Functional Changes Associated with Structural Alterations Induced by Mobilization of a P Element Inserted in the Sex-lethal Gene of Drosophila

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Manuscript received February 20, 1987
Accepted May 4, 1987

ABSTRACT

Genetic analysis of rearrangements within the multifunctional sex determining gene Sex-lethal has allowed correlation of changes in specific functions with DNA alterations. Rearrangements were isolated by mobilization of a P element which is on the 5' side of the gene, at coordinate 0. Previous work has shown that rearrangements associated with alterations in Sxl gene function are found within an 11-kb region between coordinates -11 and 0. Here it is shown that insertion of foreign DNA, per se, at coordinate 0 is compatible with wild-type gene function. However, deletion of sequences on either side of this point generates a mutant phenotype. Deletions extending distally beyond coordinate -6.5 kb result in a null phenotype, whereas smaller distal deletions or proximal deletions eliminate only some Sxl functions.

Sxl (Sxl, 1-19.2) appears to be a binary switch gene that both determines and maintains the decisions leading to sexual dimorphism (reviewed in Baker and Belote 1983; Lucchesi 1983; Cline 1985; Notthiger and Steinmann-Zwicky 1985). In females, Sxl is required throughout development for the proper levels of X chromosome dosage compensation, somatic sexual differentiation and germline development (Lucchesi and Skripsky 1981; Sanchez and Notthiger 1982; Cline 1983, 1984; Schupbach 1985). Females homozygous for null mutations in the gene die as embryos. In contrast, males do not require Sxl: males hemizygous for these null alleles, or indeed for deletions of the gene, are both viable and fertile (Maine et al. 1985a, b).

Complementation and clonal analysis have shown that Sxl is a complex locus and have suggested that this gene encodes multiple functions (Cline 1985; Maine et al. 1985b). Whereas some mutations eliminate all of the known female-specific functions, others affect only specific functions. For instance, several alleles specifically interfere with germ line development without affecting the somatic functions required for either dosage compensation or sex differentiation (Perrimon et al. 1986; T. W. Cline, unpublished data). The somatic functions themselves can be further subdivided into early functions required to initiate the commitment to a sexual pathway and later functions required to maintain and express that pathway decision (Cline 1984, 1985, 1986; Maine et al. 1985b).

Molecular studies have shown that DNA rearrangements that are associated with an Sxl mutant phenotype are localized to an 11-kb region between coordinates 0 and -11 (Figure 1) (Maine et al. 1985a). A preliminary description of the transcription pattern within this region showed it to be quite complex and to include overlapping sex- and non-sex-specific transcripts (Maine et al. 1985b). We now know that probes centromere distal to coordinate 0 detect at least nine overlapping transcripts which are all transcribed in the proximal to distal direction. Four of these transcripts are female-specific, three are male-specific and two are embryo-specific. In contrast, the region proximal to coordinate 0 contains four transcripts which are present in both sexes (E. M. Maine and H. K. Salz unpublished data).

Although it is conceivable that Sxl's multiple functions are carried out by a single gene product, the fact that Sxl has multiple overlapping transcripts suggests otherwise. As a first step in correlating function with specific transcripts within this gene, we have begun a study to determine whether any of the functions can be localized within the gene. By analyzing a series of rearrangements, we have been able to correlate structural alterations with changes in specific Sxl functions.

Using an approach similar to TsuBota and Schedl (1986), we isolated rearrangements within Sxl by mobilization of a P element already located within the gene. Once mobilized by hybrid dysgenesis, P elements can excise imprecisely generating deletions, inversions or insertions (Voelker et al. 1984; TsuBota and Schedl 1986). The P element used in this study, Sxl<sup>Pp</sup>, is the most 5' DNA rearrangement associated with a mutant phenotype (Maine et al. 1985a; E. M. Maine and H. K. Salz, unpublished data). Sxl<sup>Pp</sup> is a partial loss-of-function allele believed to be mutant in the sexual pathway maintenance function (Maine et al. 1985b).
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\[ \text{In(1)Sxl}^{af} \]

\[ \text{Sxl}^{fPb} \]

\[ \text{centromere proximal} \]

\[ 1 \text{ kb} \]

\[ \text{S1} \]

\[ \text{RX5} \]

\[ \text{RS93} \]

\[ \text{H5X/X} \]

\[ \text{RS95} \]

\[ 3.7 \]

**Figure 1.**—Restriction map of the wild-type DNA segment which includes Sxl. Shown are the site of the parental P element insertion, Sxl\(^{PP}\), the site of the breakpoint of In(1)Sxlpb\(^{af}\) and the site of the gypsy insertion associated with Sxl\(^{EB}\). The coordinate system used is that in MAINE et al. (1985a): one unit equals 1 kilobase. Also indicated are the different probes used in this study. Restriction sites are designated as follows: R, EcoR1; X, XhoI; H, HindIII; S, SalI; P, PstI; A, AvaII.

(MAINE et al. 1985b). The complex complementation behavior of this allele (T. W. CLINE, unpublished data and this report) has allowed us to design screens for dysgenic derivatives of Sxl\(^{PP}\) that are associated with either an increase or a decrease in Sxl activity. An additional screen was undertaken to isolate derivatives without bias toward Sxl function.

**MATERIALS AND METHODS**

All crosses were carried out at 25° on a standard cornmeal, dextrose, yeast medium (CLINE 1978). Genes not listed in LINDSLEY and GRELL (1968) are referenced in the text. In situ hybridizations to polytene chromosomes and genomic Southern blot analyses were performed as described by MAINE et al. (1985a).

**RESULTS**

**Structural alterations compatible with full Sxl function**

The scheme used to isolate Sxl\(^{PP}\) derivatives with an increase in the female-specific functions is illustrated in Figure 2. The partial loss-of-function allele Sxl\(^{PP} \text{Sxlpb}^{af}\) (CLINE 1984) only partially complemented Sxl\(^{PP}\). These diplo-X animals were sexually transformed and exhibited reduced viability: only 283 Sxl\(^{PP} \text{Sxlpb}^{af}\) animals were recovered in a cross that generated 1073 Sxl\(^{+}\) sisters. Thus we screened for dysgenic derivatives of Sxl\(^{PP}\) that fully associated with Sxlpb. Of 5759 derivative chromosomes screened, 386 complemented Sxl\(^{PP} \text{Sxlpb}^{af}\) (84 of these were known to be derived from independent events). Stocks were established from 13 independently derived chromosomes. Each new derivative fully complemented a deletion for Sxl, and is, therefore, functionally wild type (data not shown).

In order to determine what type of DNA rearrangements are compatible with wild-type gene function, we examined DNA from each of these lines by whole genome Southern blot analysis. Three of the revertants (Sxl\(^{PP} \text{Sxlpb}^{af}\), Sxl\(^{PP} \text{Sxlpb}^{af}\) and Sxl\(^{PP} \text{Sxlpb}^{af}\)), were associated with a precise or near precise excision of the parental P element at coordinate 0. The restriction pattern generated in digests of Sxl\(^{PP} \text{Sxlpb}^{af}\) DNA is indistinguishable from wild type, suggesting that the parental P element excised precisely (Figure 3). Sxl\(^{PP} \text{Sxlpb}^{af}\) and Sxl\(^{PP} \text{Sxlpb}^{af}\) have also lost the parental P element; however, in addition both of these revertants are associated with complex rearrangements.

The restriction pattern of Sxl\(^{PP} \text{Sxlpb}^{af}\) DNA is a composite of that for wild-type and Sxl\(^{PP}\) DNA (Figure 3). It is likely, therefore, that Sxl\(^{PP} \text{Sxlpb}^{af}\) is a duplication of Sxl: one copy still retains the P element insertion, while the other has lost it. The duplication, which extends beyond the cloned region, is not cytologically visible.

Sxl\(^{PP} \text{Sxlpb}^{af}\) is an inversion between polytene bands 5A and 6F (the cytogenetic location of Sxl). Although a single rearrangement breakpoint at coordinate +3.2 was visible by Southern blot analysis, in situ hybridization to polytene chromosomes showed that the inversion is associated with an additional rearrangement. As expected for an inversion, probes from sequences distal to coordinate +3.5 hybridized only to the 5A breakpoint and a probe spanning the inversion breakpoint (probe 3.7) hybridized to both the 5A and the 6F breakpoints. However, probes from sequences proximal to the inversion breakpoint also hybridized to both the 5A and the 6F breakpoints, demonstrating that the region between coordinates +3.5 and +16.5 is duplicated at the 5A breakpoint.
The proximal limit of the duplicated region is beyond the cloned region.

The remaining ten revertants still retain P element sequences at the site of the parental insertion (Figure 4). Seven of the insertions are smaller than the parental P element, ranging in size from ~0.1 kb to ~0.6 kb. These revertants appear to have arisen by deletion of a portion of the parental P element. In four of the seven, the deletions are entirely within the P element: both terminal repeats appear to be intact at least as proximal to coordinate 0 as judged by the presence of the AvaII sites in the 31-bp repeats. The remaining three revertants (Sxl

Two of the revertants (Sxl*

Contrary to our expectations, all three proximal deletions failed to complement the null, demonstrating that the sequences proximal to coordinate 0 are required for wild-type Sxl gene activity (Table 1). However, as described below, these deletions cause only partial loss of function.

Isolation of distal deletions: Additional rearrangements associated with a Sxl mutant phenotype were selected using the scheme illustrated in Figure 5 (crosses 1 through 4A). Since Sxl

Within the transposon that is not visible by whole genome analysis, or that this stock now contains a closely linked suppressor of Sxl

Structural alterations associated with decreased Sxl function

Isolation of proximal deletions: The results of the previous section demonstrate that insertions at coordinate 0 are generally compatible with wild-type Sxl gene function. It is possible, therefore, that sequences proximal to coordinate 0 are not required for proper gene function. This possibility may be tested by isolating deletions which extend proximally from coordinate 0 and then assay ing them for Sxl gene function.

Proximal deletions were isolated by screening for dysgenic derivatives of Sxl

These derivatives were shown to be deletions of DNA sequences extending proximally from Sxl by both genomic Southern blots and in situ hybridization to polytene chromosomes. No hybridization was observed with probes proximal to coordinate +0.6 in a genomic Southern blot using DNA made from males carrying a duplication which covers l(1)jnR1 but not Sxl [Dp(1;3)1

Further analysis by genomic Southern blots localized the distal breakpoint in each derivative to the interval between coordinates −0.8 and +0.6 (Figure 6).

The proximal deletions were then tested for complementation with a Sxl null allele, Sxl

The remaining revertant, Sxl

The proximal deletions failed to complement the null, demonstrating that the sequences proximal to coordinate 0 are required for wild-type Sxl gene activity (Table 1). However, as described below, these deletions cause only partial loss of function.
among 7347 X chromosomes screened. As expected, none of these derivatives complemented Sxl\(^{+/+}\) (Table 1).

These nine derivatives were divided into two groups based on the size of the associated DNA rearrangements (Figure 6): class A consists of six deletions that extend at least 13 kb distal to coordinate 0; class B consists of three rearrangements that are localized between coordinates -6.5 and 0.

Class A: Southern analysis of Sxl\(^{FBO}\) reveals that the deletion breakpoints lie outside of the cloned region. The proximal breakpoints of the remaining five deletions lie between coordinates -1.0 and +3.0. The distal breakpoint of only one deletion, Sxl\(^{DC2}\), lies within the cloned region, at coordinate -13. Moreover, no P element sequences are evident at the deletion breakpoints of Sxl\(^{DG2}\), Sxl\(^{GC2}\) and Sxl\(^{DC2}\) (data not shown); whereas Sxl\(^{ED1}\) and Sxl\(^{RD2}\) still retain small
remnants (~0.1 kb) of the parental P element.

Class B: In contrast to class A derivatives, class B derivatives are associated with rearrangements confined to a region located between coordinates -6.5 and 0. Two of the derivatives, Sxl<sup>DV</sup> and Sxl<sup>DAI</sup>, are deletions. Sxl<sup>DV</sup> deletes sequences from coordinates 0 to coordinate -5.0. The parental P element, however, is still present at coordinate 0 (Figure 7). The deletion associated with Sxl<sup>DAI</sup> is slightly larger than that associated with Sxl<sup>DV</sup> and extends to coordinate -6.5. In addition, the P element sequences which remain at coordinate 0 are similar to the parental P element, except that the distal AvaII site is missing (data not shown). The third derivative, Sxl<sup>l(1)R1</sup>, is an inversion of Sxl DNA extending from coordinate 0 to coordinate -5.0 (Figure 7). The inversion breakpoints are flanked by P element insertions that are identical to the parental P element.

**Functional analysis of rearrangements associated with a Sxl mutant phenotype**

The nature of the functional defects associated with these new rearrangements was initially assayed by
TABLE 1
Relative viability of Sxl heteroallelic females

<table>
<thead>
<tr>
<th>Parental allele</th>
<th>Maternally contributed tester allele</th>
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<tbody>
<tr>
<td></td>
<td>Sxl&lt;sup&gt;IPb&lt;/sup&gt;</td>
</tr>
<tr>
<td>Class A derivatives</td>
<td></td>
</tr>
<tr>
<td>Sxl&lt;sup&gt;IPb&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0/510</td>
<td>380/378 (101%)</td>
</tr>
<tr>
<td>Class B derivatives</td>
<td></td>
</tr>
<tr>
<td>Sxl&lt;sup&gt;IPb&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0/329</td>
<td>0/609</td>
</tr>
<tr>
<td>Sxl&lt;sup&gt;IPb&lt;/sup&gt;</td>
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<tr>
<td>0/488</td>
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<tr>
<td>Sxl&lt;sup&gt;IPb&lt;/sup&gt;</td>
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<td>0/314</td>
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<td>0/262</td>
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Proximal deletions

<table>
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<th>Proximal deletions</th>
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</thead>
<tbody>
<tr>
<td>Sxl&lt;sup&gt;IPb&lt;/sup&gt;</td>
</tr>
<tr>
<td>0/229</td>
</tr>
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<td>Sxl&lt;sup&gt;IPb&lt;/sup&gt;</td>
</tr>
<tr>
<td>0/188</td>
</tr>
<tr>
<td>Sxl&lt;sup&gt;IPb&lt;/sup&gt;</td>
</tr>
<tr>
<td>0/212</td>
</tr>
</tbody>
</table>

Sexual phenotype of viable heteroallelic animals: * female, † intersex, ‡ female but sterile.

Relative viability is assessed by comparing the number of observed heteroallelic animals to the number of wild-type sisters recovered in the same cross. The percent viability is in parentheses. Genetic markers allow diplo-X animals to be distinguished from haplo-X animals irrespective of their sexual phenotype. The paternally derived chromosomes are marked with y cm, except for dx Sxl<sup>IPb</sup> and dx Sxl<sup>IPb</sup>. The maternally derived chromosomes are marked as follows:

- cm Sxl<sup>IPb</sup> ct, cm Sxl<sup>IPb</sup> v, y Sxl<sup>IPb</sup> ct and y Sxl<sup>IPb</sup> v or f. These chromosomes are balanced with Bimister except for Sxl<sup>IPb</sup> which is balanced with FM6. The lethality associated with the deletion of l(l)mR1 in the proximal deletions is covered by Dp(l;Y)ct+ y+ (Johnson and Judd 1979).

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**Figure 6.**—Schematics of DNA rearrangements associated with a Sxl mutant phenotype. P element sequences are indicated by the thick black bars. Deletions of specific sequences are indicated by slashed bars. The uncertainty in the location of the deletion breakpoint of Sxl<sup>IPb</sup> is indicated by a broken thin line. Restriction sites are designated as follows: R, EcoRI; X, XhoI; H, HindIII; S, Sall; P, PstI, A, AvaII.
intragenic complementation. We used four tester alleles, each of which affects Sxl’s multiple functions differently:

—Sxl’9 seems to be defective only in the early sexual pathway initiation steps (MAINE et al. 1985b; CLINE 1986).

—SxlLS appears to be defective only in the later functions required for proper maintenance and/or expression of the female-specific pathway commitment (SANCHEZ and NOTHIGER 1982; MAINE et al. 1985b; CLINE 1986).

—Sxl2593 appears to reduce all the somatic functions in a temperature dependent fashion (MARBLL and WHITTLE 1978; CLINE, 1984; T. W. CLINE, unpublished data).

—SxlR1 seem to be primarily defective in the germline function (PERRIMON et al. 1986; T. W. CLINE, unpublished data).

**Somatic functions:** The complementation pattern generated by the tester alleles that primarily affect the somatic functions (dosage compensation and sexual differentiation) is presented in Table 1. Since dosage compensation is a vital function, complementation between SxlPb or its derivatives and the tester alleles was assessed by measuring the relative viability of the heteroallelic combination. Relative viability was determined by calculating the number of observed heteroallelic animals divided by the number of expected animals (the number of Sxl+ sisters recovered in each cross).

Complementation between SxlPb and the tester alleles demonstrates that SxlPb retains some of the somatic functions. This is shown by the fact that SxlPb and SxlPb complemented fully. In contrast, complementation with the two other tester alleles, SxlR5 and Sxl2593, was not complete; SxlR5/SxlPb females had a relative viability of 43% and were sterile; SxlR5/SxlPb animals had a relative viability of 13% and were “true intersexes.” Bristle position and morphology in the sexually dimorphic region of the foreleg is a sensitive indicator of sexual phenotype. In a “true intersex” these bristles differentiate as neither female bristles nor male sex comb teeth; instead, they appear as morphological intermediates.

Both the class A and the class B derivatives (which were isolated based on their failure to complement Sxl’9) failed to complement all the somatic tester alleles. These results indicate that these deletion derivatives most probably eliminate all known somatic functions.

In contrast, the complementation behavior of the proximal deletions shows that they retain some somatic function. Full complementation between Sxl’9 and the proximal deletions was observed. These results suggest that the proximal deletions are wild type with respect to the early pathway initiation function of Sxl. However, these deletions are not wild type with respect to the later somatic functions as demonstrated by their lack of complementation with SxlLS and their partial complementation with Sxl2593. Only 11% of the expected SxlR52/Sxl2593 heteroallelic animals were recovered, all of which were sexually transformed. These animals, although extremely male like, were “true intersexes” as judged by the morphology of the bristles in the sex comb region of the foreleg. The other two proximal deletions, SxlR12 and SxlRc1 exhibited similar phenotypes to SxlR52.

To determine whether SxlR52 retains any feminizing differentiation function, we induced homozygous somatic clones of SxlR52 in a Sxl+ background by mitotic recombination (Table 2). Mutant bristles included in the sex comb region of the foreleg were phenotypically male. These bristles were, in all cases, identical to those bristles found in clones homozygous for a Sxl deletion (SxlPb). Thus, with respect to its

<p>| Genotype of | No. of | No. and sex of |
| homogygous | homogygous | clones |</p>
<table>
<thead>
<tr>
<th>clones</th>
<th>forelegs</th>
<th>clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>y SxIPb/SL2</td>
<td>159</td>
<td>4 Male</td>
</tr>
<tr>
<td>y SxIPb</td>
<td>164</td>
<td>8 Female</td>
</tr>
<tr>
<td>y SxIPb/h</td>
<td>164</td>
<td>3 Male</td>
</tr>
</tbody>
</table>

Larvae were exposed to ca. 1500 rad of gamma radiation at 48-72 hr after oviposition. The forelegs of adult females of the genotype y Sxl M(1) c1S3 SXL- M(1) w were mounted and scored under a compound microscope for the presence of phenotypically yellow clones (Sxl/Sxl). The minute, M(1)o, was employed in the cross to increase the size of the clones (MORATA and RIPOOL 1975).
ability to direct female differentiation, Sxl

Germline function: Sxl germline function was initially assayed by complementation with Sxl\textsuperscript{P1}, a homozygous viable but sterile allele of Sxl (PERRIMON et al. 1986; T. W. CLINE, unpublished data). These females produce abnormal egg chambers which are similar in phenotype to the cystic egg chambers observed in germ cells homozygous for a Sxl null allele (SCHUPBACH 1985). Thus, it appears that Sxl\textsuperscript{P1} is defective in a Sxl gene function which is essential for normal germline development. The parental allele appears to retain this function, as shown by its ability to complement Sxl\textsuperscript{P1} (Table 3).

The complementation between Sxl\textsuperscript{P1} and a representative sample of the deletion derivatives is presented in Table 3. Both the class B rearrangements (Sxl\textsuperscript{P1}, Sxl\textsuperscript{P2}, and Sxl\textsuperscript{P3}) and the three proximal deletions partially complemented Sxl\textsuperscript{P1}: Heteroallelic females were semiferile, producing an average of 3 to 14 daughters each. The ovaries of these females did not contain any abnormal oocytes; therefore, these rearrangements complement the defect in Sxl\textsuperscript{P1} associated with the cystic egg chamber phenotype. Although the cause of the reduced fertility is not known, it is clearly not due to gonadal dysgenesis since these derivatives were previously shown to have an M cytotype (data not shown).

In contrast, the class A deletions (Sxl\textsuperscript{P70} and Sxl\textsuperscript{P22}) did not complement Sxl\textsuperscript{P1}. Ovaries of these heteroallelic females contained only cystic egg chambers identical to those of Sxl\textsuperscript{P1}.

Since these experiments indicate that the class B derivatives and the proximal deletions retain some Sxl germline function, it was of interest to determine whether these derivatives are capable of directing normal female germline development. For this purpose we induced homozygous germline clones in an Sxl\textsuperscript{+} background by mitotic recombination (Table 4). Germline clones homozygous for an allele that is able to support germline development are identified by the production of eggs and progeny from an otherwise sterile female (see Table 4 for details). Germ cells homozygous for Sxl\textsuperscript{P1} developed normally. In contrast, no functional eggs derived from germ cells homozygous for the class A, B or proximal deletions were recovered. The absence of functional eggs derived from germ cells homozygous for the proximal deletions is not due to the absence of the vital gene l(1)jnr R1, because germ cells homozygous for a mutation in this gene differentiate normally. However, we cannot rule out the possibility that the proximal deletions lack an unidentified closely linked gene that is required for germline development.

### Table 3

<table>
<thead>
<tr>
<th>Sxl\textsuperscript{P1}</th>
<th>Average No. of daughters recovered per female</th>
<th>Phenotype of egg chambers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sxl\textsuperscript{P1}</td>
<td>0 (n = 14)</td>
<td>Cystic</td>
</tr>
<tr>
<td>Sxl\textsuperscript{P22}</td>
<td>26.0 ± 1.1 (n = 16)</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Class A derivatives:
- Sxl\textsuperscript{P1} 0 (n = 16) Cystic
- Sxl\textsuperscript{P22} 0 (n = 16) Cystic

Class B derivatives:
- Sxl\textsuperscript{P70} 3.0 ± 1.0 (n = 16) Normal
- Sxl\textsuperscript{P22} 4.1 ± 0.2 (n = 16) Normal
- Sxl\textsuperscript{P3} 7.1 ± 1.1 (n = 16) Normal

Proximal deletions:
- Sxl\textsuperscript{P1} 13.5 ± 1.4 (n = 14) Normal
- Sxl\textsuperscript{P2} 13.7 ± 1.9 (n = 16) Normal
- Sxl\textsuperscript{P22} 8.2 ± 1.2 (n = 16) Normal

The data are presented as the average number of female progeny ± one standard error of the mean) produced by a single heteroallelic female. The number of heteroallelic females tested for sterility is indicated in parentheses. The heteroallelic females were generated in the following cross: homozygous + / Sxl\textsuperscript{P1} x Sxl\textsuperscript{P1} + y ov. Virgin heteroallelic females between 2 and 5 days old were mated to wild-type males and allowed to lay eggs for 6 days. After the 6-day laying period, ovaries from the heteroallelic females were examined with phase contrast optics. To avoid complications in comparing the results of the different fertility tests due to non-sex-specific lethality associated with the proximal deletions, only the number of female progeny recovered was recorded.

### Table 4

<table>
<thead>
<tr>
<th>Genotype of homologous clones</th>
<th>No. of fertile females with homozygous clones</th>
<th>No. of females tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sxl\textsuperscript{P1}</td>
<td>14</td>
<td>292</td>
</tr>
<tr>
<td>l-</td>
<td>18</td>
<td>586</td>
</tr>
</tbody>
</table>

Class A derivatives:
- Sxl\textsuperscript{P1} 0 (n = 16) Cystic
- Sxl\textsuperscript{P22} 0 (n = 16) Cystic

Class B derivatives:
- Sxl\textsuperscript{P70} 0 (n = 16) Cystic
- Sxl\textsuperscript{P22} 0 (n = 16) Cystic

Proximal deletions:
- Sxl\textsuperscript{P1} 0 (n = 16) Cystic
- Sxl\textsuperscript{P2} 0 (n = 16) Cystic
- Sxl\textsuperscript{P22} 0 (n = 16) Cystic

Germline clones were induced by mitotic recombination in females heterozygous for ovo\textsuperscript{P1} (PERRIMON and GANS 1983). Larvae were irradiated at 32 to 48 hr after oviposition. After eclosion, adult females of the genotype y em Sxl/ovo\textsuperscript{P1} ov were collected as virgin and mated to balancer males (either FM7 or Binsane) and tested for fertility. Initially females were tested in groups of six, and then individually if any eggs were observed. The phenotype of the progeny was recorded in order to distinguish between a proximal recombination event and a distal recombination event. If the Sxl allele is not defective in the germline function, then clones homozygous for y ovo\textsuperscript{P1} will produce functional eggs. These clones are produced by a mitotic recombination event proximal to both Sxl and ovo\textsuperscript{P1}. Distal mitotic recombination occurring between the two mutations will also generate a clone which will produce eggs due to the loss of ovo\textsuperscript{P1}. However, these clones are still heterozygous for Sxl. Distal clones were distinguished from proximal clones by the presence of y Sxl\textsuperscript{+} males among the resulting progeny.
DISCUSSION

In order to better understand the relationship between the structural organization of Sxl and its multiple functions, we have isolated and characterized a series of new DNA rearrangements. These rearrangements, generated by mobilization of a P element insertion located on the 5′ side of Sxl, were generally associated with structural alterations of the parental P element insertion and/or the surrounding DNA. The alterations include insertions, deletions, duplications and inversions. Deletions, which are the most useful class of rearrangements for this type of study, were generated at a frequency of ~0.1%. A similar study at the rudimentary locus yielded deletions at about the same frequency (TSUBOTA and SCHEDL 1986); however, the deletions were generally smaller than those isolated at Sxl, ranging in size from 1.0 to 6.5 kb. In this study, the smallest deletion isolated was 5.0 kb in length. One of the larger Sxl deletions, SxlPBO, removes DNA sequences on both sides of the parental P element. The extent of this deletion is not known, but the fact that it is male viable limits it to the 0.6 cm interval between the known flanking vital genes at the same frequency (TSUBOTA and SCHEDL 1986); however, the deletions were generally smaller than those isolated at Sxl, ranging in size from 1.0 to 6.5 kb. In this study, the smallest deletion isolated was 5.0 kb in length. One of the larger Sxl deletions, SxlPBO, removes DNA sequences on both sides of the parental P element. The extent of this deletion is not known, but the fact that it is male viable limits it to the 0.6 cm interval between the known flanking vital genes l(1)jnR1 and l(1)jnL1 (NICKLAS and CLINE 1983).

Sxl mutations associated with DNA alterations are localized between coordinates 0 and −11 (MAINE et al. 1985a). In this study we have isolated ten insertions at coordinate 0 that are Sxl+. These results show that the mutant phenotype associated with the P element insertion at coordinate 0 is not due to disruption of DNA sequences necessary for proper gene function. Rather, the mutant phenotype is likely to be caused by properties of DNA sequences contained within the transposon.

Since the parental P element contains, on the basis of restriction mapping, an intact promoter it is possible that transcription from within the P element is the cause of the mutant phenotype (O’HARE and RUBIN 1983). The P element is oriented such that transcription, if it occurs, is in the same direction as Sxl. Four of the wild-type derivatives appear to have lost the promoter region. However, this simple explanation may not be correct since five of the revertants appear to retain the promoter region. Thus, it seems likely that the mutant phenotype may be caused by additional properties of DNA sequences within the P element.

Even though insertions at coordinate 0 are compatible with wild-type gene function, deletions that are missing sequences on either side of coordinate 0 are not. Not all deletions, however, are null alleles; those lacking sequences proximal to coordinate 0 and those lacking sequences between coordinate 0 and −6.5 (class B) still complement partial loss-of-function Sxl alleles. Only those deletions that remove sequences at least through coordinate −13 (class A) eliminate all known functions. The class B deletions and the proximal deletions provide functions lacking in certain alleles, allowing us to correlate the presence of certain DNA sequences with the presence of certain Sxl functions. These data are summarized in Figure 8.

The validity of this analysis relies on the presumption that the mutant phenotype of the deletions results from the absence of certain DNA sequences and is not due to properties within flanking DNA sequences now adjacent to Sxl. The three proximal deletions were isolated, independently, without bias as to Sxl gene activity. Thus it is unlikely that the adjacent DNA sequences would influence Sxl activity. In contrast, the class B deletions were isolated in a screen that demanded a reduction in the somatic functions of Sxl. The mutant phenotype of the class B deletions, therefore, could be influenced by the presence of residual P element sequences at the deletion breakpoints. With this caveat in mind, we can interpret the results of the functional analysis.

Germline functions: Complementation data between the class B derivatives and SxlPBO, an allele believed to be defective specifically in the female germline function (PERRIMON et al. 1986; T. W. CLINE, unpublished data), suggests that sequences distal to coordinate −6.5 are sufficient to provide the function that is defective in SxlPBO. However, the mutant behavior of germline clones homozygous for these deletion chromosomes demonstrates that the deleted sequences are nevertheless required for wild-type Sxl activity in the germline.

Our results suggest that Sxl may have two different activities in the germline: one which is localized to sequences distal to coordinate −6.5 and another which requires sequences proximal to the same point. The absence of the function localized distal to coordinate −6.5 is associated with a cystic egg chamber phenotype. This phenotype is similar to that observed in germ cells homozygous for a Sxl null (Schupbach 1985). We have not yet determined the phenotype associated with the absence of the second function. Consistent with this interpretation is the identification of two Sxl transcripts whose abundance is dependent on the presence of the female germline (H. K. Salz, unpublished data).

Whether the sequences proximal to coordinate 0 are required for Sxl’s germline function cannot be addressed by the experiments reported here since the proximal deletions may also remove another (as yet unidentified) locus required for germline differentiation. Attempts to identify genes in the vicinity of Sxl have been limited, so far, to a search for vital genes (NICKLAS and CLINE 1983).

Somatic functions: Complementation tests indicate that deletions lacking sequences distal to coordinate 0 are phenotypic nulls with respect to all known somatic
functions. Only the proximal deletions show any evidence of intragenic complementation with the somatic tester alleles.

**Pathway initiation functions**: The proximal deletions fully complement $Sxl^{P}$, an allele believed to be defective only in the early functions required for initiation of the female sexual pathway (Maine et al. 1985b; Cline 1986). Thus, if this interpretation is correct, the sequences essential for the pathway initiation functions are within the DNA segment distal to coordinate 0.

**Dosage compensation and sex determination functions**: The complementation behavior of the proximal deletions with $Sxl^{P}$ indicates that they also retain some of the later functions required for maintenance and expression of the female specific pathway. $Sxl^{P}$ is a temperature sensitive mutation that reduces but does not eliminate $Sxl$'s somatic functions (Marshall and Whittle 1978; Cline 1984). $Sxl^{P}$ homozygotes, at 25°, are semiviable and are sexual intermediates. One copy of this allele by itself does not provide enough function for viability but one copy is viable in compound with the proximal deletions; therefore the proximal deletions must retain some of the vital dosage compensation activity.

In contrast, these deletions do not appear to retain any detectable feminizing differentiation function. The sex determination function was assayed, independently of the dosage compensation function, by examining the effect on foreleg development of $Sxl$ lesions in somatic clones. Diplo-X clones homozygous for one of the proximal deletions are phenotypically male, as are diplo-X clones homozygous for a deletion of the entire cloned region.

By correlating the presence or absence of certain DNA sequences with the presence of certain $Sxl$ functions, we have been able to map regions of the gene that are required for these functions. Our data suggest that sequences both proximal and distal to coordinate 0 are essential for somatic sexual differentiation and for normal levels of dosage compensation function. In contrast, sequences distal to coordinate 0 appear to be sufficient for the early functions required in the initiation of the female sexual pathway and also for some aspects of the germline function. Surprisingly, analysis of several cDNAs has placed the 5' end of at least some of the transcripts between coordinates 0 and +0.5 (E. M. Maine, unpublished data). A priori, one would have expected that the deletion of the 5' end of the $Sxl$ gene or a substantial portion of the RNA coding region would result in a null mutation. Instead, we find that deletions extending into the transcription unit result in partial loss-of-function mutations. Although the phenotype of these deletions could be explained by the presence of DNA sequences adjacent to $Sxl$ which are capable of acting as a promoter, we believe this is unlikely. An alternative possibility is that there are multiple promoters dispersed within the $Sxl$ transcription unit. This may, in part, account for the complex pattern of transcription and would be especially intriguing given the functional complexity of the locus.

We are grateful to D. Mohler for sending us $Sxl^{P}$ prior to publication. We thank L. Bell, C. Cronmiller, I. Greenwald and R. Steward for their helpful comments on the manuscript. We also thank all the members of the Princeton fly group (both past and present) for their advice and encouragement. This work was supported by grants from the National Institutes of Health and the March of Dimes to T. C. and P. S. H. S. was supported by an American Cancer Postdoctoral fellowship during part of this work.

**LITERATURE CITED**


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Communicating editor: A. SPRADLING