Genetic and Molecular Analysis of New Female-Specific Lethal Mutations at the Gene Sxl of Drosophila melanogaster

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ABSTRACT

We have isolated three female-specific lethal mutations at the gene Sex-lethal (Sxl): Sxl*, SxlLs and SxlL*. We have carried out the complementation analysis between these mutations and other previously reported Sxl mutations. It is possible to classify the alleles tested in this report into two complementation groups: the bc group defined by Sxl*, and SxlLs, and the LS group defined by SxlL*. The other alleles tested affect both complementation groups albeit with different degrees. Contrary to what happens with mutations at the LS group, mutations at the bc group do not affect sex determination, nor late dosage compensation nor oogenesis. Both SxlLs and SxlL* present a DNA insertion of at least 5 kb between position -10 and -11 on the molecular map, within the fourth intron. On the contrary, Sxl*, a strong mutation affecting all Sxl functions, is not associated to any detectable DNA alteration in Southern blots, so that it seems to be a “point” mutation. In agreement with their phenotypes, both SxlLs/SxlL* and SxlL* homozygous female larvae express only the late Sxl transcripts characteristic of females, while females homozygous for SxlL* express only the late Sxl transcripts characteristic of males. Moreover, SxlL* presents a lethal synergistic interaction with mutations at either da or the X:A ratio, two signals that define the initial activity state of Sxl, while SxlL* do not. These data suggest that the two complementation groups are related to the two sets of early and late Sxl transcripts, which are responsible for the early and late Sxl functions, respectively: SxlLs and SxlL* would affect the early functions and SxlL* would affect the late Sxl functions.

In Drosophila melanogaster, the gene Sxl controls the processes of somatic sex determination, dosage compensation and oogenesis. The functional state of Sxl is determined by the X:A ratio signal: in 2X;2A flies Sxl will be ON, while in X;2A flies Sxl will be OFF (CLINE 1978). Two X-elements of the X:A signal have been identified: sisterless-a (sis-a) (CLINE 1986) and a region of the achaete-scute complex that has been named sisterless-b (sis-b) (CLINE 1988) which corresponds to the gene scute-T4 (sc-T4) (TORRES and SANCHEZ 1989; PARKURST, BOPP and ISH-HOROWIEZ 1990; ERICKSON and CLINE 1991). Activation of Sxl requires also the maternal daughterless (da) product (CLINE 1978). Once the state of Sxl is defined, an event that occurs at the blastoderm stage, the X:A signal is no longer used and the activity of Sxl remains fixed (SANCHEZ and NÖTHIGER 1983; BACHILLER and SANCHEZ 1991). The capacity of the gene Sxl to function as a stable “switch” is thought to be due to a positive autoregulatory function of the Sxl gene product (CLINE 1984). This gene is controlled throughout development by alternative splicing of its primary transcript (BELL et al. 1988). The gene f (2)d is needed for the female-specific splicing of Sxl RNA, thus suggesting the involvement of f (2)d in the positive autoregulatory pathway of Sxl (GRANADINO, CAMPIZANO and SANCHEZ 1990).

The gene Sxl produces two temporally separate sets of transcripts. The early set is composed of three transcripts found only around the blastoderm stage, presumably, in female embryos (SALZ et al. 1989). The late set is formed by three other transcripts, present in both females and males, which appear slightly later in embryogenesis and persist throughout the remainder of development and in adult life. The three female late transcripts overlap extensively and share most exons, but differ at their 3’ ends. The three male transcripts are similar to their female counterparts, except for the presence of an additional exon (exon 3), which contains a translation stop codon; consequently, the male late transcripts give rise to presumably inactive truncated proteins (BELL et al. 1988). Two Sxl transcripts are associated with the development of the female germline, one of them being also present in the soma (SALZ et al. 1989).

Two sets of Sxl mutations have been isolated. One set is formed by loss-of-function mutations, generically named as SxlL, which are characterized by their reces-
sive female-specific lethal phenotype (CLINE 1978; MARSHALL and WHITTLE 1978; SANCHEZ and NÖTHIGER 1982; this report). The other set is formed by gain-of-function mutations, generically named at SxlM, which are characterized by their dominant male-specific lethal phenotype (CLINE 1978; MAINE et al. 1985a). Molecular analysis of Sxl mutations (MAINE et al. 1985; SALZ, CLINE and SCHEDL 1987; this report) has allowed us to establish a correlation between changes in Sxl specific functions and DNA alterations.

We have isolated two X-ray-induced (Sxlβ and Sxlβ) and one EMS-induced (Sxlδ) female-specific Sxl mutation. Here we report, first, the complementation analysis of these new mutations and other known Sxl mutations for the processes controlled by Sxl: sex determination, dosage compensation and oogenesis. Second, the DNA map is given of the new Sxlβ, Sxlβ and Sxlδ mutations. Finally, we analyzed the late Sxl transcripts of females mutant for different Sxl alleles.

MATERIALS AND METHODS

Flies were cultured on standard food at 25°, unless otherwise stated. For a description of the mutations and chromosomes see LINDSLEY and GREALY (1968) and LINDSLEY and ZIMM (1986, 1985, 1987, 1990).

Induction, isolation and genetic mapping of Sxl mutants: Sxlβ and Sxlδ were induced by X-ray treatment (3000 rad) of males, while Sxlδ was induced by treatment of males with EMS according to the procedure of LEWIS and BAKER (1968). We used X chromosome isogenic strains for mutagenesis. The isolation criteria was the female-specific lethal phenotype (CLINE 1978; MAINE et al. 1985a). Molecular analysis of Sxl mutations (MAINE et al. 1985; SALZ, CLINE and SCHEDL 1987; this report) has allowed us to establish a correlation between changes in Sxl specific functions and DNA alterations.

We have isolated two X-ray-induced (Sxlβ and Sxlδ) and one EMS-induced (Sxlδ) female-specific Sxl mutation. Here we report, first, the complementation analysis of these new mutations and other known Sxl mutations for the processes controlled by Sxl: sex determination, dosage compensation and oogenesis. Second, the DNA map is given of the new Sxlβ, Sxlβ and Sxlδ mutations. Finally, we analyzed the late Sxl transcripts of females mutant for different Sxl alleles.

Crosses for the clonal analysis of the Sxlβ mutations in the germline: y w Sxlβ fβ+/FM6 // Sxlδ oc v fβ+/FM6 // Sxlδv/FM7 // y Sxlβ fβ+/FM6 and y Sxlδ oc v F M6 females were crossed with ovoDf Y males. The Sxlβ/ovoDf females were crossed to appropriate males to test mosaicism in the germline.

Clonal analysis of Sxlδ mutations in the soma: The irradiation dose was 1000 rad and was supplied by a Philips X-ray machine at a rate of 300 rad/min. The adult flies were kept in a mixture of ethanol-glycerol (3:1) for several days, and subsequently mounted for analysis under a compound microscope. The sexual phenotype of the marked clones was assessed in sexually dimorphic regions of the fly: sex comb region of foreleg and external derivatives of the genital disc. For a description of the morphology of these structures see BRYANT (1978).

Clonal analysis of Sxlδ mutations in the germline: The progeny of the different crosses were irradiated at 24-48 hr after oviposition. The irradiation dose was 1000 rad applied by a Philips X-ray machine at a rate of 300 rad/min.

The clonal analysis in the germline is based on the use of ovoDf. This is a dominant female-sterile mutation, which affects autonomously the development of the germline, while having no effect on the somatic tissues involved in oogenesis (PERRIMON 1984). Germ cells of the sterile ovoDf Sxl females that suffered X-ray-induced mitotic recombination between their two X chromosomes will render, in the next cell division, two daughter cells: one will be homozygous for ovoDf, which will not develop, and the other will be homozygous for Sxl, whose capacity to develop is being analyzed. When the irradiated females eclosed, they were mated to appropriately marker stocks to test mosaicism in the germline.

Effect of Sxlβ on the lethal phase of female larval hemizygous for Shaker: We crossed y Sxlβ Df(Sh+)FM6, y w B; Dp(Sh+) / TM1, Me red females with y Sxlβ Y; red/red males. Df(Sh+) stands for Df(1)F1C7 and Df(Sh+) stands for Df(1)F1C7. The female progeny will show the white or the yellow phenotype if they are, respectively, heterozygous or homozygous for Sxlβ. In this last case, they will be red if they carry the Df(Sh+) (two doses of Sh+) and red if not (one dose of Sh+). Thus, the existence of yellow-red larvae indicates that the Df(Sh+)/ females do not die as embryos. The white and the red phenotype was monitored in the Malpighian tubules, and the yellow phenotype in the mouthparts.

Analysis of enzymatic activities: We analyzed the activities of the X-linked enzymes fumarase (FUM), 6-phosphogluconate dehydrogenase (6PGDH) and glucose-6-phosphate dehydrogenase (6GPDH) and the activities of the autosomal enzymes alcohol dehydrogenase (ADH) and NADP-isocitrate dehydrogenase (IDH-NADP). We followed the experimental procedure of LUCCHESI and RAWLS (1973) for 6GPDH, 6PGD and IDH-NADP; the experimental procedures of JACOBSON, MURPHY and HARTMAN (1970) for ADH, and the experimental procedure of WHITNEY and LUCCHESI (1972) for FUM. We used a spectrophotometer Kontron Uvicron 810. The larvae were homogenized in a solution described by BELOTE and LUCCHESI (1980). To produce the larvae we crossed y w Sxlβ FM6 females with y w Sxlβ w Y males. The female larvae homozygous for Sxlβ showed the yellow-white phenotype, while the Sxlβ heterozygous females showed a wild-type phenotype. The Sxlβ males showed the yellow phenotype, while the Sxl+ males (FM6) showed a wild-type phenotype. The yellow phenotype was monitored in the mouthparts and the white

the maternal da products: y d da'/CyO females were crossed, at 29°, with males carrying the Sxl mutations from the stocks detailed above.

Crosses for the clonal analysis of the Sxlδ mutations in the germline: y w Sxlδ fδ+/FM6 // Sxlδ oc v fδ+/FM6 // Sxlδv/FM7 // y Sxlδ fδ+/FM6 and y Sxlδ oc v F M6 females were crossed with ovoDf Y males. The Sxlδ/ovoDf females were crossed to appropriate males to test mosaicism in the germline.

Clonal analysis of Sxlδ mutations in the soma: The irradiation dose was 1000 rad and was supplied by a Philips X-ray machine at a rate of 300 rad/min. The adult flies were kept in a mixture of ethanol-glycerol (3:1) for several days, and subsequently mounted for analysis under a compound microscope. The sexual phenotype of the marked clones was assessed in sexually dimorphic regions of the fly: sex comb region of foreleg and external derivatives of the genital disc. For a description of the morphology of these structures see BRYANT (1978).
phenotype in the Malpighian tubules. Females and males were separated by the different size of their gonads.

**Analysis of sgs-4 and sgs-3 transcripts:** Total RNA from each type of larvae was extracted following the experimental procedure of Case and Danefold (1978). Levels of sgs-4 and sgs-3 transcripts was quantitated by the dot-blot technique using as probes pRH0.75 and adm124E8, respectively (Meyrowitz and Hogness 1982). McGinnis, Shermoen and Bekendorf (1983), kindly supplied by S. K. Bekendorf. RNA blotting and hybridization were performed as described by Maniatis, Fritsch and Sambrook (1982). To quantify the degree of hybridization we measured the spots of the film with a Molecular Dynamics Computing Densitometer, model 300A.

**DNA analysis of Sxl mutants:** Total nucleic acid from adult males was extracted as described (Meyrowitz and Hogness 1982) and treated with RNase (2 mg/ml) for 2 hr at 37° (Breen and Lucchesi 1986). Restriction digests of genomic DNA were fractionated on 0.7–0.8% agarose gels and subsequently transferred to nitrocellulose filters. The filters were hybridized with 32P-labeled nick-translated DNA probes AS1, AS2A and S1-p15. AS1 and AS2A were kindly supplied by T. W. Cline. S1-p15 refers to the 4.8-kb fragment (Xhol-XhoI), coordinates −10.8 to −6) from AS1 that we subcloned in the pBHR2 plasmid vector. Hybridization was carried out as described in Maniatis, Fritsch and Sambrook (1982).

**Northern analysis of Sxl transcripts:** RNA preparation from frozen larvae or adult flies, electrophoretical fractionation of total RNA and blotting to nylon membranes were performed as described elsewhere (Maniatis, Fritsch and Sambrook 1982; Campuzano et al. 1986). Hybridization with [32P]RNA probes was carried out as previously described (Granadino, Campuzano and Sanchez 1990). Genomic fragments from AS1 (Maine et al. 1985) were subcloned in pGem-1 and pGem-2, and used as templates for preparing the male (g2) and the non-sex specific (h’) RNA probes, respectively (see Figure 4).

**RESULTS**

**Effect of Sxl mutant alleles on female viability and their complementation pattern:** The results of the complementation test are shown in Table 1. None of the Sxl mutant alleles complements Sxl+ (bc mutant allele), which is a deficiency for Sxl (Salz et al. 1987). All of the Sxl mutations in homoyzgous produce variable degrees of lethality in female flies, with the only exception of Sxl+/+ (homozygous). This is the weakest mutant allele which, by itself, has no effect on female viability (Cline 1980). Sxl+/ and Sxl+/+ are the most moderate alleles, and they are lethal with any of the other Sxl mutations, except Sxl+/+/. The different viability degree of Sxl+/+ with Sxl+/ (2%) and with Sxl+/ (35%) qualifies Sxl+/ as the strongest mutant allele. Sxl+ and Sxl+ do not complement each other. They do not complement neither Sxl+/ nor Sxl+/+. However, Sxl+ and Sxl+ fully complement Sxl+/+, as Sxl+ does. This defines two complementation groups within the Sxl gene for female vital functions: one group is defined by the Sxl+/+ mutation (LS complementation group) and the other is defined by the Sxl+/, Sxl+/ and Sxl+/ mutations (bc complementation group). The rest of the Sxl mutations affect both complementation groups albeit with different degrees. Sxl+ and Sxl+ affect the LS group more than the bc one. On the contrary, Sxl+ and Sxl+ affect the bc group more than the LS one.

**Analysis of dosage compensation in females homozygous for Sxl+ or Sxl+:** The female-specific phenotype of Sxl+ and Sxl+ suggests that females homozygous for these mutations die because of a disarrangement in their dosage compensation process, as is the case with other female-specific lethals at the gene Sxl (Lucchesi and Skripsky 1981). As a first approach, we analyzed the effect of Sxl+ and Sxl+ on dosage compensation in larvae: 70% of the Sxl+ and Sxl+ homozygous individuals die as embryos, and 28% as larvae. Therefore, the test only assay the individuals that survived the early lethal period. We followed three different experimental approaches.

The first experimental approach was one followed by Breen and Lucchesi (1986). In Sxl+/ or Sxl+ homozygous female larvae, we measured the amount of the X-linked transcript coded by the gene sgs-4, which is dosage compensated (McGinnis, Shermoen and Bekendorf 1983), relative to the amount of RNA encoded by the autosomal gene sgs-3 (Meyrowitz and Hogness 1982). These two RNAs are coordinately expressed in salivary glands from the middle of the third larval instar to the prepupal stage. If Sxl+ and Sxl+ alter the dosage compensation process, the level of the sgs-4 transcript should increase, compared to the sgs-3 transcript, in the females homozygous for these Sxl mutations. The measurement of the amounts of sgs-4 and sgs-3 transcripts was carried out by means of the dot-blot technique (for details see MATERIALS AND METHODS). The results are shown in Figure 1. Females homozygous for Sxl+ have a higher value for the sgs-4/sgs-3 ratio than heterozygous females; however, this difference is not significant (P > 0.05). With respect to the Sxl+ mutation, the sgs-4/sgs-3 value of the homozygous females, which is similar to the one found for the males, is significantly (P < 0.05) lower than that of the heterozygous females, contrary to expectation if Sxl+ was affecting dosage compensation. This suggests that the Sxl+ and Sxl+ mutations do not increase the transcription of the X chromosome in homozygous female larvae.

The second experimental approach consisted in measuring the specific activity of enzymes coded by dosage-compensated X-linked genes relative to enzymes coded by autosomal genes. The X-linked enzymes tested were FUM, 6PGDH, and G6PDH, and the autosomal enzymes were ADH and IDH-NADP. The analysis was carried out for the Sxl+ mutation. The enzymatic activities were measured in crude extracts of larvae. If Sxl+ was affecting dosage compensation, an increase in the enzymatic activities of the X-linked enzymes, compared to the autosomal ones,
TABLE 1

Complementation analysis for the viability of females double heterozygous for different Sxl mutant alleles

<table>
<thead>
<tr>
<th>Sxl allele</th>
<th>7B0</th>
<th>f1</th>
<th>fd</th>
<th>fn7M1</th>
<th>M1fm3</th>
<th>f2593</th>
<th>fhu1</th>
<th>fLs</th>
<th>fb</th>
<th>fc</th>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
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<td>(321)</td>
<td>(561)</td>
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<td>(561)</td>
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<td></td>
</tr>
<tr>
<td>fn7M1</td>
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<td>(618)</td>
<td>(1118)</td>
<td>(626)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>M1fm3</td>
<td>(352)</td>
<td>(1385)</td>
<td>(1505)</td>
<td>(488)</td>
<td>(137)</td>
<td></td>
<td></td>
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<tr>
<td>f2593</td>
<td>(199)</td>
<td>(393)</td>
<td>(1093)</td>
<td>(529)</td>
<td>(263)</td>
<td>(395)</td>
<td></td>
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<td></td>
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<tr>
<td>fhu1</td>
<td>(161)</td>
<td>(369)</td>
<td>(341)</td>
<td>(704)</td>
<td>(495)</td>
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<tr>
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<td>(383)</td>
<td>(1930)</td>
<td>(236)</td>
<td>(310)</td>
<td>(264)</td>
<td>(228)</td>
<td>(858)</td>
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<td>fb</td>
<td>(210)</td>
<td>(488)</td>
<td>(1108)</td>
<td>(257)</td>
<td>(312)</td>
<td>(266)</td>
<td>(142)</td>
<td>(438)</td>
<td>(366)</td>
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<tr>
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<td>(1850)</td>
<td>(257)</td>
<td>(291)</td>
<td>(447)</td>
<td>(226)</td>
<td>(327)</td>
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<td>(254)</td>
</tr>
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<td>f9</td>
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<td></td>
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</tr>
</tbody>
</table>

* Percentage of experimental females with respect to control sister females.
† Number of control flies.

FIGURE 1.—Analysis of the level of sgs-4 and sgs-3 transcripts in male and female larvae homozygous (Ho) or heterozygous (Ht) for Sxl<sup>b</sup> or Sxl<sup>l</sup>. The bars represent the 95% confidence intervals. The experiment was performed four times. A statistical analysis of the results showed nonsignificant differences for the sgs-4/sgs-3 ratio between the four replicas of the experiment. We then pooled all the data to get a better estimate for the sgs-4/sgs-3 ratio value.

should be expected, in consequence of a hypertranscription of the two X chromosomes. The results are shown in Figure 2. No significant (P > 0.05) differences were observed between the four genotypes (homozygous and heterozygous females and males) for FUM and G6PDH. On the contrary, significant (P < 0.05) differences were observed between these four genotypes for 6PGDH; however, the reduced level of that enzyme in homozygous compared to heterozygous females is contrary to expectation if Sxl<sup>b</sup> was affecting dosage compensation. All the results are the same regardless of which autosomal enzyme (ADH or IDH) was used for reference. Thus, also with this experimental test we have not detected alterations in the dosage compensation process of female larvae homozygous for Sxl<sup>b</sup>.

The two sets of experiments that we performed are based on the capacity to quantitate certain products (transcripts or enzymes) by means of in vitro tests (hybridization with a probe or enzymatic reactions). The fact that we did not detect significant changes in the level of X chromosome transcription, caused by the bc mutations, cannot be attributed to a limited sensitivity of the experimental test that we used, since they have been successfully applied to determining variations in the level of X chromosome transcription due to mutations affecting dosage compensation (Lucchesi and Manning 1987). We still performed a third experimental test. This is an in vivo test based on the capacity of the organism to survive depending on the level of X chromosome transcription. This test takes advantage of the haploinsufficient character of the X-linked gene Shaker (Sh): both Df(Sh)/Y males and Df(Sh)/+ females die as embryos (Ferrus et al. 1990). J. L. De La Pompa and A. Ferrus (personal communication) found that the lethal phase of Df(Sh)/+ females is retarded to larval stage if a hypertranscription
of the X chromosomes was assured. They found that Sxl^{[1]} DJSh/Sxlf^{[1]} sh+ females die as larvae, because the Sxl^{[1]}/Sxlf^{[1]} constitution causes a hypertranscription of the X chromosomes (Lucchesi and Skripsky 1981), so that more Sh^+ product is produced from the single Sh^+ allele. Our reasoning was that if the Sxlf^6 mutation is affecting dosage compensation, an increase of the X chromosome transcription will occur, and then the lethal phase of DfSh/+ females homozygous for Sxlf^6 is expected to be retarded to the larval stage. We set up crosses to produce females of genotype Sxlf^6 DfSh/Sxlf^6 sh^+, which can be identified as the larval stages (see MATERIALS AND METHODS). We did not find any of these female larvae among a total of 685 larvae that we analyzed. Thus, homozygosity for Sxlf^6 does not change the lethal phase of DfSh/+ females, which still die as embryos.

Within the limits of resolution of the techniques that we have applied, we conclude that the Sxl^b and Sxl^f mutations seem to have no obvious effect on late dosage compensation.

**Effect of Sxl mutant alleles on female sexual development and their complementation pattern: Sxl^b** and Sxl^f fully complement Sxl^{[1]}^{[1]}. All the viable flies double heterozygous for Sxl^b or Sxl^f and the other Sxl mutations develop as wild-type females, with the exception of Sxl^{[1]^{[1]}}/Sxl^b and Sxl^{[1]^{[1]}}/Sxl^f flies, which occasionally present a male spot in the fifth or sixth tergites. Moreover, Sxl^{[1]^{[1]}} or Sxl^{[1]^{[1]}} homozygous females survive as males and Sxl^{[293]} flies as intersexes, while Sxl^{[3]^{[3]}} produce female flies (Marshall and Whittle 1978; Cline 1980; 1984; this report). None of the alleles that produce sexual transformation fully complements each other. Thus, they define a single complementation group for Sxl functions involved in female sexual development.

Females homozygous for Sxl^{[2]}, Sxl^{[2]} or Sxl^b are not viable. The clonal analysis of Sxl^{[2]} (Cline 1979) and Sxl^{[2]} (Sanchez and Notiher 1982) has revealed that these two mutations affect the female sexual development. We have carried out the clonal analysis of Sxl^{[2]}. All clones homozygous for this mutation developed male structures instead of female ones, thus demonstrating that Sxl^{[2]} does also affect the female sexual development (Table 2). The Sxl^b and Sxl^f homozygous flies that survive are normal females. It is possible, however, that we are missing the effect of Sxl^b and Sxl^f on sexual development due to its association with a lethal phenotype, or to complementation in the case of viable combinations with other alleles. To assess the role of Sxl^b and Sxl^f in sexual determination, we have carried out their clonal analysis. None of the clones homozygous for these mutations displayed sexual transformation (Table 2), contrary to the observation with other female-specific Sxl mutations analyzed so far. A similar result has been reported for Sxl^{[1]} (T. Cline, unpublished results, cited in Maine et al. 1985b). These results could indicate that either Sxl^{[1]} and Sxl^f do not affect the Sxl feminating functions, or that they affect Sxl functions only needed at the beginning of development, when the state of activity of Sxl is defined. For this reason, we carried out the clonal analysis of Sxl^b and Sxl^f by irradiation at the blastoderm stage. All of the Sxl^{[1]} clones (8 in forelegs and 14 in the external terminalia) and all of the Sxl^{[2]} clones (9

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**TABLE 2**

Clonal analysis of Sxl mutations following irradiation at 24–48 hr of development

<table>
<thead>
<tr>
<th>Sxl allele tested</th>
<th>No. of forelegs</th>
<th>Female phenotype</th>
<th>Male phenotype</th>
<th>No. of external terminalia</th>
<th>Female phenotype</th>
<th>Male phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sxl^{[1]}</td>
<td>298</td>
<td>0</td>
<td>6</td>
<td>100</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Sxl^{[1]}</td>
<td>781</td>
<td>19</td>
<td>0</td>
<td>413</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Sxl^{[1]}</td>
<td>1112</td>
<td>21</td>
<td>0</td>
<td>456</td>
<td>21</td>
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</table>

The genotypes of the irradiated females were: y Sxl^{[1]} p66/M(1)O^9, y Sxl^{[1]} p66/M(1)O^9 and y Sxl^{[1]} p66/M(1)O^9.
in forelegs and 14 in the external terminalia) developed female structures.

It can be argued that we are losing some of the Sxl\(^{16}\) or Sxl\(^{16}\) clones and only the “escaper” ones survive, those that for unknown reasons express higher levels of Sxl\(^{+}\) activity and consequently develop female structures. We believe that this is not the case, since even clones homozygous for the very strong amorph mutation Sxl\(^{16}\) (CLINE 1979) or for a deficiency of Sxl (Sxl\(^{180}\), induced at blastoderm, our unpublished results) can survive, differentiating male structures. Moreover, the frequency of clones homozygous for Sxl\(^{16}\) or Sxl\(^{16}\) does not differ significantly (P > 0.05) from those homozygous for Sxl\(^{16}\), a stronger mutation (this report). We also irradiated at 24-48 hr of development female larvae of genotype y\(^{f6}\)/M(1)O\(^{y6}\), finding that the frequency of y\(^{f}\) clones in the forelegs of these females is similar to that found in the clonal analysis of Sxl\(^{16}\), Sxl\(^{16}\) and Sxl\(^{16}\) (data not shown). We conclude that the bc mutations do not affect the Sxl-feminizing functions.

The Sxl\(^{2593}\) mutation appears to reduce all the somatic functions in a temperature-dependent fashion (MARSHALL and WHITTLE 1978; CLINE 1984). Interestingly, the temperature-sensitive period of the Sxl\(^{16}/\) Sxl\(^{2593}\) combination occurs early in development (CLINE 1985). Sxl\(^{16}\) seems to affect the early Sxl functions (MAINE et al. 1985b; CLINE 1985). Sxl\(^{2593}\) may also specifically affect these Sxl functions, and its effect on late Sxl functions may be a consequence of the alteration of early functions needed for Sxl stable activation (SALZ et al. 1989). We carried out a clonal analysis of Sxl\(^{2593}\) to test if this mutation affects the late Sxl functions. We irradiated y Sxl\(^{2593}\) sn\(^{1}\)/f\(^{96}\) females at 24-48 hr of development. We focussed the analysis on the forelegs considering only the yellow-singed/forked twin clones. The yellow-singed partner is homozygous for Sxl\(^{2593}\). We found a total of 22 y sn\(^{1}\)/f\(^{96}\) twin clones. The y sn\(^{1}\) partner always developed sex combs. These were formed by pure male teeth, or a mixture of pure male and true intersexual teeth. These results demonstrate that Sxl\(^{2593}\) directly affects the late Sxl functions required continuously during development of the female cells.

In summary, mutations at the bc complementation group do not affect the Sxl-feminizing functions, contrary to mutations at the LS complementation group.

**Effect of the Sxl mutant alleles on female fertility and their complementation pattern:** The Sxl\(^{16}\) mutation autonomously affects the development of the germline, while Sxl\(^{m761}\) and Sxl\(^{m136}\) do not (CLINE 1983, 1984; SCHÜPBACH 1985; STEINMANN-ZWICKY, SCHMID and NÖTHIGER 1989; NÖTHIGER et al. 1989). We carried out the clonal analysis of Sxl\(^{LS}\), Sxl\(^{16}\), and Sxl\(^{2593}\) in the germline. The results are shown in Table 3. Germ cells homozygous for Sxl\(^{16}\) or Sxl\(^{16}\) do not develop into oocytes, while germ cells homozygous for Sxl\(^{2593}\) give rise to functional eggs. Table 3 also presents the data on the clonal analysis of Sxl\(^{16}\) and Sxl\(^{16}\) in the germline. It could be argued that the Sxl\(^{16}\) or Sxl\(^{16}\) homozygous females that survived were escapers that expressed high levels of Sxl\(^{+}\) function and consequently were fertile. Germ cells homozygous for Sxl\(^{16}\) or Sxl\(^{16}\), however, are able to produce functional eggs.

The fertility test was carried out with those females which did not present a sexual transformation big enough to disregard the possibility of its fertility. All the viable combinations of Sxl\(^{16}\) or Sxl\(^{16}\) with the other Sxl alleles are fertile, except with Sxl\(^{m761}\). Sxl\(^{m136}\) has a similar behavior but in combination with Sxl\(^{m761}\) produces some females which are fertile and others which are sterile. Table 4 shows the analysis of the gonads and the internal derivatives from the genital disc of sterile females, as well as transformed ones, carrying different Sxl mutations. The first three allelic combinations have testis, though atrophied, showing undifferentiated cells unable to produce sperm. These aberrations are not due to the absence of the Y chromosome, because XO males show normal testis that produce nonmotile elongated sperm. There are cases in which the yellow sheath typical for testis does not cover the testis completely, but just the apical region of them. The internal genital structures are male-like, which are indistinguishable from the normal male ones. Some Sxl\(^{LS}/\)Sxl\(^{2593}\) females lack gonads, and others show female-like genital structures.

The Sxl\(^{16}/\)Sxl\(^{m761}\) and the Sxl\(^{16}/\)Sxl\(^{m761}\) females lack gonads. The Sxl\(^{m136}/\)Sxl\(^{m761}\) females may present two, or no ovaries in a 18:3:13 rate. The fertile females always contain two ovaries, while the sterile females contain one or two ovaries which look normal, but oviposition never takes place, even though these fe-

### Table 3

<table>
<thead>
<tr>
<th>Genotype of homozygous clones</th>
<th>No. of females tested</th>
<th>No. of females with homozygous clones</th>
</tr>
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<tbody>
<tr>
<td>Sxl(^{16})</td>
<td>1126</td>
<td>0</td>
</tr>
<tr>
<td>Sxl(^{16})^{LS}</td>
<td>1309</td>
<td>0</td>
</tr>
<tr>
<td>Sxl(^{m761})</td>
<td>849</td>
<td>22</td>
</tr>
<tr>
<td>Sxl(^{16})</td>
<td>728</td>
<td>6</td>
</tr>
<tr>
<td>Sxl(^{m136})</td>
<td>530</td>
<td>5</td>
</tr>
</tbody>
</table>

The genotypes of the irradiated females were: y Sxl\(^{m136}/\)ovo\(^{y6}\)^{v}, y Sxl\(^{m136}/\)ovo\(^{y6}\)^{v} Sxl\(^{2593}\) c\(^{m}\)\(^{m}\)/ovo\(^{y6}\)^{v}, y Sxl\(^{m136}/\)ovo\(^{y6}\)^{v} and y Sxl\(^{m136}/\)ovo\(^{y6}\)^{v}. The different frequency of females with homozygous clones for Sxl\(^{16}\) or Sxl\(^{16}\) relative to Sxl\(^{m761}\) is presumably due to the map position of the markers used for the clonal analysis (see MATERIALS AND METHODS), and to the different irradiation conditions used for the clonal analysis of Sxl\(^{16}\) and Sxl\(^{16}\), as a consequence of using a different X-ray machine. The genotypes of the irradiated females were: y Sxl\(^{m136}/\)ovo\(^{y6}\)^{v}, y Sxl\(^{m136}/\)ovo\(^{y6}\)^{v} Sxl\(^{2593}\) c\(^{m}\)\(^{m}\)/ovo\(^{y6}\)^{v}, y Sxl\(^{m136}/\)ovo\(^{y6}\)^{v} and y Sxl\(^{m136}/\)ovo\(^{y6}\)^{v}. The different frequency of females with homozygous clones for Sxl\(^{16}\) or Sxl\(^{16}\) relative to Sxl\(^{m761}\) is presumably due to the map position of the markers used for the clonal analysis (see MATERIALS AND METHODS), and to the different irradiation conditions used for the clonal analysis of Sxl\(^{16}\) and Sxl\(^{16}\), as a consequence of using a different X-ray machine.

### Table 4

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SxIM1fm3 stores full viability of experimental females (data not shown). The strong group is formed by the complementation group and the weakest group, according to the strength of their female-specific lethal interaction. The strong group includes the scute mutant alleles and mutations at $Sxlf^z$. In all cases the replacement of the $Sxlf^z$ copy by the constitutive $Sxlf^{M1}$ allele restores full viability of experimental females (data not shown).

Table 5 shows the results of the interaction between the $Sxlf$ mutant alleles and a reduced level of maternal $da$ product. Females heterozygous for any of the tested $Sxlf$ alleles, except $Sxlf^{L5}$, exhibit a reduced viability. These alleles are the same that interact with an altered $X:A$ ratio signal.

**Molecular mapping of $Sxlf$ mutations**: Southern blot analysis of genomic DNA from $Sxlf^{L6}$ and $Sxlf^{R}$ males digested with different restriction enzymes and probed with $\lambda S2A$ (see Figure 4) displayed no detectable differences in restriction fragment pattern, when compared with wild-type strains Oregon-R (OR-R) and Canton-S (CS), and with yellow $^{m}$ (y$^{+}$) (an isogenic strain for the X chromosome that was used to induce the $Sxlf^{P}$ and $Sxlf^{R}$ mutations) (data not shown). In contrast, genomic DNA samples from the mutant strains have gross alterations in their restriction fragment pattern when probed with $\lambda S1$.

Figure 5A shows that the restriction pattern of OR-R (lane a) and of y$^{+}$ (lane e) DNA digested with PstI and hybridized with $\lambda S1$ is in agreement with the restriction map of MAINE et al. (1985a), except an extra band of more than 14 kb (marked with a point) that does not fit with that map and that is also present in flies deficient for $Sxlf$ (our unpublished data). In CS (lane b) the 9.5-kb band is replaced by one of more than 19 kb, due to the presence of the B104 transposable element in CS (at position −15) (MAINE et al. 1985a and Figure 5A) with at least a PstI restriction size. In the $Sxlf^{P}$ (lane d) and $Sxlf^{R}$ (lane c) mutants, the 9.5-kb PstI fragment is missing, whereas two new fragments appear (marked with an arrowhead) one of 2.0 kb and one of about 13 kb that partially overlaps with the 14-kb band. This modification is consistent with a DNA insertion of at least 5 kb, with a PstI restriction site, located to the right of the PstI site at coordinate −9. The digestion with XhoI and hybridization with $\lambda S1$ (Figure 5B) allows location of the insertion within the 1.8-kb XhoI-PstI fragment at coordinates −10.8 and −9, respectively, since the 4.8 XhoI-XhoI fragment (−10.8, −6) is missing in the mutants. To define better the molecular lesion of $Sxlf^{P}$ and $Sxlf^{R}$ we have used this 4.8-kb XhoI-XhoI fragment (plasmid S1-p15, see MATERIALS AND METHODS) as a probe. OR-R (lane a) and y$^{+}$ (lane e) DNA digested with PstI and hybridized with S1-p15 (Figure 5C) display the expected fragments of 5.9 and 9.5 kb, while CS (lane b) displays the 5.9- and 10-kb fragments. In the case of $Sxlf^{P}$ (lane d) and $Sxlf^{R}$ (lane c) we found the expected 5.9-kb fragment, and a new fragment of 2.0 kb, that appeared when $\lambda S1$ was used as probe, while the other new fragment of 13 kb is not detectable. This is explained if the 5-kb foreign DNA fragment is inserted very close to the XhoI restriction site at coordinate −10.8 and the PstI restriction site of the insertion map next to its left end.

### Table 4

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of flies</th>
<th>Gonads</th>
<th>Internal derivatives from the genital disc</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Sxlf^{2953}$</td>
<td>10</td>
<td>0</td>
<td>20 0</td>
</tr>
<tr>
<td>$Sxlf^{194}$</td>
<td>6</td>
<td>0</td>
<td>12 0</td>
</tr>
<tr>
<td>$Sxlf^{2593}$</td>
<td>6</td>
<td>0</td>
<td>6 6</td>
</tr>
<tr>
<td>$Sxlf^{15}$</td>
<td>74</td>
<td>90</td>
<td>0 58</td>
</tr>
<tr>
<td>$Sxlf^{176}$</td>
<td>12</td>
<td>2</td>
<td>0 22</td>
</tr>
<tr>
<td>$Sxlf^{1}$</td>
<td>13</td>
<td>0</td>
<td>0 26</td>
</tr>
</tbody>
</table>

* Both types of tissues coexist in the same fly.
* One among the 90 flies contained some male gonadal tissue.
* Both females contained some male gonadal tissue.

males are fertilized. These three allelic combinations contain female-like genital structures.

In summary, $Sxlf^{P}$, $Sxlf^{R}$ and $Sxlf^{2953}$ do not severely affect the $Sxlf$ functions required in the germline to follow the oogenic pathway, while $Sxlf^{L5}$ and $Sxlf^{4d}$ do so.

**Female-specific lethal synergistic interaction between $Sxlf$ mutant alleles and mutations at sisterless-a, scute or daughterless genes**: As mentioned above, the state of activity of $Sxlf$ is defined by the $X:A$ signal and the maternal $da$ product. As a characterization test for the $Sxlf$ mutant alleles we have analyzed the response of these alleles to alterations in either the $X:A$ signal or in the $da$ gene.

Figure 3 shows the results of the interaction between the $Sxlf$ mutant alleles and a deficiency for sisterless-a ($sis-a$), the $Df(1)N71$, or a deficiency for scute ($scu$), the $Df(1)svr$. As mentioned in the Introduction, both $sis-a$ and $scu$ genes behave as numerator elements of the $X:A$ signal. In general, both deficiencies present a similar specificity of interaction with the different $Sxlf$ alleles. Among these we can basically distinguish two groups, according to the strength of their female-specific lethal interaction. The strong group is formed by the $bc$ complementation group, together with the $Sxlf^{2953}$ allele and the strongest alleles $Sxlf^{1}$ and $Sxlf^{4d}$. The weak group is formed by the $L5$ complementation group and the $Sxlf^{2953}$, $Sxlf^{176}$, $Sxlf^{2953}$ and $Sxlf^{15}$ alleles. In all cases the replacement of the $Sxlf^{*}$ copy by the constitutive $Sxlf^{M1}$ allele restores full viability of experimental females (data not shown).

### Table 4

Analysis of the gonads and the internal derivatives from the genital disc of females carrying different $Sxlf$ mutations

<table>
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<td>10</td>
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<td>$Sxlf^{176}$</td>
<td>12</td>
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<tr>
<td>$Sxlf^{1}$</td>
<td>13</td>
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</table>

* Both types of tissues coexist in the same fly.
* One among the 90 flies contained some male gonadal tissue.
* Both females contained some male gonadal tissue.
Relative viability (%) of females double heterozygous for Sxl mutant alleles and Df(1)svr or Df(1)N71

### FIGURE 3.
Lethal synergistic interaction between Sxl mutant alleles and mutations affecting the X:A ratio signal. For crosses see MATERIALS AND METHODS.

### TABLE 5
Analysis of the female-specific lethal synergistic interaction between the maternal product of daughterless and the Sxl mutant alleles

<table>
<thead>
<tr>
<th>Sxl mutant allele tested</th>
<th>No. of male flies used for viability reference</th>
<th>Relative viability (%) of Sxl⁺/Sxl⁺ females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sxl⁺⁺</td>
<td>195</td>
<td>55</td>
</tr>
<tr>
<td>Sxl⁺⁺⁺</td>
<td>317</td>
<td>73</td>
</tr>
<tr>
<td>Sxl⁺⁺⁻</td>
<td>261</td>
<td>55</td>
</tr>
<tr>
<td>Sxl⁺⁺⁻⁻</td>
<td>521</td>
<td>36</td>
</tr>
<tr>
<td>Sxl⁺⁺⁻⁻⁻</td>
<td>469</td>
<td>65</td>
</tr>
<tr>
<td>Sxl⁺⁺⁻⁻⁻⁻</td>
<td>394</td>
<td>111</td>
</tr>
</tbody>
</table>

The experiment was performed at 29°C because of the temperature-dependence for the lethal effects involving the interaction between the maternal da product and Sxl mutations (CLINE 1980).

The digestion with XhoI and hybridization with S1-p15 (Figure 5D) show that in Sxl⁺⁺ (lane d) and Sxl⁺⁺⁻⁻ (lane c) the 4.8-kb fragment is replaced by one of 9.5 kb, which overlaps with the 9.2-kb fragment and was hardly detectable in Figure 5B. These results and those obtained by a double digestion with PstI and XhoI followed by hybridization with the S1-p15 subclone (Figure 5E) are consistent with the insertion of a foreign DNA fragment of around 5 kb inserted near the XhoI restriction site at position -10.8 in the Sxl⁺⁺ and Sxl⁺⁺⁻⁻ mutants (see Figure 4). Although Sxl⁺⁺ and Sxl⁺⁺⁻⁻ arose in different mutations, both appear to contain a similar insertion.

With respect to Sxl⁻⁻, no detectable alterations in the restriction fragment pattern were observed, either with λS1 or with λS2A. Thus, Sxl⁻⁻ seems to be a "point" mutation.

### Analysis of late Sxl transcripts in mutants for this gene
We have analyzed by Northern blots the late Sxl transcripts from female-specific lethal mutations at the gene Sxl. Two Sxl probes have been used: the h¹ probe, that contains some of the exons common to both sexes, and the g² probe, that contains the male-specific exon (BELL et al. 1988) (see Figure 4).

Figure 6A shows that, when the h¹ probe was used, the Sxl⁺⁺⁻⁻ female larvae (lane 3) present only the three characteristic female Sxl transcripts of 4.2, 3.3 and 1.9 kb, while the Sxl⁺⁺⁻⁻ male larvae (lane 4) present the three Sxl transcripts of 4.4, 3.6 and 2.0 kb typical of males. In lanes 1 and 2 are shown the transcripts from female and male OR-R larvae, respectively. We must stress that the Sxl⁺⁺⁻⁻ female larvae present exclusively the female Sxl transcripts, as confirmed when the g²-probe was used (Figure 6B). Male Sxl transcripts are only present in the OR-R (lane 4) and the Sxl⁺⁺⁻⁻ (lane 2) male larvae. With respect to the Sxl⁺⁺⁻⁻ female and male larvae, they express the Sxl transcripts characteristic of males (Figure 7A, hybridization with the h¹ probe, and Figure 7B hybridization with the g² probe).

On the other hand, the Sxl⁺⁺⁻⁻ female larvae express the typical female Sxl transcripts and none of the male transcripts (Figure 8A, hybridization with the h¹ probe, and Figure 8B hybridization with the g² probe). These results agree with the complementation behavior shown by these mutations.

The Sxl⁺⁺⁻⁻ homozygous females die very soon in development. For this reason, to determine the type of Sxl transcripts generated by that mutant allele we have analyzed the transcripts expressed by the Sxl⁺⁺⁻⁻/ Sxl⁺⁺⁻⁻ female larvae, using as control female larvae homozygous for Sxl⁺⁺⁻⁻. This mutation is viable in females (CLINE 1980; this report), while in combination with Sxl⁺⁺⁻⁻ only 30% of the females survive and the rest die as larvae (Table 1). The Sxl⁺⁺⁻⁻ female and male larvae present the female and male Sxl transcripts, respectively (Figure 9A hybridization with the h¹ probe, and Figure 9B hybridization with the g² probe). However, the Sxl⁺⁺⁻⁻/ Sxl⁺⁺⁻⁻ female larvae express both female and male Sxl transcripts (Figure 9A hybridization with the h¹ probe, and Figure 9B hy-
Sxl Mutant Alleles

**DISCUSSION**

The complementation analysis between different Sxl mutant alleles shows that the new alleles Sxl^{10} and Sxl^{15} belong to a different complementation group from most other previously described Sxl alleles: they define together with Sxl^{10} the bc group of alleles, whereas the Sxl^{15} allele defines the LS group. The rest of the Sxl mutant alleles tested in this report, including the new allele Sxl^{10}, affect both complementation groups albeit with different degrees. Sxl^{10} and Sxl^{15} affect more the LS group than the bc group. On the contrary, Sxl^{10} and Sxl^{15} affect more the bc group than the LS group. Other Sxl alleles have been identified that specifically affect the develop-

**FIGURE 4.**—DNA rearrangements associated with Sxl^{10} and Sxl^{15} mutations. We followed the restriction map of MAINE et al. (1985). Restriction sites are designated as follows: R, EcoRI; X, XhoI; H, HindIII; S, SalI; P, PstI. λS1, λS2A and S1-p15 were the probes used for Southern blots. Schematic representation of the male and female Sxl cDNAs and the two probes, g^2 and h^1, used for Northern blots (BELL et al. 1988).

**FIGURE 5.**—Genomic Southern blots from Sxl^{10} and Sxl^{15} mutant flies. Lane a, OR-R; lane b, CS; lane c, Sxl^{10}; lane d, Sxl^{15} and lane e, y^+.


The g^2 probe. (We suggest that the male Sxl transcripts come from the Sxl^{10} mutant allele.)
FIGURE 6.—Late SxI transcripts in female larvae mutant for SxIfl.
To generate the larvae we crossed y cm SxIfl homozygous females with males of the same genotype. The ratio of female to male larvae is 0.76 and that of female to male adults is 0.30. Lane 1, wild-type females; lane 2, wild-type males; lane 3, SxIfl homozygous females; and lane 4, SxIfl males. A, filter probed with h'; B, filter probed with g'. C, hybridization of filter 6B with the actin probe DM-AS. The distortion in the migration of the lower molecular weight RNAs is due to the presence of rRNA in the RNA preparation.

FIGURE 7.—Late SxI transcripts in female larvae mutant for SxIfl. To generate the larvae we crossed y w SxIfl and v f"/FM6 females with y w SxIfl and v f"/FM6 males. The mutant larvae were recognized by the yellow phenotype of the mouthparts and the white phenotype of the Malpighian tubules. Female and male larvae were separated by the different size of their gonads. Lane 1, SxIfl homozygous females; lane 2, SxIfl males; lane 3, wild-type females, and lane 4, wild-type males. A, filter probed with h'; B, filter probed with g'; C, hybridization of filter 7B with the actin probe DM-AS.

*men* of the female germline (Perrimon et al. 1986; Salz et al. 1987).

The SxIfl mutation results from the insertion of the transposable element gypsy between positions 0 and -2 on the molecular map, within the first intron (Maine et al. 1985). The bc mutations result from the insertion of foreign DNA between positions -10 and -11 on the molecular map, within the fourth intron (this report). We believe that the two complementation groups are related to the two sets of early and late SxI transcripts, which are responsible for the early and late SxI functions, respectively.

The analysis of the late SxI transcripts in female larvae homozygous for SxIfl shows that these females express only the SxI transcripts typical of females. Female larvae homozygous for SxIfl, on the contrary,
express the Sxl transcripts characteristic of males and none of the females. This would explain why Sxl^{b} and Sxl^{e} do not affect the processes of somatic sex determination, late dosage compensation and oogenesis (this report), while Sxl^{LS} does (SÁNCHEZ and NÖTHERG 1982; MAINE et al. 1985b; CLINE 1986; this report). Moreover, the Sxl^{b}/Sxl^{LS} female larvae express exclusively the female Sxl transcripts, in accordance with the complementation behavior of these mutations: in these females, the Sxl^{b} allele would provide the late Sxl functions, while the Sxl^{LS} allele would provide the early Sxl functions. The fact that Sxl^{b}/Sxl^{LS} females express only the late female Sxl transcripts indicates that the primary Sxl^{LS} transcripts are capable of following the female-specific splicing in the presence of normal late Sxl^{+} products from the Sxl^{b} allele. These female Sxl^{LS} transcripts might contain a presumably nondetectable alteration by Northern blots, so that they would code for nonfunctional Sxl products; since clones homozygous for Sxl^{LS}, induced during development of Sxl^{LS}/+ larvae, develop male structures instead of female ones (SÁNCHEZ and NÖTHERG 1982).

There is evidence supporting the sex specificity of the early Sxl transcripts (D. COULTER, cited in SALZ et al. 1989; TORRES and SÁNCHEZ 1991). The gene Sxl seems to contain a sex-specific and a non-sex-specific promoter. The sex-specific promoter would function in females early in development producing the early Sxl transcripts, as a consequence of the response of the gene Sxl to the X:A ratio signal. The non-sex-specific promoter functions later and throughout development of both females and males giving rise to the primary Sxl RNA, that subsequently will be differently spliced in females and males. The early promoter seems to be downstream of the late promoter and located upstream of the male-specific exon (SALZ et al. 1989). Then, the production of the early Sxl transcripts would be regulated by transcriptional control and subsequent splicing to eliminate the male-specific exon (SALZ et al. 1989). The Sxl^{b} and Sxl^{e} mutations may affect the early Sxl transcripts by interfering with their transcriptional control and/or their splicing. This would explain the female-lethal synergistic interaction between these mutations and the maternal da product, and mutations at either sis-a or sc (Table 5 and Figure 3), which impair early Sxl expression.

In this context, the comparison of the Sxl^{b} and Sxl^{e} mutations relative to the Sxl^{M} mutations become relevant. The molecular analysis of the Sxl^{M} mutations has revealed the existence of the B104 transposable element inserted around the PstI restriction site at coordinate −9 (MAINE et al. 1985a), next to the region where the insertion in the Sxl^{b} and Sxl^{e} mutants is located (see Figure 4). However, these mutations present an opposite phenotype. The gain-of-function Sxl^{M} mutations express constitutively the female Sxl functions involved in sex determination and dosage compensation, thus causing their dominant male-specific lethal phenotype (CLINE 1978). A possibility is that constitutive Sxl expression in these Sxl^{M} mutants is a consequence of cis-activation of Sxl by promoter and/or enhancer sequences contained in the B104 transposable element (BELL et al. 1988). In this respect, it is worth mentioning that the B104 element is specifically transcribed at the beginning of development (SCHERER et al. 1982). On the contrary, the Sxl^{b} and Sxl^{e} mutations are characterized by their recessive female-specific lethal phenotype, similar to the loss-of-function Sxl alleles. Although the nature of the 5-kb insertion of these mutants remains unknown, it may be possible that this insertion disrupts sequences involved in the early activation of Sxl. We cannot discard, however, the possibility that these mutations affect the correct splicing of the early Sxl RNAs, thus reducing the amount of the correct Sxl products.

SALZ et al. (1989) suggested that the early transcripts may be involved in directing the first female-specific splicing of the late transcript. Besides, the early transcripts have to provide the vital Sxl-dependent function already operating early in development, such as dosage compensation of the genes expressed at the blastoderm stage (CLINE 1984; GERGEN 1987). Since be mutant female larvae express the late Sxl transcript characteristics of females (Figure 6), we propose that these mutations do not prevent the first female-specific splicing of the late Sxl transcripts by the early Sxl products, but they affect some early female Sxl vital function. This function could be responsible for the dosage compensation process required early in development and different from the late dosage compensation process carried out by the msl genes (BELOTE and LUCCHESE 1980; CLINE 1984), or it could be a still unknown process.

One possibility is that different early Sxl products are responsible for the different early Sxl functions: the Sxl^{b} and Sxl^{e} mutations would affect the early products involved in the early female Sxl vital function, but would not affect the products involved in directing the first female-specific splicing of the late Sxl transcripts. Alternatively, in case that both early Sxl functions are carried out by common products, the Sxl^{b} and Sxl^{e} mutants may produce a low amount of early Sxl transcripts, sufficient to direct the first female-specific splicing of the late transcripts, but insufficient for the early female vital function of Sxl. The analysis of the early Sxl transcripts in the Sxl^{LS} homozygous females will help us to understand the relationship between the early Sxl transcripts and the early Sxl functions.

The Sxl alleles affecting the late Sxl functions form
a single complementation group for both sex determination and dosage compensation. However, no correlation exists between the effect of the different Sxl alleles (by themselves or in allelic combination) on these processes. Also no correlation is found between the effects of Sxl mutations in the germline and soma development. This lack of correlation suggests that the different late Sxl functions might be carried out by the different late Sxl products, or combinations of them.

Sxl-dependent somatic function for development of the germline: Females of genotype Sxl<sup>+/Sxl<sup>fl<sub>M7</sub></sup></sup> or Sxl<sup>+/Sxl<sup>fl<sub>M7</sub></sup></sup> lack gonads. We have observed, however, germ cells at the blastoderm stage and in the larval ovaries of these females (data not shown). Thus, the absence of gonads in these females cannot be attributed to the lack of germ cells, as it happens in Cornision Interministerial de Ciencia y Tecnologia (CICYT). B. G. for their comments on the manuscript, and to M. C. PARTEEARROYO assistance, and to M. CORDERO for her help with the statistical

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