35 vials that were set up contained 20 females and 2 males, and these vials were transferred daily. Putative duplications were crossed to *TM3/TM6* or *Minute (l(3)63bf^1/TM3 Sb* or *l(3)63Bf^2/TM3Sb)* and lines balanced over *TM3Sb* or *TM6B* were established from these crosses.

DpT33^{R}f19^L females of the genotype *In(3LR)f19/In(3LR)T33* were crossed to *l(3)63Bf^2* males. Single *Sb$^+$*, *Minute$^+$* offspring were mated to *TM3Sb/TM6* flies and the resulting progeny were used to set up a stock which was checked cytologically.

Isolation of point mutations: Several different parental strains were used in these screens, including *ri e*, *ri p^p*, *In(3L)P* and *hsADH61C Ifm(3)7* (this chromosome was originally used for another screen, but was used in these screens because a large number of F$_1$ progeny were available). Males aged 3–4 days in the absence of females were mutagenized for 24 hr in vials with 0.0125 M ethyl methanesulfonate (EMS) or 0.005 M diepoxybutane (DEB) in a 1% sucrose solution soaked into Kimwipes. The males were then crossed to *TM3Sb/TM6B* or *Ly/TM3* females and the cross was transferred to new vials every day. The males were discarded after 3 days and the females were then transferred every other day for 4 more days. Single male progeny carrying the mutagenized chromosome over *Ly* or *TM3Sb* were crossed to females of the genotype *Dp(3L)BK116/Df(3L)HR218* and their progeny were examined for the

absence of the *Df(3L)HR218/Sb$^+$* or *Df(3L)HR218/Ly$^+$* progeny. Flies carrying the mutagenized chromosome over *Dp(3L)BK116* chromosome were then crossed to *TM3Sb/TM6B* and a balanced stock was isolated. In some screens, F$_1$ females were crossed to deficiency-bearing males, and the progeny were checked for the absence of the *Minute*, nonbalancer class.

Nomenclature of mutations: All of the mutations that map to the to the 63 region have been named according to the system of LINDSLEY and ZIMM (1985). These mutations are listed in Table 3. Several mutations were isolated that did not map to the 63 region but were lethal over deletions in the 63 region (see below). These mutations are named by their original isolation number.

Deletion mapping, complementation tests and lethal phase analysis: Chromosomes carrying mutations and deletions were placed in stocks over *TM6B*. Complementation tests or deficiency mapping crosses were done in vials by crossing at least two males of one genotype to two females of the second genotype and scoring for *Tb$^+$* adults. Lethal phase determination was also performed in this manner with the *TM6B* marker *Tb* being used to infer the genotype of larvae or pupae.

Labeling of proteins from single embryos: Bottles containing 50–100 flies were set to collect embryos. Two-hour collections were performed using Petri plates containing standard media that had been lightly covered with 5% acetic acid. Embryos were then aged 4 hr so that they would be 6–8 hr old during the labeling. Embryos were placed in glass depression slides with a few drops of water and the top of the depression slide was covered with a cover slip. The slide was then placed on a slide warmer set at 37° and embryos were heat shocked for 45–60 min. After heat shock, embryos were dechorionated in 50% (v/v) bleach and rinsed 10 times with TB1. Single embryos were put in microtiter wells containing 4 μl of TB1. Embryos were broken with a tungsten needle and labeled by addition of 1 μl of a solution containing 2–3 μCi [^{35}S]methionine (Amersham). Embryos were then incubated for 30–45 min, and 25 μl of sodium dodecyl sulfate (SDS) sample buffer (1% SDS, 10% glycerol, 10 mM Tris-HCl, pH 7.0, 1% β-mercaptoethanol) were added to terminate the labeling. Microtiter dishes were incubated for 10 min at 65° to solubilize proteins. The sample was then added to an Eppendorf tube, 70 μl of acetone was added, and proteins were precipitated by centrifugation for 30 min in an Eppendorf microfuge at 10,000 × *g*. Pellets were solubilized in 15 μl SDS sample buffer and run on a 12% SDS-polyacrylamide gel. Gels were dried and autoradiographed for 1–3 days.

Construction of transformation vector: The *hsp82* gene was originally cloned into an *Adh* containing vector pSXΔ1A (ASHBURNER 1989). A *Bam*HI-*Eco*RI fragment containing *hsp82* sequences 900 bp to 150 bp upstream of the start site of transcription from the plasmid 330.1 (BLACKMAN and MESELSON 1986) was ligated into pSXΔ1A that had been cut with *Bgl*II and *Eco*RI. The resulting vector (pbrx) was linearized with *Eco*RI and an 8-kb *Eco*RI-*Eco*RI fragment containing the region 150 bp 5′ to 3 kb 3′ of *hsp82* (from the plasmid 301.1, HOLMGREN *et al.* 1981) was ligated into it yielding pbx19. A 7.5-kb *Bam*HI-*Bgl*II fragment from pbx19 containing 900-bp 5′ and 2.5-kb 3′ *hsp82* sequence and about 50 bp of pSXΔ1A vector sequence was ligated into the *Bam*HI site of the CaSpeR *P* element vector (PIROTTA 1988). Appropriate restriction digests were used to determine the orientation of the *hsp82* gene within CaSPer. The final transformation construct pW82, contains the 5′ region of the *hsp82* gene next to the 3′ *P* element end of the CaSpeR vector.

TABLE 1

Rearrangements isolated in this study

Name	Cytology/new order	Comments
Df(3L)HR119	DF(3L) 63C6-63E	
In(3L)HR128	IN (3L) 63A-D	
Df(3L)HR218	Complex	*Minute*
Df(3L)HR232	DF(3L) 63C1-D1	
Df(3L)HR277	DF(3L) 63B6-64B12	
Df(3l)HR298	DF(3L) 63B1-64A6	*Minute*
Tp(3L)HR321	TP? 63D	Insertion of several bands of unknown origin into 63D.
Df(3L)HR370	DF(3L)63A1-D10	*Minute*
Df(3L)M21	DF(3L)62F-63D	*Minute* DEB induced on a *ri pᵖ* chromosome

P element transformation: *w* females were crossed to *w; ry P(Δ2-3ry+)* 99B (ROBERTSON *et al.* 1988) males in egg collection containers and offspring were collected at 1-hr intervals. Embryos were dechorionated manually on double stick tape using watchmakers forceps and were desiccated for 2–4 min in Petri dishes containing Drierite. Desiccated embryos were covered with halocarbon oil to prepare for injection.

Needles for injection were pulled from glass capillary tubes using a Kopf vertical needle puller. Needles were filled with a solution containing the pW82 plasmid at a concentration of 1 mg/ml and were used for injection aided by a Narishige injection apparatus.

Surviving embryos were placed in single vials. Of about 1000 embryos injected 41 survived to adulthood and 32 were fertile. Adults were crossed to *w/w; +/+* or *w/w; TM3/TM6B* flies, and offspring were checked for presence of nonwhite eye color. Three flies gave rise to transformed offspring. Two flies gave rise to four transformed offspring each while the third gave rise to approximately 100 transformed offspring representing at least two independent insertions. Single lines were set up by crossing transformed flies to *w/w; T(2, 3)apˣᵃ/SM5 ;TM3/+*. *Ap⁺* offspring were then crossed to *w/w; +/+; +/+* to determine linkage, and crossed *inter se* to establish stocks.

In situ hybridization: *In situ* hybridizations were done essentially as described by GALL and PARDUE (1971) under the following conditions and modifications. PW82, the transformation vector, was labeled with [³H]CTP by nick translation; 30,000–60,000 cpm were added per slide. The RNAse step was omitted. Slides were developed after a 1–2-week exposure time as described by PARDUE (1986).

RESULTS

Isolation of deletions: HOFFMANN and CORCES (1984) constructed a transgenic strain, 28AC, carrying a *ry⁺* marked *P* element insert at 63D. This line was used to isolate deletions and other rearrangements in the 63 region. 28AC males were irradiated with 3500–4000 R and crossed to *ve st ry* females; 140,000 progeny were screened for *ry⁻* eye color and 21 fertile *ry⁻* progeny were recovered. Of these 21 stocks, 9 had cytologically detectable rearrangements. Of the 12 cytologically normal lines 10 were homozygous viable and 2 were homozygous lethal but were viable over all lethal rearrangements.
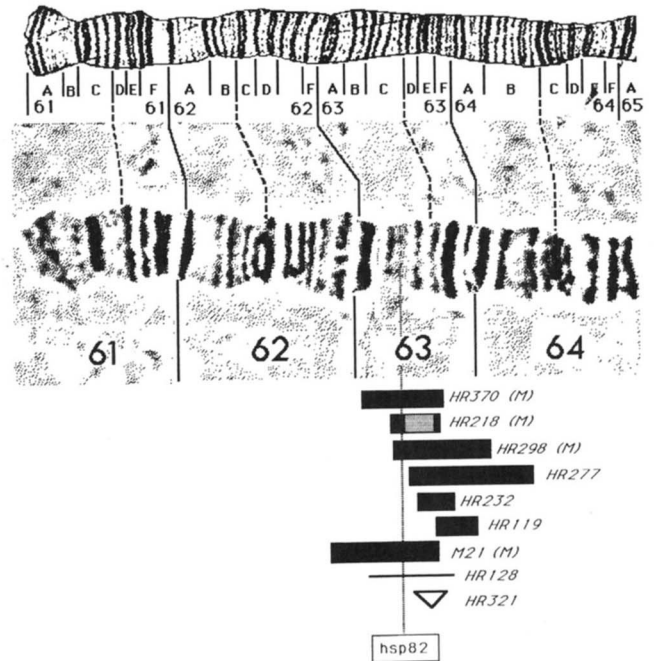

FIGURE 1.—Chromosomal rearrangements in the 63 region. Solid bars indicate deletions. The line indicates an inversion The triangle indicates an apparent insertion event. Shaded area in *Df(3L)HR218* represents uncertainty in cytology (see text). M indicates a deletion uncovering the *Minute* locus.

These latter 12 lines were not characterized further.

Cytological and genetic analysis of the rearrangements: The salivary gland chromosomes from the *ry⁻* strains were examined for rearrangements. Six lines showed deletions, two showed inversions (one of which was lost) and one was an apparent insertion. These results are summarized in Table 1 and diagrammed in Figure 1. These strains were then checked for the presence or absence of the heat shock puff at 63B which is due to the *hsp82* gene. The results of these studies are shown in Figure 2. Three of the deletions *Df(3L)HR370, Df(3L)HR298 and Df(3L)HR218* removed the heat shock puff, while the other rearrangements had no effect on the puff. In addition to the deletions isolated in this screen, another deletion, *Df(3L)M21*, which was isolated in a screen described below, also removed the heat shock puff (data not shown).

The four deletions which removed the heat shock puff were also *Minute*. LINDSLEY *et al.* (1972) identified a *Minute* locus in this region by use of segmental aneuploids and localized it to 63A-63D. Our results place the *Minute* between 63B6 (the left breakpoint of the *Minute* deficiency *Df(3L) HR298* and 63C1 (the left breakpoint of the *Df(3L)HR232* deletion which is non-*Minute*. The *Minute* deletions showed very weak female fertility and it was not possible to maintain stocks containing *Minute* deletions over third chromosome balancers. Therefore, they were maintained over duplications which cover the region.
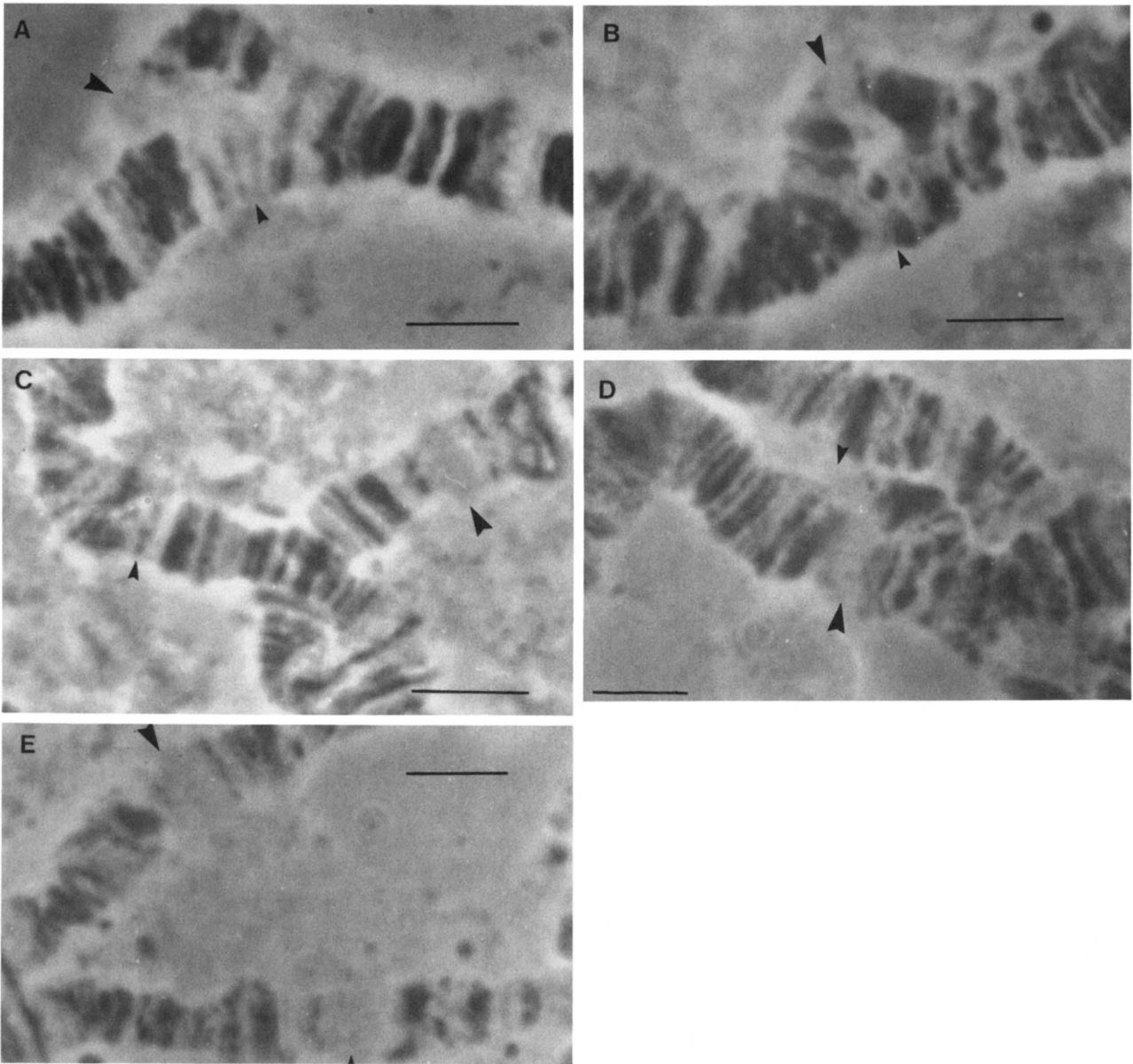
FIGURE 2.—Squashes of salivary glands from heat shocked deletion heterozygotes. Third instar larvae were heat shocked for 25 min at 37° and salivary glands squashes were prepared as described in MATERIALS AND METHODS. In each squash the large arrowhead indicates the wild-type chromosome and the small arrowhead indicates the deficiency chromosome. A, *Df(3L)HR298/+*; B, *Df(3L)HR370/+*; C, *Df(3L)HR218/+*; D, *Df(3L)HR232/+*; E, *Df(3L)HR277/+*.

The rearrangements were crossed *inter se* to determine whether they overlapped genetically as they appeared to cytologically. Except for *Df(3L)HR218* all of the deletions were lethal over the other deletions. *Df(3L)HR218* was viable over the deletions *Df(3L)HR277*, *Df(3L)HR119* and *Df(3L)HR232* (but not *Df(3L)HR298* or *Df(3L)HR370*). Our original cytology suggested that *Df(3L)HR218* might be a deficiency from 63B6-D1. If this cytology were correct, then flies of the genotype *Df(3L)HR232/Df(3L)HR218* would be completely deficient for all loci in the region from 63C1 to 63D. It is possible that there are no vital loci in this region, although that seems surprising. It is more likely that *Df(3L)HR218* is a complex re-

arrangement which removes the *ry* element at 63D and some material in the 63AB region but retains some material in the region between 63C and D. The bands in this region are very faint so such a complex aberration could not easily be detected cytologically.

The inversion *In(3L)HR128* is homozygous lethal and is lethal over *Df(3L)HR370* and *Df(3L)M21* but not over any of the other deletions. This suggests that the distal breakpoint at 63A is in an essential gene while the proximal one is a simple break in the *P* element and does not effect any nearby vital loci.

Isolation of duplications: We sought to isolate duplications in the 63C region for two reasons. First, the duplication which we originally used to cover the

Minute, Dp(3L)BK116 (duplicated for 63A-64C), was a tandem duplication and occasionally broke down by homologous recombination. Therefore it was useful to obtain other duplications in order to maintain deletion bearing stocks. Secondly, we hoped to use these duplications to construct a finer map of the *Minute* and other mutations in the region

In order to generate duplications, we used a variation on the *Minute* duplication screen devised by GRELL (1969) and BRODERICK and ROBERTS (1982) in which radiation induced duplications are scored by their ability to cover the *Minute* phenotype. In our screen, we irradiated female flies that were heterozygous for a wild type chromosome and an inversion, *In(3L)P*, which has its distal breakpoint at 63B8-9. (CRAYMER (1984) reported that the distal breakpoint was between 63B8 and 63B11. The *In(3L)P* breakpoint is distal to the heat shock puff which has been localized to 63B9 (SEMESHIN *et al.* 1985). Therefore, the distal breakpoint must map within 63B8 and 63B9). This breakpoint is between the breakpoints of *Df(3L)HR298* (63B6) and *Df(3L)HR232* (63C1). We predicted that it would be possible to generate duplications containing only part of the 63 region by (1) breakage and rejoining of sister chromatids of the *In(3L)P* chromosome (see Figure 3A) or (2) breakage and rejoining in both the *In(3L)P* chromosome and the wild-type chromosome (see Figure 3B). If such duplications contained material from the proximal end of the duplication then the *Minute* would map proximal to 63B8-9 (*In(3L)P* breakpoint), whereas if the duplicated material originated from the distal region of the inversion the duplication would map distal to 63B8-9.

In this screen 700 females were irradiated and crossed to *Minute* bearing males and approximately 32,000 progeny were scored for coverage of the *Minute* phenotype. Eight duplications which covered the *Minute* phenotype were isolated in this screen. The duplication bearing lines were examined cytologically and the results summarized in Table 2. All duplications which contained material from the *In(3L)P* chromosome contained duplications of the proximal region. This was true for three tandem duplications induced on *In(3L)P* and two duplications which were of the type diagrammed in Figure 3B. Thus the *Minute* locus must map proximal to 63B8. One tandem duplication was derived solely from wild type chromosomes (*Dp(3L)pdp19*) and one insertional duplication originated from wild type (*Dp(3L)pdp23*).

One of the duplications, *Dp(3L)pdp7*, had another useful feature: deletions could be recombined onto this duplication giving deficiencies and partial duplications. For example, recombination of the deletion *Df(3L)HR370* onto *Dp(3L)pdp7* gives a chromosome deleted from 63A-63D but duplicated from 63B8-9
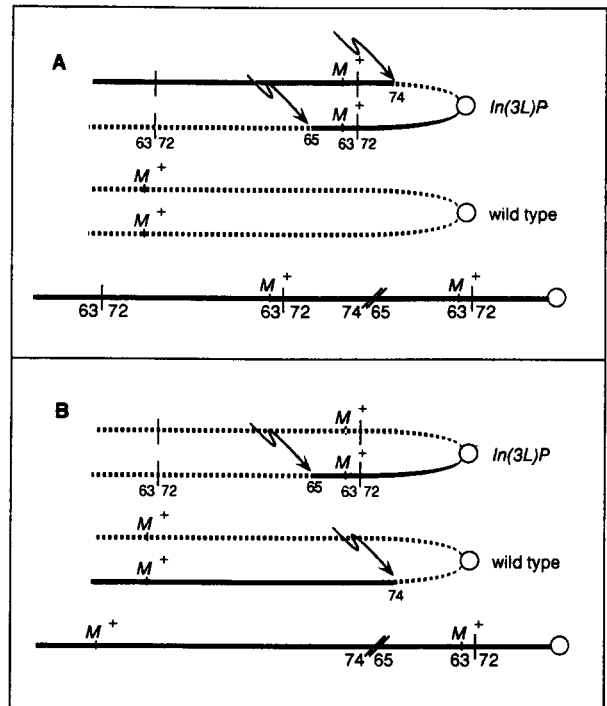

FIGURE 3.—Duplications from *In(3L)P*. In **A**, breaks occur in both sister chromatids of *In(3L)P* (1) resulting in a tandem duplication on the inversion chromosome (2). In **B**, one break occurs on a wild-type chromosome and one breaks occurs on *In(3L)P* (1) resulting in an insertional duplication (2). The *Minute* locus (*M*) is shown close to the proximal breakpoint of *In(3L)P*. Note that duplications of the *Minute* will duplicate the *In(3L)P* breakpoint closest to the *Minute* locus itself. It is also possible to generate breaks in sister chromatids of the wild type chromosome yielding tandem or insertional duplications on the wild type chromosome.

to 65, and thus is effectively deleted from 63A to 63B8-9. This chromosome, designated *Df(3L)HR370* +*Dp(3L)Pdp7*, is useful in mapping point mutations distal or proximal to the *In(3L)P* breakpoint.

One other duplication was generated in another way using the method of MULLER (1930). Females heterozygous for the inversions *In(3LR)T33* (64C-97/98; J. J. BONNER, unpublished) and *In(3LR)fl9* (62A-98A, CRAYMER 1984) were crossed to *Minute* males. A recombination event between the two inversions can result in the chromosome whose order is now 61–64C|97–62|98–100 and thus duplicated for the *Minute* locus. This duplication can not break down by recombination and thus is useful for maintaining balanced deficiency/duplication stocks.

Embryo labeling experiments: Our cytological data showed that *Df(3L)HR218, Df(3L)HR298* and *Df(3L)HR370* removed the heat shock puff. However, the breakpoint of *Df(3L)HR218* is sufficiently close to the puff that it is formally possible that the effects on the heat shock puff are a position effect and that this deletion does not actually remove the gene. In order to determine whether *Df(3L)HR218* was deleted for the *hsp82* gene, we examined whether embryos which were homozygous for the *Df(3L)HR218* deficiency

TABLE 2

Duplications of the 63 region

Name	New order	Type
pdp2	61-63B8||72-63B|65-63B8||72-100	A
pdp5	61-63B8||72-63B8||72E|63C-63B8||72-100	A
pdp7	61-72|65B-63B8||72-100	B
pdp11	61-74A|65C-63B8||72-100	B
pdp14	61-63B||72-63B8||72-74A|63E-63B||72-00	A
pdp19	61-63D|62D-100	Tandem on wild type
pdp23	61-63B8||72-63B8||72-100F|61A-63E	Transposition of 61-63E onto tip of 3R
t33^{L}F19^R	61-64C|97-62|98-100	Synthetic duplication from recombination between inversions
BK116	61A-64C|63A-100	Tandem on wild type

Type A duplications are sister chromatid exchanges on *In(3L)P* (see Figure 3A). Type B duplications are exchanges between wild type and *In(3L)P* chromosomes (see Figure 3B). Double lines indicate In(3L)P breakpoints, single lines indicate irradiation induced breakpoints.
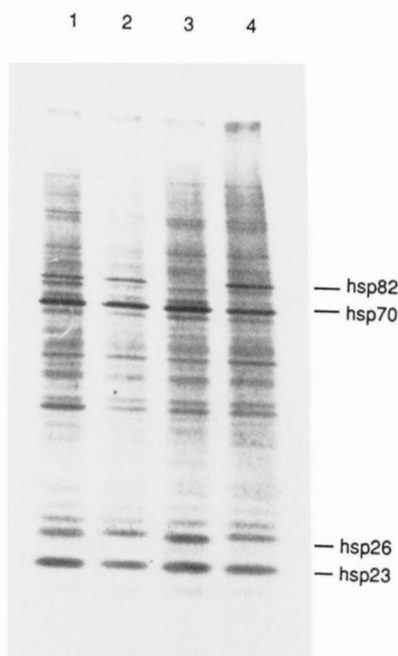

FIGURE 4.—Protein synthesis in single heat-shocked embryos from *Df(3L)HR218/Dp(3L)BK116* parents. Embryos were heat shocked and labeled as described in MATERIALS AND METHODS. Proteins from individual embryos were resolved on a 12% SDS-polyacrylamide gel electrophoresis gel and exposed to film for autoradiograpy. Lane 3 shows an embryo which fails to synthesize hsp82 but shows an otherwise normal pattern of heat-shock proteins. Thus this embryo is likely to be homozygous for *Df(3L)HR218*. The embryos shown in lanes 1, 2 and 4 have normal heat-shock profiles and thus are *Df(3L)HR218/Dp(3L)BK116* heterozygotes or homozygous for *Dp(3L)BK116*.

could synthesize the hsp82 protein. In this experiment, embryos were collected from adult flies of the genotype *Df(3L)HR218/Dp(3L)BK116*. We had no way of determining the genotype of homozygous embryos, so we labeled single embryos and predicted that 25% of these embryos should fail to synthesize hsp82.

Single embryos were labeled after heat shock as described in MATERIALS AND METHODS. Figure 4 shows examples of such experiments. As predicted, we could find embryos which failed to synthesize hsp82. From a total of 35 embryos analyzed in this manner 7 failed

to synthesize hsp82, while showing normal synthesis of other heat-shock proteins. This is a frequency of 20%, close to the predicted frequency of 25%. These embryos were not simply deficient in their ability to heat shock or synthesize proteins as the rest of their protein profile is indistinguishable from their wild-type siblings except for the lack of hsp82 synthesis. Less than 5% of embryos from genotypes not carrying hsp82 deletions failed to synthesize hsp82, and most of these embryos showed very poor synthesis of all heat-shock proteins. Similar results were obtained with *Df(3L)HR298*, *Df(3L)HR370*, and *Df(3L)M21* (not shown). These results also indicate that there is no other *hsp82* gene (*i.e.*, which makes a large heat shock protein) and that there is no translatable maternal *hsp82* message at this stage of development. This latter result is consistent with the findings of ZIMMERMAN, PETRI and MESELSON (1983) who noticed that while unfertilized eggs had large quantities of *hsp82* message, there was little or no *hsp82* message at cellular blastoderm.

Isolation of mutations in the 63 region: In order to isolate lethal and visible mutations in the 63 region, the F$_2$ screens shown in Figure 5 were used. We used EMS since it has been shown to be an efficient mutagen and DEB, since this mutagen has been shown to give small deletions (OLSEN and GREEN 1982) that could easily be detectable as alterations on Southern blots or on polyacrylamide gels. In the first screen, deletions *Df(3L)HR218*, *Df(3L)HR370* and *Df(3L)HR298* were used but in subsequent screens only *Df(3L)HR218* was used since it is the smallest deletion which removes the *hsp82* gene. Screens were done at 29° to isolate possible temperature sensitive mutations and in one screen a heat shock (35°, 2 hr) was applied after 4–5 days of development to isolate possible heat shock sensitive mutations (though none of this type were found).

Since females carrying *Minute* deletions, such as *Df(3L)HR218*, were virtually sterile, F$_1$ males could not be crossed to females of the genotype deletion/
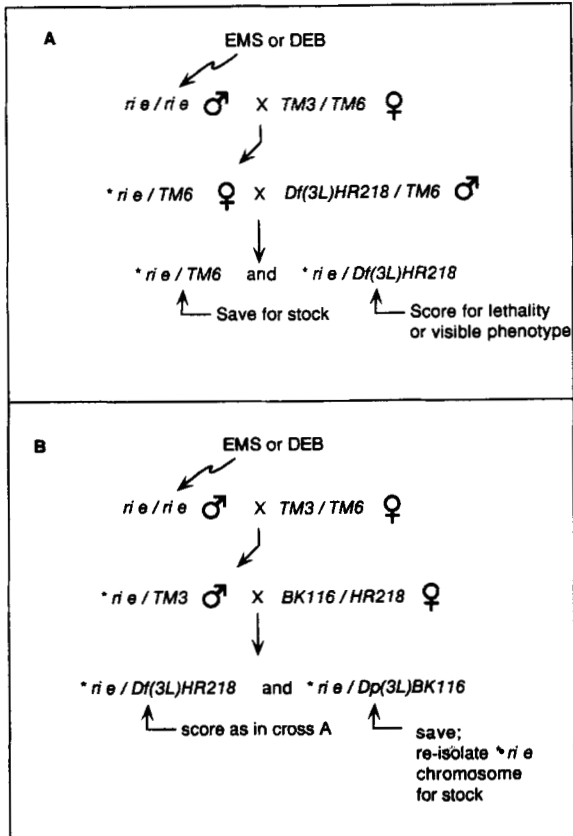
FIGURE 5.—F₂ screens for point mutations in the 63 region. In
A, females bearing mutagenized chromosomes are mated to dele-
tion bearing males. The asterisk indicates a mutagenized *ri e* chro-
mosome. In **B**, males carrying mutagenized *ri e* chromosomes were
crossed to females carrying *Df(3L)HR218* and a duplication which
covers the *Minute*.

Balancer. Instead, F_1 females were crossed to defi-
ciency bearing males or F_1 males were crossed to
females carrying the deletion over *Dp(3L)BK116*. The
former method was found to be inefficient since single
F_1 females often gave too few progeny to score loss of
the *Minute* class reliably. *Minute* individuals were typ-
ically weak, and often appeared in less than Mendelian
proportions; large numbers of progeny were there-
fore required to identify lethal mutations reliably. A
further reason not to use the F_1 females is that F_1
females bearing new alleles of the haplo-insufficient
female sterile locus (which turned out to be the *Min-
ute*) would provide few progeny indeed.

We performed one small scale F_2 screen to generate
possible multilocus deletions by looking for new DEB
induced alleles of one of the complementation groups
(*63Bb*). This screen gave fewer problems than the F_2
screens discussed above since no *Minute* deletions were
used. We were able to obtain one new allele of this
complementation group and one new large deletion
(*Df(3L)M21*), but we failed to obtain any small multil-
ocus deletions.

In all, 9017 EMS mutagenized chromosomes and
6361 DEB treated chromosomes were screened for

mutations. Forty lethal mutations were recovered in
these screens. In addition to mutations isolated by
these F_2 screens, several mutations isolated in other
laboratories were were tested as well. These mutations
were chosen because their recombination map posi-
tion or *in situ* hybridization location suggested that
they might fall in the 63 region. The mutations and
their locations are *l(3)SG3* (4.3), *l(3)SG4* (10.7), *l(3)SG5*
(12.1), *l(3)SG6* (12.4) (all mutations from SHEARN *et
al* 1971). *l(3) neo9* (63C) and *l(3)neo10* (63C) (COOLEY,
KELLY and SPRADLING 1988) and *dib* (12) (JÜRGENS *et
al.* 1984). Of the above mutations, only *l(3)SG3* and
dib were lethal over any of the deletions tested.

We were unable to find loci which showed heat
shock dependent lethality or which showed multiple
temperature sensitive alleles as one might predict for
a mutation in a heat-shock protein. As discussed below
one locus did show some small differences in lethal
periods at elevated temperatures.

Mapping and complementation of mutations: The
mutations generated fell into 14 complementation
groups which were mapped by deletion and comple-
mentation analysis. The results of this analysis is
shown in Table 3 and Figure 6. It was important to
ensure that the mutations actually mapped to the
region rather than being second site mutations which
interacted with the deficiency. Second site interaction
is fairly common when a *Minute* mutation is used
(LINDSLEY and GRELL 1968; LEICHT and BONNER
1988). To determine whether or not a mutation
mapped in the 63 region the scheme shown in Figure
7 was used. Several mutations were isolated which fell
outside the region; these will be discussed in more
detail below. Of the mutations which fell in the region,
all of the DEB-induced mutations fell into a single
complementation group, whereas the EMS-induced
mutations showed a more random distribution. All of
the mutations which mapped in the region acted as
point mutants; there were no multilocus deletions or
mutations which gave complex complementation pat-
terns.

Correlation of genetic and cytological data: In
several cases the initial cytology was some what impre-
cise due to the faint banding pattern of the 63 region.
Therefore, it was useful to determine whether the
genetic data were consistent with the cytology and if
the genetic data could resolve any uncertainties in the
cytology. There were several points which we hoped
to address. First, we did not know the positions of the
distal breakpoints of *Df(3L)HR298* and *Df(3L)HR218*.
Two complementation groups were isolated which
were lethal over *Df(3L)HR370* and *Df(3L)HR218*
but not *Df(3L)HR298*. (see Figure 8). This shows
that *Df(3L)HR218* removes material distal to the
Df(3L)HR298 breakpoint. These two complementa-
tion groups were lethal over the synthetic deletion

TABLE 3

Genes of the 63 region

Gene	Allele	Chromosome	Mutagen	Synonym	Lethal phase
l(3)63Aa	1-2	*ri e*	EMS		Early larval
l(3)63Ab	1-4	*ri e*	EMS		Early larval
l(3)63Ba	1	*ri e*	EMS		Early larval
l(3)63Bb	1-2, 7-8	*ri e*	DEB		Late larval/early pupal
	3-5	*ri e*	EMS		Late larval/early pupal
	6	*ri pᵖ*	DEB		Late larval/early pupal
l(3)63Bc	1-4	*ri e*	EMS		See Figure 8
	5	*In(3L)P*	EMS		See Figure 8
l(3)63Bd	1-3	*ri e*	EMS		See Figure 9
	4	*In(3L)P*	EMS		See Figure 9
l(3)63Be	1-3	*ri e*	EMS		Late larval
l(3)63Bf				*M(3)LS3*	
	1-2	*ri e*	EMS		Embryonic/early larval
l(3)63Bg				*l(3)SG3*	
	1	*mwh red e*	EMS	*m47*	Pupal
	2	*ri e*	EMS		Pupal
	3-4	*In(3L)P*	EMS		Pupal
l(3)63Bh	1	*ri e*	EMS		Pharate adult
l(3)63Bi	1	*ri e*	EMS		Late larval/early pupal
l(3)63Da	1	*hsADH61C lfm(3)7*	EMS		Late larval
l(3)63Ea	1	*hsADH61C lfm(3)7*	EMS		Pupal
l(3)63Eb	1	*hsADH61C lfm(3)7*	EMS		Pupal
dib	1	*st e*	EMS	*10F27ᵃ*	Embryonic

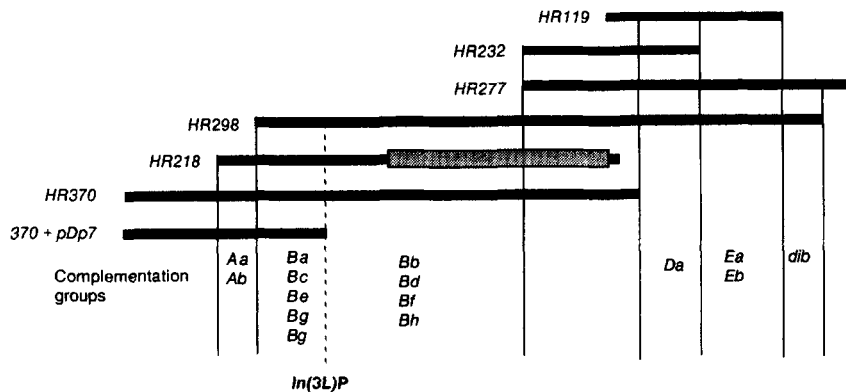ᵃ Three alleles of this mutation exist, but only one has been tested.

FIGURE 6.—Deletion map of mutations in the 63 interval. Dark bars show extent of deletions. Gray area in *HR218* represents uncertainty in cytology (see text).

Df(3L)HR370 + *Dp(3L)Pdp7* placing them to the left of the *In(3L)P* breakpoint (63B8–9). *DF(3L)HR298* removes several loci that are removed by *Df(3L) HR370* + *Dp(3L)Pdp7*. Therefore the distal breakpoint of *Df(3L)HR298* falls between the distal breakpoint of *Df(3L)HR218* and the *In(3L)P* breakpoint and the original designation of 63B6 for the breakpoint of *Df(3L)HR298* is probably close to being correct.

The second point we hoped to resolve was the uncertainty in the cytology of *Df(3L)HR218*. Our genetic results show that *Df(3L)HR218* removes genes which map to the left and to the right of the *IN(3L)P* breakpoint (see Figure 8). However, it does not remove any lethals proximal to 63C1 since *Df(3L)HR218* is viable over *Df(3L)HR232* and *Df(3L)HR277*. Therefore, *Df(3L)HR218* is a complex rearrangement, possibly an inversion involving 63D and 63A-B and a deletion of material in the 63B region.

The final point we hoped to address was the relative positions of the distal breakpoints of *Df(3L)HR232* and *Df(3L)HR277*. We were unable to find any mutations which fell between the breakpoints of these deletions so we can not resolve this issue.

Characterization of complementation groups in the 63 region: Analysis of lethal periods has been performed for complementation groups in the 63B region. These results are summarized in Table 3, and Figures 8 and 9. In Table 3 the lethal phases represent the lethal phases for hemizygous and *trans*-heterozygous combinations of alleles. In many cases accessory lethals have not been removed, so the lethal phase of homozygotes is not considered in these experiments. For all but two loci, the lethal phases were identical for all allelic combinations and for hemizygotes. The lethal phase analysis for the two exceptions is shown in Figures 7 and 8. In these two figures, lethal phases
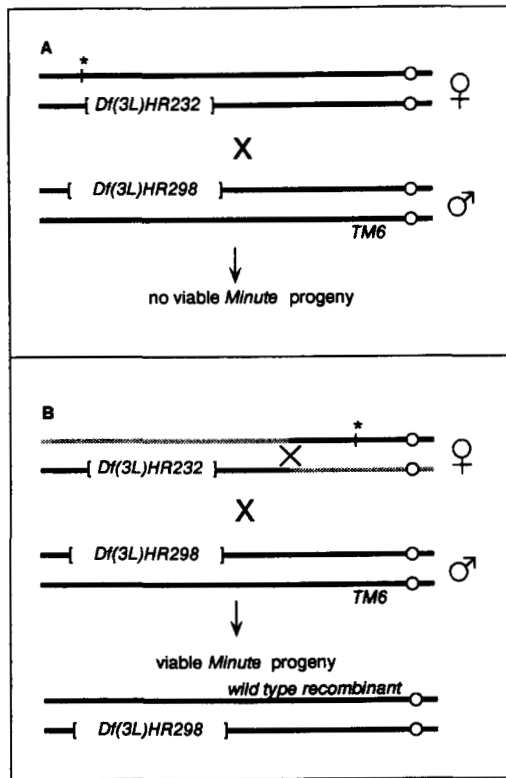
FIGURE 7.—Mapping scheme to determine whether lethal mutations mapped to 63 region. In **A**, the mutation (indicated with an asterisk (*) maps to the 63 region. A female heterozygous for *Df(3L)HR232* and the mutation will only very rarely generate recombinants that carrying neither the mutation nor the *Df(3L)HR232* chromosome. Thus this female will generate few gametes that contain chromosomes that are viable over *Df(3L)HR298*. In **B** the mutation lies outside the 63 region. Recombination between the mutation and *Df(3L)HR232* can yield a chromosome which is wild type and thus will survive over *Df(3L)HR298*.

FIGURE 8.—complementation matrix for alleles of *l(3)63BC*. PA indicates that individuals die as pharate adults. S indicates that flies are sterile in both sexes. Allele number designations are from Table 3.

for all combinations of alleles except for homozygotes is presented. More detailed descriptions of the lethal phases and other genetic characteristics for several loci are discussed below.

63Bb: This group represents the largest complementation group identified with eight members. All of the DEB-induced mutations (including one that was isolated in the F₂ screen to identify new alleles of this locus) fall into this complementation group. Thus, this

FIGURE 9.—complementation matrix for alleles of *l(3)63BD*. EP indicates early pupal lethality. L3 indicates lethality in L3. Allele number designations are from Table 3.

locus may represent a hot spot for DEB-induced mutations. In addition, three EMS induced alleles have been isolated. As indicated in Table 3, individuals carrying mutations at this locus tend to die as early pupae, but many die at larval stages. Mutant animals have imaginal discs, but these discs show no signs of metamorphosis after pupa formation. The 63Bb product may be required for metamorphosis, but it seems unlikely that its sole function is in metamorphosis since many individuals fail to pupariate.

63Bc: This group represents the second largest complementation group with five members. All members of this complementation group can survive to pharate adulthood both as hemizygotes or *trans*-heterozygotes. Dissected pharate adults show no obvious morphological defects. Flies that are heterozygous for allele *63Bc⁵* and any other allele of this locus will eclose, but the surviving adults are usually sterile and show a spread wing phenotype. Sterile males make sperm, but the sperm is not motile, while sterile females lay few eggs which tend to be small and are not fertilized. The lethal phase analysis for alleles of this locus is presented in Figure 8.

63Bd: This group includes four members, all EMS induced. The lethal phenotype is similar to *63Bb*. This complementation group shows some temperature sensitivity in phenotype in that genotypes that will pupate at 22° fail to do so at 29°. This phenotype is interesting with respect to the possibility that *63Bd* might represent the *hsp82* locus; however, evidence presented below suggests that this group is not the hsp82 complementation group. The lethal phase analysis for alleles of this locus is shown in Figure 9.

63Bf: This complementation group represents the *Minute* locus previously named *M(3)LS3* by LINDSLEY *et al.* (1972). Two EMS-induced alleles of this locus were isolated. Both alleles show *Minute* phenotypes that are virtually indistinguishable from the *Minute* phenotype exhibited by the *Minute* deletions: flies carrying these *Minutes* grow slowly, show very low female fertility and are lethal over several mutations which interact with the *Minute* deletions (discussed below).

63Bg: This complementation group is equivalent to the locus *l(3)SG3* (SHEARN *et al.* 1971). These authors

isolated one allele of this locus, *M47*; we isolated three additional alleles. The phenotype of *M47* as described by SHEARN *et al.* (1971) was abnormal differentiation of eye-antennal and leg discs but normal differentiation of wing and haltere discs. Upon examination of this allele and other alleles of this locus we found that the lethal pupae showed some eye development, but the eyes tended to be extremely small while the legs often were relatively normal looking except for not being completely extended. Often (even in the case of the *m47* allele) the wings were not fully developed; they tended to be much smaller than usual. When the mutations were hemizygous with deletions, many mutant animals showed no evidence of metamorphosis at all. Thus, these mutations are likely to be hypomorphic, although we can not rule out the possibility that the difference in phenotype between the hemizygous and homo- or heterozygous mutations is an effect of genetic background.

dib: This mutation was identified by JÜRGENS *et al.* (1984) as an embryonic lethal mutation which fails to show any cuticle differentiation. We localized this mutation to between 63E9 (the proximal breakpoint of *Df(3L)HR119*) and 64A8 (the proximal breakpoint of *Df(3L)HR298*). Hemizygous *dib* animals were embryonic lethal, but we did not examine the phenotype more closely.

Second site interactions: Several mutations were isolated which were lethal over the *Minute* deletions but could be separated by recombination from the 63 region. A few of these mutations were extremely weak stocks that were eventually lost. Several were reasonably robust and four of these were examined in more detail as discussed below. We do not have accurate map positions for these mutations but our mapping tests suggest that they are all at least 40 cM from the 63 region. All of these mutations were EMS induced except for D224A6 which was DEB induced.

1156: This mutation is a larval lethal as a homozygote, but in combination with the deficiencies will survive to pupation and will occasionally show pharate adults. *1156* mutation shows similar phenotypes in combination with deficiencies and with both alleles of the *63Bf* locus. This mutation is not lethal over several other *Minutes* tested, including *M(3)i*, *M(3)65F*, *M(3)S36* *M(3)76*, *M(3)95A*, and *M(2)S7*. *M(3)63B* is somewhat more severe than the other *Minutes* tested; this fact may explain the apparent specificity of interaction with *1156*. Alternatively, this genetic interaction may result from a particular interaction of the gene products.

E10-808: This mutation is embryonic or early larval lethal as a homozygote but shows pupal lethality over either deletions or point *Minutes* of *M(3)LS3*. This mutation also shows a similar lethal period in combination with *M(3)i*.

D224A6: This mutation is similar to E10-808 in that it is an early larval lethal as a homozygote, but is pupal lethal over deletions or point *Minutes*.

E19-214: This mutation is embryonic lethal but in combination with either *Minute* deletions or point *Minutes* shows a *Serrate*-like wing phenotype. This phenotype is cold sensitive showing virtually complete penetrance at 22° but is highly suppressed at 29°. Allelism of this mutation was tested for several mutations with similar phenotypes including *cp*, *Ser* and *Bd*. *E19-214* is not allelic to any of these loci; although it interacts strongly with *Bd* (*Bd/E19-214* flies have severely reduced wings) and enhances the phenotype of *Ser*, the *E19-214* locus does not map near *Ser* or *Bd*. *E19-214* also shows the Serrate wing phenotype in combination with *M(3)i* although the penetrance is not as high in this combination as with *M(3)LS3*.

Attempts to identify *hsp82* mutations: Four loci, *63Bh*, *63Bd*, *63Bf* and *63Bh*, were candidates for *hsp82* mutations by their cytological locations (63B8-C1). In order to determine whether any of these loci represented the *hsp82* complementation group, *P* element rescue was attempted. A vector containing an *hsp82* gene with 900 bp 5′ and several kb 3′ was constructed. The 5′ region of this vector has been shown to give both constitutive and heat-shock inducible expression when fused to the structural gene for bacterial β-galactosidase (XIAO and LIS 1989).

This *P* element vector was injected into Drosophila embryos and four independent lines were isolated. The inserts were mapped by *in situ* hybridization to 39BC, 58CD, 94CD and 96B (data not shown). All of the inserts are homozygous viable and fertile except for the insert at 94CD which is homozygous lethal. Both of the third chromosome insertions were crossed onto *Df(3L)HR370* and *Df(3L)HR218*.

Expression of the transgene was tested in an embryo labeling experiment. This experiment was performed as described for the previous embryo labeling experiment except that the deletion bearing chromosome, *Df(3L)HR218*, also contained the insert at 94CD. Thus, all the embryos should be able to synthesize hsp82. Consistent with this prediction, 16/16 embryos labeled hsp82 (data not shown).

The transgenic flies were then used to test rescue in the crosses outlined in Figure 10A. Note that in this cross, it is not only possible to determine whether or not the lethality of a complementation group is rescued but also whether or not the lethal phase is altered. Thus, for example, it could be determined whether the genotype *D32-187/Df(3L)HR218* which normally dies as an early pupa, shows any sign of metamorphosis. A second set of crosses was used to try to rescue *trans*-heterozygous combinations of alleles. In this case one or both copies of the second
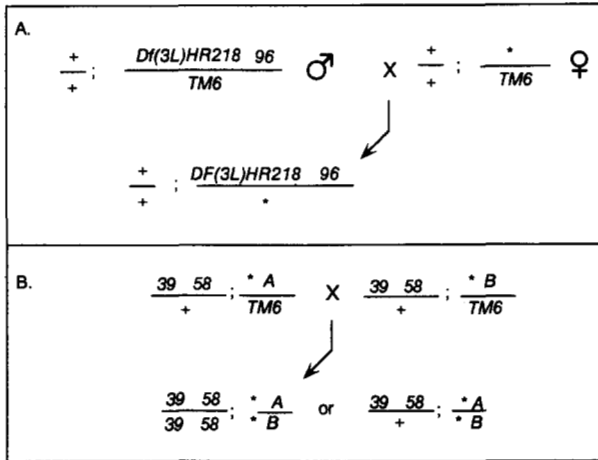
FIGURE 10.—Rescue crosses. In **A**, females carrying mutation to be tested (designated by an asterisk) were crossed to males carrying deletion chromosomes onto which the hsp82 bearing *P* element inserted at 96B had been crossed. Offspring were scored for adult non-*TM6* progeny indicating rescue. Non-*TM6B* larvae and pupae were scored for improvement in phenotype indicating partial rescue. These crosses were also performed with flies carrying second chromosomal inserts in addition to the insert at 96B). In **B**, *A and *B indicate different alleles of the same locus. In this cross a chromosome containing *P* elements at 39BC and 58CD was used. Offspring from cross B were checked for survival or improvement of phenotype of non-TM6 progeny as in A.

chromosome *P* element was used. These crosses are diagrammed in Figure 10B.

Results from attempts to rescue: Neither complementation group *63Bb* nor *63Bd* showed any obvious rescue by the *P* elements. Complementation group *63Bh* initially showed what looked like a few rescued progeny, however, later tests showed that this rescue was not dependent on having a *P* element but was due to some other factor in the genetic background. This mutation (one allele) dies as a fairly normal looking pharate adult, thus perhaps it is not surprising that a slight change in genetic background should allow for some low level of survival. Increasing the dose of *hsp82* did not increase the rate of survival further suggesting that the survival was not due to the transgene.

Several tests were done to determine whether or not the *Minute* locus represented the *hsp82* complementation group. First, the *Minute* bristle phenotype of flies carrying one, two or three extra copies of hsp82 was examined and found to be no less severe than the original *Minute* deletion or mutation Secondly, the female fertility was not rescued by the transgene. Finally the transgene was used to determine whether or not flies which were *trans*-heterozygotes for second site lethal mutations and the *Minute* could be rescued. None of the lethal interactions with second site mutations was affected by the transgene.

In addition to the transgenes that we constructed, we also attempted rescue experiments with flies containing the *D. pseudocbscura hsp82* gene. This gene is

about 99% identical at the amino acid level (BLACKMAN and MESELSON 1986) and is expressed in *D. melanogaster* (C. LAIRD and E. JAFFE, personal communication). We were unable to detect any rescue with this *P* element strain either. The results from these two sets of experiments suggest that we have not isolated mutations in the *hsp82* gene.

DISCUSSION

We have initiated a genetic and cytological study of the 63 region, a previously unstudied interval of the third chromosome. We have isolated overlapping deletions which cover the region from 62F to 64B; however only a subregion (63B) was studied extensively. Within this smaller section, which encompasses 13 bands, 11 lethal complementation groups have been identified.

Our screens also enabled us to map three previously identified mutations: (1) *dib*, an embryonic lethal mutation (JÜRGENS *et al.* 1984) which had been initially mapped to 62D–64C was placed between 63F and 64A. (2) *l(3)SG3*, a pupal lethal mutation which had been mapped by recombination to position 4.3, was mapped between 63B6 and 63B8. (3) *M(3)LS3*, which had been mapped to 63A–63D using segmental aneuploid stocks, was mapped to 63B8–C1.

Mapping of the *Minute* mutation was facilitated by duplications isolated in a variation on the *Minute* screen. This screen generated chromosomes which were duplicated for one end of an inversion. One such duplication was also useful in mapping other mutations in this region. We believe that screens of this type should be of general utility in isolating small duplications with a defined endpoint.

One primary reason for undertaking this cytogenetic analysis was to isolate mutations in the *hsp82* gene. Currently, we have no evidence that any of our complementation groups represent the *hsp82* gene. There are several factors that may explain our inability to isolate or identify such mutations in this screen.

First, we may not have completely saturated the region for mutations. Calculations based on the Poisson distribution suggest that there may be one or two loci which were undetected in these screens These calculations may underestimate the number of loci if there are some genes which are less mutable than average (see LEFEVRE and WATKINS 1986).

Second, our screening procedure may not be efficient in isolating *hsp82* mutations. The F_2 screens were only useful in isolating "tight" mutations; many F_1 males gave only a few *Minute* offspring since flies carrying *Minute* deletions grow slowly and tend to be somewhat weak. Thus, leaky mutations may have been thrown out. If EMS (which causes mainly single base pair changes) generates predominantly semilethal mutations in hsp82, we could have missed such mutations.

DEB, which has been shown to induce small deletions (OLSEN and GREEN 1982), should have been able to produce complete knockouts of *hsp82*. However, for some reason DEB showed extreme gene specificity and was not able to mutate many genes in this region. Irradiation or P element mutagenesis may have been more efficient in isolating *hsp82* mutations although these mutagens generally give a lower mutation rate than EMS or DEB.

Third, it is possible that *hsp82* may not be absolutely essential either for normal growth or during heat shock. We predicted that *hsp82* would be an essential gene since it is highly conserved and is essential in at least one other organism (yeast; BORKOVITCH *et al.* 1989). It is possible that in Drosophila, the function of *hsp82* is not absolutely required for viability. Alternatively, the function of *hsp82* may be performed by another protein. If so, then this gene must not be heat shock inducible, and/or must reside in the same chromosomal region as *hsp82*, since embryos homozygous for *Df(3L)HR218* fail to synthesize any heat shock proteins in this size range. There also have been no reports identifying cognate *hsp82* genes in *D. melanogaster*.

Fourth, it is possible that we have isolated *hsp82* mutations but have not been able to prove that any of the complementation groups represent *hsp82*. We have attempted rescue with multiple copies of two different P element constructs (containing either the *D. melanogaster* or *D. pseudobscura hsp82* gene) but have not been able to show rescue with either strain. We have also tried to identify *hsp82* mutations by alterations in mobility on one or two dimensional gels but have found no evidence for such changes (data not shown). Therefore, although we can not absolutely rule out the possibility that one of the complementation groups is *hsp82*, none of our tests gives any indication to support this possibility.

In summary, we have screened the 63 region for mutations in the *hsp82* gene and in other genes. Although we have not isolated hsp82 *mutations* we have identified and mapped a number of loci in the 63 interval. Our cytogenetic studies should be useful in further genetic and molecular analysis of genes in this region.

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