Dissection of chromosome region 89A of Drosophila melanogaster by local transposition of P elements

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In the chromosome region 89A of Drosophila melanogaster, a few meiotic genes have been suggested to exist besides c3G and rec. We carried out local mutagenesis using a strain carrying a P element-insertion (plwB) at 89A, and obtained new genetic variants. Two are female sterile mutations, an allele of the homeless locus (hls167) and a new mutation tibi (tbi), and three are lethal mutations at the serpent locus. The tbi mutation is a paternally-rescuable maternal-effect-lethal. Destabilization of the P elements revealed that these mutations were caused by P element-insertions, and produced 12 deletion lines. These lines were then used for systematic complementation tests. The results showed that: (1) hls, tbi and at least three lethal genes in addition to c3G, rec and l(3)89Aa are located within the deletion of Df(3R)c3G2 (89A2-3; 89A4-5); (2) the gene order is rec, tbi, hls (from centromere to telomere), and both c3G and l(3)89Aa are possibly located proximally. We cloned 117 kb of DNA from this region by plasmid rescue and chromosome walking, and mapped several of the breakpoints of the deletions. These analyses delimited the rec gene to within 21 kb of the cloned DNA, although the c3G gene could not be located on the molecular map.

INTRODUCTION

Finding crossover suppressor in chromosome 3 of Gowen (c3G) mutation in Drosophila melanogaster was the first demonstration that meiotic recombination is genetically controlled (Gowen and Gowen, 1922; Gowen, 1933). Since then, many mutations affecting meiosis have been discovered and analyzed in Drosophila (reviewed in Baker and Hall, 1976; Hawley, 1993). The c3G mutant exhibits almost no meiotic recombination in females, and is the only mutation that completely lacks a synaptonemal complex (Smith and King, 1968). The recombination defective (rec) mutation is another meiotic mutation; it severely reduces recombination frequency (Grell, 1984). Both c3G and rec are located in the polytene chromosome subdivision 89A (Lewis, 1948; Nelson and Szauter, 1992). In addition, the mutator gene (mu), which shows slight reduction in recombination frequency and enhancement of nondisjunction besides its mutator activity, is also located to this region (Gold and Green, 1974). Grell (1984) pointed out that this chromosome segment may harbor a variety of recombination-related genes. However, no attempts have been made to search for other meiotic genes in this chromosome region.

We performed mutagenesis around the 89A region using P element local transposition and destabilization techniques (Tower et al., 1993, Zhang and Spradling, 1993) in order to analyse the gene organization and to find other meiotic genes in this region. We obtained two female sterile mutations with P element-insertions, and 12 deletions. We also cloned 117 kb of DNA from this region by chromosome walking. A fine genetic and molecular map of this region was constructed by complementation tests and molecular analyses of the breakpoints of the deletions.

MATERIALS AND METHODS

Fly strains. The strain #687 was used for the local transposition experiments. It carries a single plwB (Wilson et al., 1989) insertion in 89A as previously reported by Oguma et al. (1995) and Matsubayashi et al. (1995). The following strains were used for allelism tests: c3G2, rec1 bxw/Y;TM3, rec2 bxw/Y;TM3, ru h ts st c u srp52 sr e ca/TM3, mor1/TM6B, y;cv-c Df(3R)Po2/TM2;spa52, y; cv-c Df(3R)c3G2/TM2;spa52 and p+ Df(3R)sbd105 b x s r e/LVM. These strains were obtained from Bloomington Stock Center (Bloomington, Indiana, USA) and Mid-America Stock Center (Bowling Green, Ohio, USA). See Lindsley and Zimm (1992) for descriptions of the mutations. A female sterile mutation, homeless (hls), has been described recently.
(Gillespie and Berg, 1995), and the strains, ry^{306} hls^{2987} e/TM3 and y w; hls^{127}/TM3, were provided from C. Berg. The extent of the deletions on the polytene chromosome map is schematically represented in Figure 1.

Flies were reared on a cornmeal-yeast-glucose medium at 25°C. Mating experiments were also conducted in the same conditions.

**Construction of local transposition lines.** It was reported that a new \( P \) element can be inserted in the vicinity of the original element when a transposase source such as \( \Delta 2-3 \) (99B) is present (Robertson et al., 1988; Tower et al., 1993; Zhang and Spradling, 1993). The crossing scheme for the local transposition using \( \Delta 2-3 \) is shown in Figure 2. To elevate the efficiency of the local transposition, the females carrying both plwB and \( \Delta 2-3 \) were crossed to white males (Zhang and Spradling, 1993). G2 flies with more pigmented eyes were selected and singly backcrossed to the white strain. Flies heterozygous for \#687 (one copy of the mini-\( w^+ \) gene) deposit less eye pigment than do homozygotes (two copies of the mini-\( w^+ \) gene), reflecting the number of plwB insertions in the genome. Thus a fly which happened to have an additional insertion by local transposition would be expected to have more strongly pigmented eyes than the original \#687 heterozygotes.

G3 females with darker eyes were again backcrossed to the white males. If the additional insertion was not closely linked with the insert of \#687, these elements should be separated by recombination. If this was the case, we would detect two types of flies in terms of eye pigmentation within the G4 and could discard the line. The lines

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**Fig. 1.** Cytological map of the 89A region, shown schematically. The 89A region contains 13 bands. Horizontal lines below the chromosome show the size of deletions.

**Fig. 2.** Mating scheme to generate local transposition of the \( P \) element. Only sex chromosomes and third chromosomes are indicated. Triangles on the chromosome indicate \( P \) elements.
showing no variation in the eye color at G4 were kept as local transposition lines. The local transposition lines are all indicated with the symbol # preceding the line number (see Table 1).

**X chromosome primary nondisjunction.** To measure X chromosome primary nondisjunction frequency, females to be examined were mass mated to Bar (B) males. Nondisjunction frequency (%) was calculated as follows: (No. of B females + No. of B males) × 2 × 100 / [No. of B females + No. of B′ males + (No. of B females + No. of B males) × 2].

**Complementation tests.** Flies from lethal lines, heterozygous for a balancer, were mated with each other. When flies without a balancer chromosome emerged at the F1, we concluded that the two lethals were nonallelic. Complementation tests with the meiotic mutants c3G or rec were performed by analyzing the frequency of primary nondisjunction of the X chromosome. It has been demonstrated that females homozygous for c3G, rec or rec show 25 to 30% nondisjunction (Hall, 1972; Grell, 1984). When females heterozygous for c3G or rec showed 20% or higher nondisjunction, the mutation under study was classified as allelic.

**Destabilization of plwB.** Males of the local transposition lines with Δ2-3 were individually mated to w; SM5; TMS/ap lines. When a fly among the progeny possessed less pigmented eyes than those of the original heterozygotes, the fly was selected to establish a destabilization line.

To get a deletion including the c3G locus, females of the selected local transposition lines that were heterozygous for TMS, Sb Δ2-3 were mass mated with w; c3G/c3G males. Female progeny showing less pigmented eye color than the original were singly mated with B males to examine nondisjunction. Male progeny from a female showing a high rate of nondisjunction were used to establish the deletion lines. Destabilization lines are all indicated with superscript R on the line number from which they were derived (see Table 1).

**Cytology.** The salivary gland chromosomes were inspected by making squash preparations of larval salivary glands. Chromosome banding was interpreted with the aid of the revised polytene chromosome map of Lefevre (1976).

**Molecular procedure.** Routine molecular procedures described in Sambrook et al. (1989) were used unless otherwise specified. Genomic DNA was prepared following the method of Pirrotta (1986). A genomic library of Canton S adult flies was made with the λEMBL3 vector (Frischauf et al., 1983) according to the method described in Shrimpton et al. (1986). DNA of λ phages was prepared as described in Santos (1991) with slight modifications. Probes for Southern blot analyses and in situ hybridization were labeled with digoxigenin 11-dUTP by random priming and were detected using a nonradioactive DNA detection kit (Boehringer Mannheim). In situ hybridization to polytene chromosomes was done according to the method of Engels et al. (1986) except for probe labeling and detection, both of which were done using a nonradioactive DNA detection kit (Boehringer Mannheim). Plasmid rescue was carried out as described in Pirrotta (1986). Competent cells for plasmid rescue were prepared according to the method of Inoue et al. (1990). The following P1, YAC, cosmid clones located in 89A (FlyBase; Ashburner and Drysdale, 1994) were obtained from E. Nitasaka for (P1), I. Duncan for (YACs) and I. S. Kiamos for (cosmids): P1 clones, DS00223, DS00250, DS00496, DS01325, DS01590, DS02195, DS02273, DS03310, DS04078, DS04124, DS04384, DS04447, DS04728, DS05546, DS0668, DS05850, DS06650, DS06724, DS06731, DS07044, DS08400; YAC clones, DYN10-42, DYN11-16, DYN15-10A/B, DYN12-08, DYN14-86; cosmid clones, 72E1, 142G12, 69B2.

**RESULTS**

**Isolation of mutants by local transposition around the 89A region.** Following the mating scheme illustrated in Figure 2, we constructed 131 local transposition lines. Of these, five lines were homozygous lethal, six lines were homozygous semilethal, and two lines were female sterile. The other 118 lines were homozygous viable and fertile. None of them showed X chromosome nondisjunction of more than 1% (data not shown). Complementation tests showed that two lethals (#33 and #149) and two female steriles (#160 and #167) fell within the deficiency Df(3R)sbd105 (88F9-89A1; 89B9-10). Three other lethals and six semilethals were fully complemented with the deficiency, so they were not investigated further. Complementation tests among the four mutant lines demonstrated that the two lethals failed to complement each other, but that the female steriles, #160 and #167, do complement each other. We further tested them using

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**Table 1. Pedigree of the lines established by local transposition and destabilization**

<table>
<thead>
<tr>
<th>Original line</th>
<th>Local transposition</th>
<th>Mutation associated</th>
<th>Designation</th>
<th>No. of lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>#687</td>
<td>#33 srp&lt;sup&gt;20&lt;/sup&gt;</td>
<td>33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>#57 srp&lt;sup&gt;19&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>#149 srp&lt;sup&gt;16&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>#160 tbi</td>
<td>160&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>#167 his&lt;sup&gt;97&lt;/sup&gt;</td>
<td>167&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>678&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
deficiencies $Df(3R)Po2$ (89A1-2; 89A11-13) and $Df(3R)c3G2$ (89A2-3; 89A4-5), and the mutants $c3G$, rec, $hls$, $l(3)89Aa$, serpent ($srp$) and moira ($mor$). The results were as follows: the lethal mutation of the lines #33 and #149 were alleles of the $srp$ locus (hereafter named $srp^{1}$ and $srp^{2}$); the female sterile mutation in #167 was an allele of $hls$ (hereafter named $hls^{167}$), and a mutation in #160 also mapped within $Df(3R)c3G2$, but was fully complemented by $c3G$, rec and $l(3)89Aa$. Thus the mutation in #160 was considered to be novel and named $tbi$ (immature).

In addition to the above lines, we obtained two lethal lines, #57 and 687R104 from a separate screen. Line #57 was associated with an allele of $srp$ ($srp^{3}$), and 687R104 carried a deletion (see below).

**Characterization of the $tbi$ and $hls^{167}$ mutations.**

The mutation $tbi$ shows a peculiar fertility (or viability) phenotype. As shown in Table 2, females homozygous for $tbi$ produced almost no progeny when crossed to males homozygous for the mutation, but produced progeny which were all heterozygotes when crossed to males heterozygous for $tbi$. On the other hand, when the heterozygous females were crossed to homozygous $tbi$ males, progeny whose genotypes were either $tbi/+\$ or $tbi/tbi$ survived. Interestingly, when heterozygous males and females were crossed, both heterozygotes and homozygotes for $tbi$ were produced, but the number of $tbi$ homozygotes was significantly reduced. The results indicate that the $tbi$ gene is expressed both maternally and zygotically, and lack of the maternal product is compensated by the zygotic product. Conversely, the lack of zygotic expression can be compensated by the maternal product. Thus, this gene is a typical case of mutation known as a paternally-rescueable maternal-effect-lethal (Gans et al., 1975; Lindsley and Zimm, 1992). The reduction of viability is more apparent in $tbi$ hemizygotes ($tbi/\ Df(3R)c3G2$) than homozygotes, suggesting that the $tbi$ allele produced in this study is a hypomorph. The lethal phase of the homozygotes is polygenic; many of embryos fail to hatch but some develop to the pupal stage and then die. A few escapers also emerge (Table 2). In addition to this maternal effect, $tbi$ affects the development of wings. The homozygotes at a high frequency have deformed wings, but the degree varies from slightly notched to ug-like wing phenotype.

The $hls^{167}$ mutation seems to affect only female fertility, since homozygotes show no apparent reduction in viability and homozygous males are fully fertile. Homozygous females oviposited eggs with abnormal respiratory appendages. This abnormality was also been described by Gillespie and Berg (1995) for their $hls$ mutation. However, hemizygotes ($Df/hls^{167}$) oviposited almost no eggs, indicating $hls^{167}$ to be a hypomorph.

**Destabilization of the local transposition lines.**

As described above, we obtained two $P$ element-induced mutations within the region of the small deficiency, $Df(3R)c3G2$, in which meiotic genes $c3G$ and rec are known to be located. In order to excavate other genes, if any, and analyse the gene arrangements in this chromosomal region, we then destabilized $P$ elements of the local transposition lines $tbi$, $hls^{167}$, $srp^{3}$, and $srp^{149}$.

From the line #160 which has a plwB insertion at the $tbi$ locus in addition to the original #687 insertion, 25 independent destabilization lines (designated as 160P) were derived. All lines were selected as having less pigmented eye color than the original. Of the 25, 12 were lethal, 11 showed the same phenotype in viability as $tbi$, and two reverted fertile. The reversion to fertility indicates that the $tbi$ sterility or low viability is caused by a plwB insertion. From #167, 20 independent lines (designated as 167P) were also established. Of these, five were lethal, 14 were female sterile, and one was homozygous viable and fertile. The reversion to viability and fertility also indicates that the female sterility of $hls^{167}$ is due to an insertion of plwB. Of the 14 female sterile lines, 167P3 showed the same ovary morphology in homozygotes as did deficiency heterozygotes, suggesting that it is a null allele. Most of the egg chambers were arrested at around Stage 8.

Destabilization of #33 produced revertants whereas no revertant from #149, indicating that $srp^{1}$ was caused by a plwB insertion but $srp^{149}$ might be not. The lines 33P24 and 33P45 turned out to be deletions (see below), and both did not complement $c3G$. The pedigree of the derivatives from #687 is shown in Table 1.

**Genetic analysis of gene arrangement in the region.**

Destabilization produced 20 lethal lines (12 from #160, five from #167, two from #33, and one from #687). In order to characterize these lethal mutations and determine the gene order, complementation tests were carried out against $Df(3R)c3G2$ and the mutations $c3G$, rec, $l(3)89Aa$, $srp$, $mor$, $hls^{167}$, and $tbi$. All lethal lines were not complemented by $Df(3R)c3G2$. The results are summarized in Table 3. They have been classified into six groups which are represented by $167^{n1}$, $160^{n8}$, $167^{n11}$, $160^{n25}$, $160^{n34}$, and $33^{n3}$,
Dissection of chromosome region 89A of Drosophila

respectively. Groups 160R5, 160R14, 167R11, and 33R24 all are deletions for multiple loci. For example, 160R5 complemented hls167 but failed to complement both tbi and rec. Similarly, 167R11 complemented rec, but not both hls167 and tbi. Furthermore 160R34 has a deletion including all rec, tbi, and hls. Assuming all the deficiencies to be the simple two-break type, the order of the genes should be rec, tbi, and hls, although the direction is not determined from these complementation data (see below). Since no deletion separated c3G and l(3)89Aa from the group of these three genes, we were unable to analyze further by complementation tests (see discussion).

All 12 lethal lines derived from #160, which is associated with tbi, were divided into two types in terms of viability. Ten of them as represented by 160R1, but excluding 160R8 and 160R32, showed significantly reduced viability when heterozygous for tbi (160R#/tbi viability as low as that of tbi/Df). Remember that tbi is a hypomorph and shows very low viability as described above (see Table 2). Therefore, this suggests that the null mutation of tbi is responsible for the lethality. On the other hand, the viabilities of 160R8/tbi and 160R32/tbi were the same as that of tbi homozygotes. These two lethal lines both retain the same hypomorphic phenotype as the original line #160. Considering the data, we assume that 160R8 and 160R32 are not a null mutation of tbi but a deficiency of another lethal gene designated as l(3)89Ab located next to tbi or other site in Df(3R)c3G2. Interestingly 160R5 behaves as does 160R1 in terms of viability over tbi, but does not complement rec, suggesting that rec is closely linked to tbi (Fig. 3).

Similarly we identified two different complementation groups among the group represented by 167R17 as summarized in Figure 3. Members of this group (4 lethals; see Table 3) were examined further by complementation tests within them, and divided into two complementation groups, since 167R37 complemented 167R17, 167R5, and 167R36 (Table 4). The deletions induced by P element-destabilization in either direction, distal or proximal to hls, suggests that lethal genes are located on both sides of hls. The lethal gene associated with 160R8 is not the same gene as that associated with 167R17 nor 167R37. Therefore the lethals associated with 167R17 and 167R37 were designated as l(3)89Ac and l(3)89Ad, respectively. It was shown that hls∆125,

### Table 3. Complementation groups at the 89A region

<table>
<thead>
<tr>
<th>Group (No. of Lines)</th>
<th>Df(3R)sbd105</th>
<th>Df(3R)Po2</th>
<th>Df(3R)c3G</th>
</tr>
</thead>
<tbody>
<tr>
<td>167R17 (4)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>160R8 (10)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>167R11 (1)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>160R5 (1)</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>160R34 (2)</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>33R24 (2)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+ indicates that the lethal mutation complements for the mutant phenotype; lethality, sterility or nondisjunction.
– indicates that complementation for the mutant phenotype was not observed.

Fig. 3. Complementation map of the 89A region. At the top, each complementation group is separated by vertical lines. The next horizontal line represents the chromosome, with the telomere to the right. The sites of insertion of P elements in strains #160, #167, #687, and #33 are indicated by triangles. Underneath the line representing the chromosome, the size of a deletion is indicated by a horizontal line. We assume all the deficiencies to be the simple two-break type. Note the large size of deletions Df(3R)c3G2 and Df(3R)Po2, which differ in their right breakpoints by about ten polytene bands, as shown in Figure 1. Both c3G and l(3)89Aa map to the left of rec, as discussed in the text. Parentheses show the number of additional lines belonging to the group.
which might carry the deletion of hls and additional viability genes (Gillespie and Berg, 1995), failed to complement 167R17 but complemented 167R37 for viability. The lethal line 167R11 possesses a larger deficiency including tbi, hls, and three lethals, l(3)89Ab, l(3)89Ac, and l(3)89Ad, but the rec locus.

In the course of this experiment, the TM6B balancer chromosome was found not to complement c3G for the non-disjunction phenotype. The primary nondisjunction frequency in TM6B/c3G females was 23.8%, and 35.0% in TM6B/Df(3R)sbd105 females, but no nondisjunction was observed in TM6B/rec females. The TM6 balancer, from which TM6B was derived, fully complemented c3G. Thus, TM6B might have acquired an unidentified c3G allele upon its establishment.

Molecular analysis of the 89A region. Since the plwB element possesses the Bluescript vector sequence, we performed plasmid rescue from #687, and isolated a DNA fragment, p687X. We then used the cloned DNA as an initial probe for screening a Canton S genomic library, and subsequently walked in both directions from it. A continuous 117 kb DNA sequence was cloned and mapped by restriction enzymes.

Insertion sites of the plwB in #687, #167, and #160 and break points of some deletions were determined by genomic Southern blot analyses; they are summarized in Figure 4. The local transposition line #167 possesses two plwB insertions as expected: one is at the same site as #687, which we set as 0 for the molecular map of the 89A region, and the additional insertion is at −21 kb relative to the first insertion. Similarly, #160 carries a second insertion at −23 kb. The distance between the secondary insertions of #160 and #167 is about 2 kb. Genetic data showed that these insertions were responsible for the mutations tbi and hls167, respectively.

The chromosomes of 33R24 and 33R44 possessed deletions for all of the cloned region. The breakpoints of the 160R34 deletion were at 0 and −48 kb. A breakpoint of Df(3R)c3G2 is somewhere between +30 and +40 kb, but the other breakpoint is beyond the cloned region. The proximal and distal breakpoints of 167R11 were somewhere between −28 and −33 kb, and between −5 and +6 kb, respectively. Chromosome walking to the left, further than −48 kb, using λ and cosmid libraries has not been successful. No P1, YAC, and cosmid clone reported as of 89A origin hybridized with the DNA fragment at −48 kb. Based on the data of breakpoints of deletion and the gene-order indicated by the complementation tests, we could place the rec gene on the restriction map between −28 and −49 kb.

Cytological examination. The cytological breakpoints of 33R24 and 33R44, which are the largest deletions obtained

### Table 4. Complementation groups among those carrying lethal mutations

<table>
<thead>
<tr>
<th></th>
<th>167R17</th>
<th>167R11</th>
</tr>
</thead>
<tbody>
<tr>
<td>167R5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>167R36</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>160R34</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates that they complement for lethality.
– indicates that they fail to complement for lethality.
Line 160R34 belongs to 160R32 group shown in Table 3.
through the destabilization experiments, were both 89A2-3; 89A12-89B1 (Fig. 1). However 160934, the second largest deficiency we obtained, was too small to allow analysis of its breakpoints at the polytene chromosome level.

We then tried to determine the centromere-telomere direction of the cloned DNA by in situ hybridization to polytene chromosomes using a few cloned DNA as probes between p54S2 and p7OH (Fig. 4), which are separated by 95 kb. All probes labeled faint bands between 89A3-7, and it was hard to decide the relative positioning of the two locations.

In order to overcome this problem, we obtained several male-recombinants occurring at the P element-insertion site of #687 using induction by Δ2-3. The direction of the molecular map as shown in Figure 4 was determined by analyses of the derived recombinant chromosomes using a polymorphic molecular marker found at –48 kb of the cloned region (data not shown).

**DISCUSSION**

**Local mutagenesis with P element.** We tried to uncover new genes in the 89A chromosome region in which two meiotic mutants c3G and rec had already been mapped. It was previously demonstrated that a P element transposes preferentially into nearby sites from its original insertion site when mobilized by Δ2-3 (99B); this process is called “local transposition” (Tower et al., 1993; Zhang and Spradling, 1993). Using this technique, we constructed 251 lines from the line #687, which carries a plwB element at 89A. Local transposition and subsequent destabilization experiments yielded two reproductive mutations (tbi and hls), four lethals (srp, l(3)89Ab, (3)89Ac, and (3)89Ac), and 12 deficiencies, which were divided into six different groups within 48kb of the 89A2-5 polytene bands. Small deletion products are very useful for fine genetic as well as molecular mapping. We could delimit the rec gene within 21 kb of DNA. Another interesting meiotic gene, c3G, is likely located just outside of the proximal end of the region (see below). We are currently working with cDNA and germ line transformation to identify precise location of the gene.

Destabilization of P elements produced a variety of deletions. Molecular analyses of the breakpoints of 160934, 167R11, 687R104, 33R24, and 33R44 demonstrated that the breakpoints did not necessarily coincide with the location of P element-insertion (Figs. 3 and 4). Cooley et al. (1990) reported that the chromosomal region sandwiched with two P elements can be deleted when destabilized by the presence of Δ2-3 transposase. The difference between their results and ours could be due to the chromosome length between the two given P elements. Cooley et al. (1990) used only a single pair of P elements inserted in an interval of over 30 polytene bands in section 93, while most of the chromosomes used in our study have insertions within less than 30 kb. Our results may suggest that small deletions are produced with one breakpoint at the insertion site and the other in the genomic DNA in the vicinity to the insertion.

**Characteristics of the tbi gene.** The newly identified tbi gene is classified as a paternally-rescuable maternal-effect-lethal (Gans et al., 1975; Lindsley and Zimm, 1992). This type of genes is expressed both maternally and zygotically, and expression at either stage is enough for the zygote to develop to the adulthood. Lack of maternal expression in females homozygous for tbi results in lethality when their eggs are fertilized with a sperm carrying the tbi mutation.

Zygotic gene expression does not occur at the stages prior to the cellular blastderm (Zalokar, 1976; Lamb and Laird, 1976; McKnight and Miller, 1976; Anderson and Lengyle, 1979). Thus the maternal product of tbi is estimated to be required at a stage when both maternal and zygotic genetic information coexist. A further interesting aspect of tbi is that expression is repressed (i.e., the mutant phenotype is enhanced) in the absence of the Y chromosome, as in the case of position effect variegation (unpublished data). Molecular analysis of tbi using transformants to understand the nature of this type of mutation and the regulation of its expression is currently ongoing.

**Gene organization of the 89A region.** In the 89A polytene map region, meiotic genes c3G and rec, and mu have all been located, and they all function in the recombination process (Gold and Green, 1974). Grell (1984) pointed out that the 89A region may contain a number of recombination related genes. In our experiments, a female sterile mutation, hls1tbi, and a maternally functioning gene, tbi, have been discovered, although novel meiotic genes were not found. At least three genes, rec, tbi, and hls, reside within 50 kb of DNA defined by the 160934 deficiency (Fig. 4). Both meiotic genes and female sterile genes should be expressed in ovaries or in female germ line cells. It is thus plausible that the region contains many genes expressed in the female germ line.

In addition to the above three genes, we identified at least three lethals whose genetic function is totally unknown. It is of course possible that these lethals are not all independent genes, but alleles of a single complex locus. For example, Gillespie and Berg (1995) showed that the transcripts of hls were detected in ovaries and also in the rest of carcasses, and small deletions (less than 2 kb) in the putative hls coding region result in the lethality. This suggests that a null allele of hls should be lethal, although hls1 does not seem to decrease viability.

At present, the location of c3G has not been determined. It could be located to either side, proximal to rec or distal to the #687 insertion site, since the complementation test described above was not sufficient to reach a conclusion.
However, screening for deletions in the c3G locus by destabilization of #33, which carries two plwB insertions at the sites of #687 and the srp locus, gave only two deficiencies, 33R24 and 33R44, despite an extensive survey. If c3G is located distally to the plwB insertion site of #687, we would have expected to obtain deletions between the two P-insertion sites (Fig. 3). The two deletions we obtained are larger than those derived from #160 and #167. Furthermore, the proximal breakpoints of the deletions 33R24, 33R44, and Df(3R) c3G2 are beyond the cloned DNA region and all of them fail to complement c3G. These results suggest that the c3G locus is located proximal to the rec gene. We have also examined genomic DNA of the strain c3G1 by Southern hybridization using cloned DNAs from 0 to +40 kb as probes, but did not find any results indicating the existence of an insertional DNA. Since c3G2 is a spontaneous mutation (Gowen and Gowen, 1922), it could well be associated with a transposable DNA element (Inoue and Yamamoto, 1987; Green, 1988). These preliminary results also suggest that the c3G locus is in the region proximal to the rec gene.

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