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

Helen K. Salz, Thomas W. Cline and Paul Schedl

Department of Biology, Princeton University, Princeton, New Jersey 08544

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.

SEX-LETHAL (*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; NOTHIGER 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 NOTHIGER 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 germline 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somatic functions required for either dosage compensation or sex differentiation (PERRIMON *et al.* 1986; T. W. CLINE, unpublished data).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^{fPb}, 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^{fPb} is a partial loss-of-function allele believed to be mutant in the sexual pathway maintenance function (MAINE

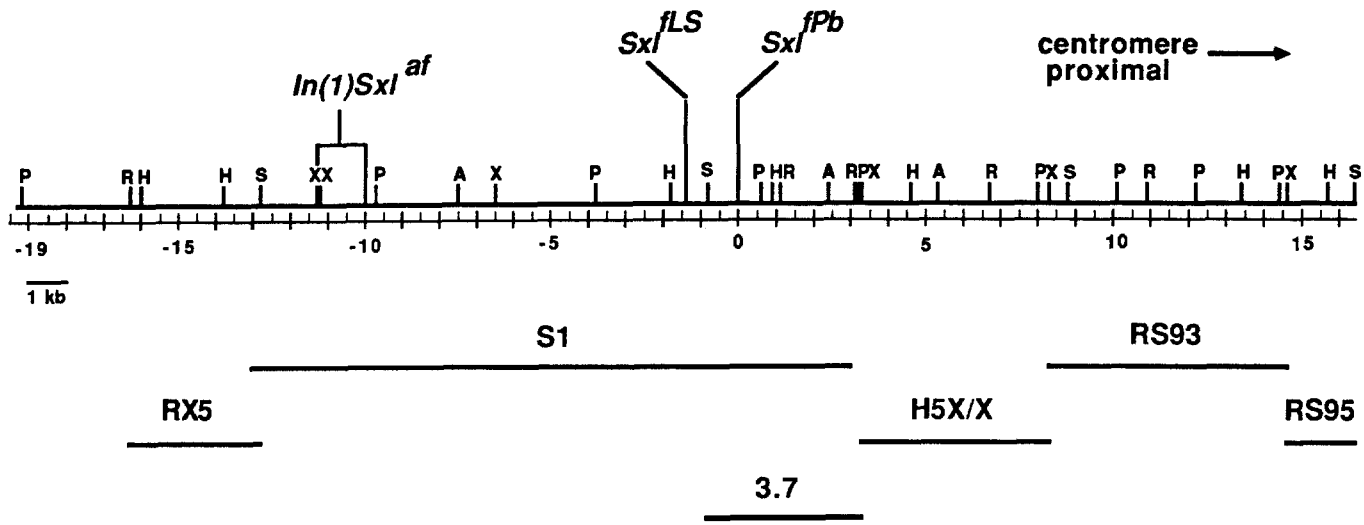

FIGURE 1.—Restriction map of the wild-type DNA segment which includes *Sxl*. Shown are the site of the parental *P* element insertion, Sxl^{fPb}, the site of the breakpoint of *In(1)*Sxl^{af} and the site of the gypsy insertion associated with Sxl^{fLS}. The coordinate system used is that in MAINE *et al.* (1985a): one unit equals 1 kilobase. Also indicated are the different probes used in this study. Restriction sites are designated as follows: R, *Eco*RI; X, *Xho*I; H, *Hin*dIII; S, *Sal*I; P, *Pst*I; A, *Ava*II.

(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^{fPb} 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^{fPb} derivatives with an increase in the female-specific functions is illustrated in Figure 2. The partial loss-of-function allele $Sxl^{fm7,M\#1}$ (CLINE 1984) only partially complemented Sxl^{fPb}. These diplo-*X* animals were sexually transformed and exhibited reduced viability: only 283 $Sxl^{fPb}/Sxl^{fm7,M\#1}$ animals were recovered in a cross that generated 1073 Sxl^{+} sisters. Thus we screened for dysgenic derivatives of Sxl^{fPb} that fully complemented $Sxl^{fm7,M\#1}$. Of 5759 derivative chromosomes screened, 386 complemented $Sxl^{fm7,M\#1}$ (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 ($Sxl^{+fPb\text{-}11}$, $Sxl^{+fPb\text{-}12}$ and $Sxl^{+fPb\text{-}13}$), were associated with a precise or near precise excision of the parental *P* element at coordinate 0. The restriction pattern generated in digests of $Sxl^{+fPb\text{-}13}$ DNA is indistinguishable from wild type, suggesting that the parental *P* element excised precisely (Figure 3). $Sxl^{+fPb\text{-}11}$ and $Sxl^{+fPb\text{-}12}$ have also lost the parental *P* element; however, in addition both of these revertants are associated with complex rearrangements.

The restriction pattern of $Sxl^{+fPb\text{-}12}$ DNA is a composite of that for wild-type and Sxl^{fPb} DNA (Figure 3). It is likely, therefore, that $Sxl^{+fPb\text{-}12}$ 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.

$Sxl^{+fPb\text{-}11}$ 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.

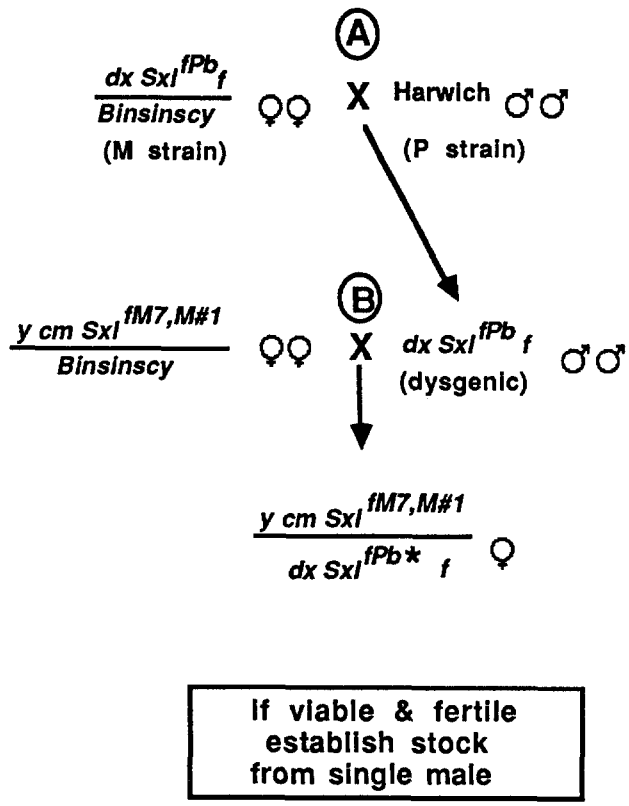

FIGURE 2.—Mutation scheme used to isolate gain-of-function dysgenic derivatives of Sxl^{fPb}.

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 judged by the presence of the *Ava*II sites in the 31-bp repeats. The remaining three revertants (Sxl^{+fPb-2}, Sxl^{+fPb-4}, Sxl^{+fPb-7}) have lost the centromere-proximal *Ava*II site (Figure 3B). The absence of this *Ava*II site suggests that the centromere-proximal terminal repeat may be completely deleted.

Two of the revertants (Sxl^{+fPb-9} and $Sxl^{+fPb-10}$) are associated with *P* element insertions which are 2.5 kb in length, and hence, larger than the parental *P* element (Figure 3A). The insertion in Sxl^{+fPb-9} appears to be a reverse tandem duplication of the parental *P* element. The insertion in $Sxl^{+fPb-10}$ appears to be a replacement of the parental *P* element by a larger, more complete *P* element, as evident from the presence of restriction sites not present in the parental *P* element (data not shown).

The remaining revertant, Sxl^{+fPb-8}, seems to be identical to the parental *P* element (data not shown). It is possible that a small internal change has occurred within the transposon that is not visible by whole genome analysis, or that this stock now contains a closely linked suppressor of Sxl^{fPb} (data not shown).

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 assaying them for *Sxl* gene function.

Proximal deletions were isolated by screening for dysgenic derivatives of Sxl^{fPb} that failed to complement mutations in the nearest proximal vital gene, *l(1)jnR1*, which is 0.3 cM away from *Sxl* (NICKLAS and CLINE 1983). The scheme used to isolate these derivatives is illustrated in Figure 5 (crosses 1 through 4B). Of the 2787 dysgenic derivative lines tested, 120 were found to contain X-linked lethals. Of these, three failed to complement *l(1)jnR1*.

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* [$Dp(1;3)^{13al}$+$Df(1)Sxl^{nl}$, MAINE *et al.* 1985a] (data not shown). Hybridization of these same probes to polytene chromosomes homozygous for each deletion and heterozygous for a duplication of both *Sxl* and *l(1)jnR1* [$Dp(1;3)^{sn13al}$, LEFEVRE 1981], yielded similar results. The probes hybridized, as expected, to the duplication on the third chromosome, but not to the deficiency bearing *X* chromosomes (data not shown). 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, $Sxl^{f\#1}$ (Table 1). 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^{fPb} fully complemented Sxl^{f9} (Table 1), derivatives could be isolated that have lost this ability. Nine such derivatives, all resulting from independent events, were recovered

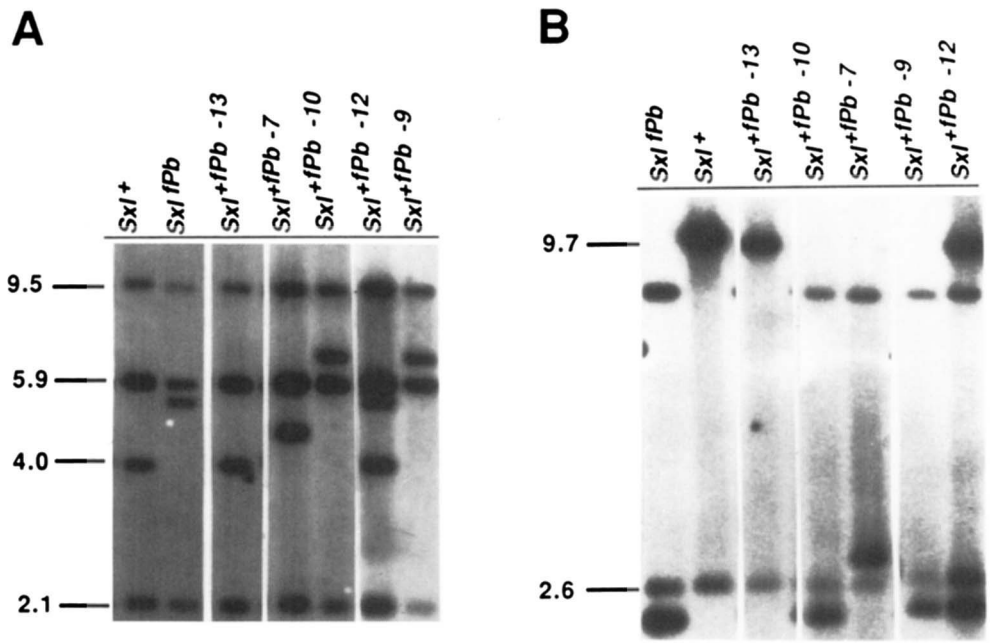

FIGURE 3.—Genomic Southern blots indicating DNA rearrangements that are compatible with full Sxl^+ gene function. Sizes of (wild-type) DNA fragments are indicated in kilobases. (A) *Pst*I digests of genomic DNA made from homozygotes of the indicated genotypes probed with recombinant S1. (B) *Ava*II digests of genomic DNA made from homozygotes of the indicated genotypes probed with recombinant 3.7.

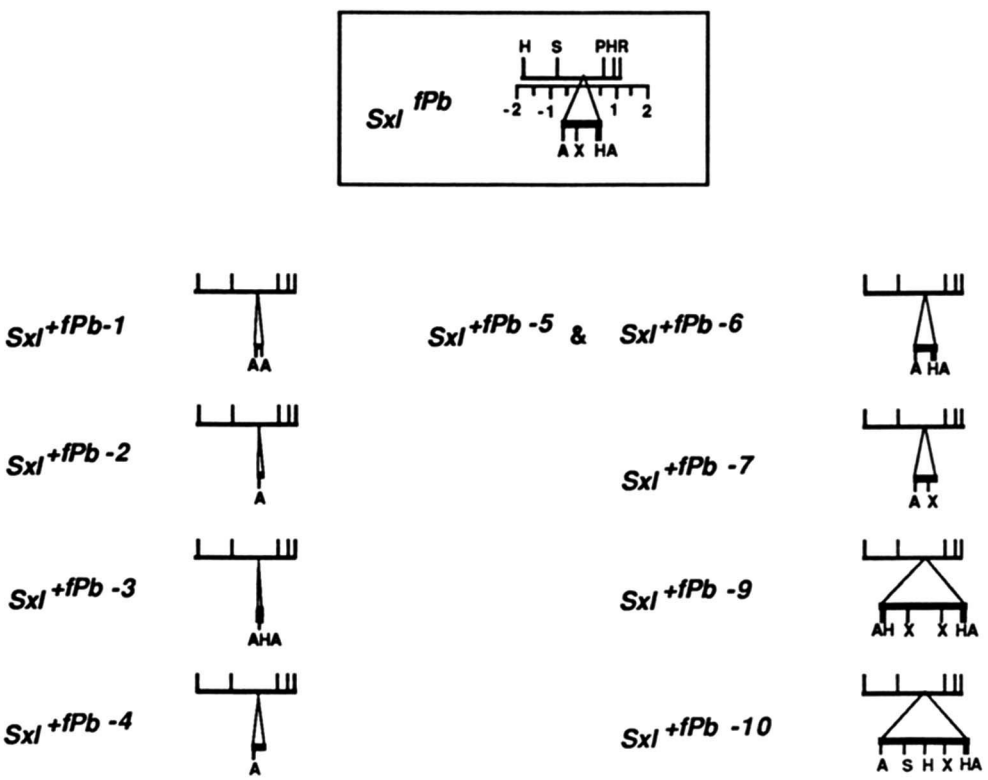

FIGURE 4.—Schematics of DNA rearrangements compatible with full Sxl^+ function. The DNA rearrangement associated with the mutant parental allele, Sxl^{fPb} is indicated within the box. Restriction sites are designated as follows: R, *Eco*RI; X, *Xho*I; H, *Hin*dIII; S, *Sal*I; P, *Pst*I; A, *Ava*II.

among 7347 *X* chromosomes screened. As expected, none of these derivatives complemented Sxl^{fm1} (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^{f7BO} 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^{f7C2}, lies within the cloned region, at coordinate −13. Moreover, no *P* element sequences are evident at the deletion breakpoints of Sxl^{f3G2}, Sxl^{f6C2} and Sxl^{f7C2} (data not shown); whereas Sxl^{fED1} and Sxl^{fED2} still retain small

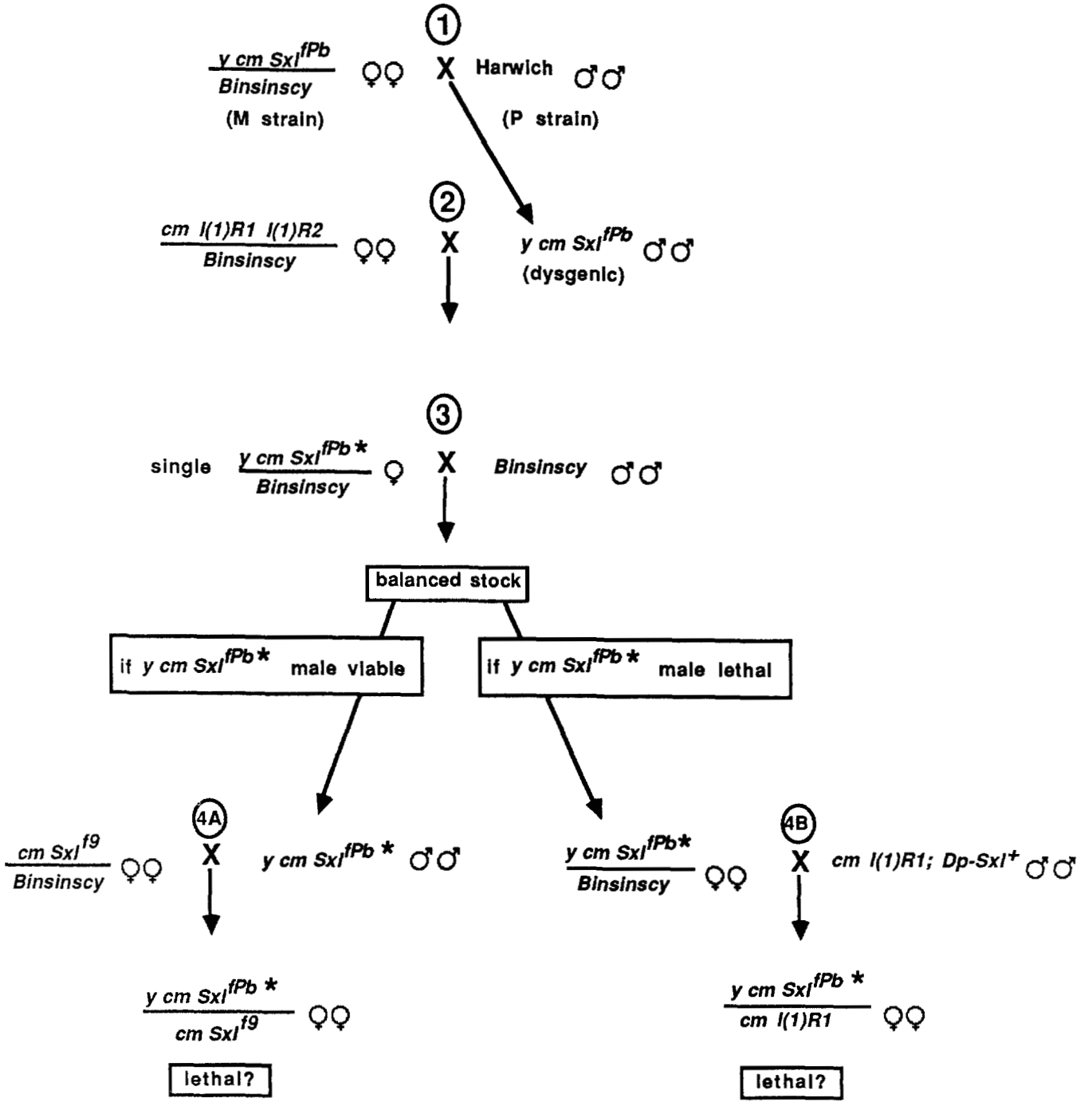

FIGURE 5.—Mutation scheme used to isolate dysgenic derivatives of Sxl^{fPb} associated with a *Sxl* mutant phenotype. Two of the derivatives, Sxl^{f3G2} and Sxl^{f6C2}, were isolated from a parental allele marked with *dx* and *f* instead of *y* and *cm*.

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^{f7AV} and Sxl^{f7AI} are deletions. Sxl^{f7AV} 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^{f7AI} is slightly larger than that associated with Sxl^{f7AV} 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 *Ava*II site is missing (data not shown). The third derivative, Sxl^{fODH}, 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

	Maternally contributed tester allele			
	Sxl^{fm1}	Sxl^{f9}	Sxl^{fLS}	Sxl^{2593}
Parental allele				
Sxl^{fPb}	0/510	380/378 (101%)[a]	29/230 (13%)[b]	161/377 (43%)[c]
Class A derivatives				
Sxl^{f7B0}	0/329	0/609	0/397	0/211
Sxl^{f3G2}	0/488	0/692	0/324	0/233
Sxl^{f6C2}	0/591	0/572	0/170	0/259
Sxl^{f7C2}	0/329	0/356	0/273	0/402
Sxl^{fED1}	0/314	0/166	0/270	0/326
Sxl^{fED2}	0/262	0/185	0/253	0/264
Class B derivatives				
Sxl^{f7AI}	0/465	0/469	0/327	0/429
Sxl^{f7AV}	0/335	0/527	0/276	0/260
Sxl^{f-}_{ODH}	0/113	0/151	0/366	0/264
Proximal deletions				
Sxl^{fRJ2}	0/229	55/58 (95%)[a]	0/180	11/209 (5%)[b]
Sxl^{fRL2}	0/188	75/69 (109%)[a]	0/262	24/212 (11%)[b]
Sxl^{fRC1}	0/212	57/44 (130%)[a]	0/408	28/231 (12%)[b]

Sexual phenotype of viable heteroallelic animals: [a] female, [b] intersex, [c] 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^{f3G2} *f* and *dx* Sxl^{f6C2} *f*. The maternally derived chromosomes are marked as follows:

cm Sxl^{fm1} *ct*, *cm* Sxl^{f9} *v*, *y* Sxl^{2593} *ct and y* Sxl^{fLS} *v oc f*. These chromosomes are balanced with *Binsinscy* except for Sxl^{fLS} which is balanced with FM6. The lethality associated with the deletion of *l(1)jnR1* in the proximal deletions is covered by *Dp(Y;1)ct*$^{+}$ *y*$^{+}$ (JOHNSON and JUDD 1979).

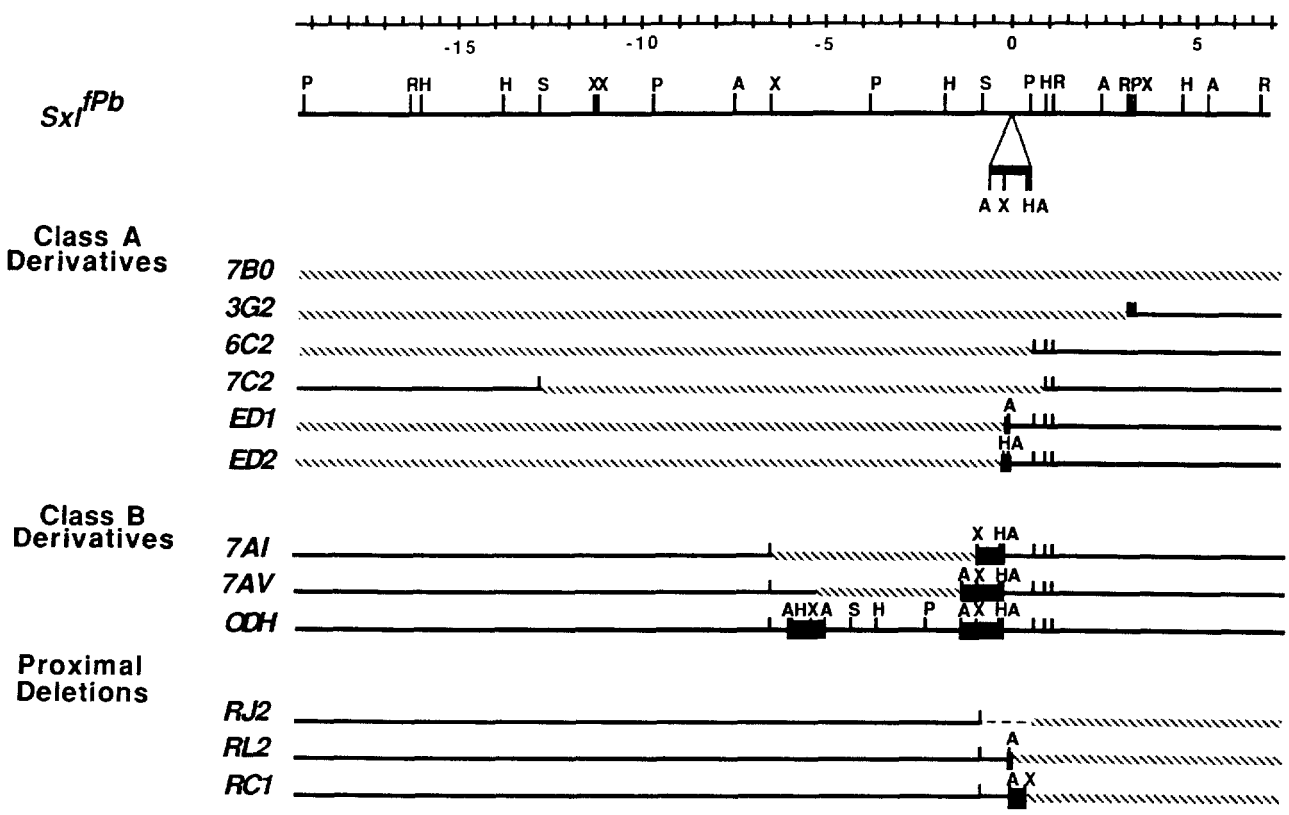

FIGURE 6.—Schematics of DNA rearrangements associated with a *Sxl* mutant phenotype. *P* element sequences are indicated by the *thick black bars*. Deletions of specific sequences are indicated by *slashed bars*. The uncertainty in the location of the deletion breakpoint of Sxl^{fRJ2} is indicated by a *broken thin line*. Restriction sites are designated as follows: R, *Eco*RI; X, *Xho*I; H, *Hin*dIII; S, *Sal*I; P, *Pst*I, A, *Ava*II.

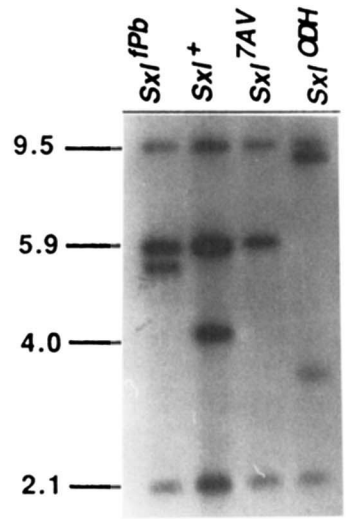

FIGURE 7.—Genomic Southern blots indicating DNA alterations associated with a *Sxl* mutant phenotype. Sizes of (wild-type) DNA fragments are indicated in kilobases. *Pst*I digests of genomic DNA made from males hemizygous for the indicated *Sxl* allele probed with recombinant S1.

intragenic complementation. We used four tester alleles, each of which affects *Sxl*'s multiple functions differently:

—Sxl^{f9} seems to be defective only in the early sexual pathway initiation steps (MAINE *et al.* 1985b; CLINE 1986).

—Sxl^{fLS} 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).

—Sxl^{2593} appears to reduce all the somatic functions in a temperature dependent fashion (MARSHALL and WHITTLE 1978; CLINE, 1984; T. W. CLINE, unpublished data).

—Sxl^{fs1} 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 Sxl^{fPb} 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 Sxl^{fPb} and the tester alleles demonstrates that Sxl^{fPb} retains some of the somatic functions. This is shown by the fact that Sxl^{f9} and Sxl^{fPb} complemented fully. In contrast, complementation with the two other tester alleles, Sxl^{fLS} and Sxl^{2593}, was not complete; Sxl^{2593}/Sxl^{fPb} females had a relative viability of 43% and were sterile; Sxl^{fLS}/Sxl^{fPb} 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.

TABLE 2

Sexual phenotype in the foreleg of somatic clones homozygous for a proximal deletion

Genotype of homozygous clones	No. of forelegs	No. and sex of clones
$y\ Sxl^{fRL2}$	159	4 Male
$y\ Sxl^{+}$	164	8 Female
$y\ Sxl^{f7BO}$	164	3 Male

Larvae were exposed to *ca.* 1500 rad of gamma radiation at 48–72 hr after oviposition. The forelegs of adult females of the genotype $y\ Sxl\ M(1)o^{+}/y^{+}\ Sxl^{+}\ M(1)o$ 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 RIPOLL 1975).

Both the class A and the class B derivatives (which were isolated based on their failure to complement Sxl^{f9}) 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^{f9} 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 Sxl^{fLS} and their partial complementation with Sxl^{2593}. Only 11% of the expected Sxl^{fRL2}/Sxl^{2593} 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, Sxl^{fRJ2} and Sxl^{fRC1} exhibited similar phenotypes to Sxl^{fRL2}.

To determine whether Sxl^{fRL2} retains any feminizing differentiation function, we induced homozygous somatic clones of Sxl^{fRL2} 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 (Sxl^{f7BO}). Thus, with respect to its

TABLE 3

Fertility of Sxl^{x}/Sxl^{fs1} heteroallelic females

Sxl^{x}	Average No. of daughters recovered per female	Phenotype of egg chambers
Sxl^{fs1}	0 (n = 14)	Cystic
Sxl^{fPb}	26.0 ± 1.1 (n = 16)	Normal
Class A derivatives		
Sxl^{f7BO}	0 (n = 16)	Cystic
Sxl^{f7C2}	0 (n = 16)	Cystic
Class B derivatives		
Sxl^{f7AV}	3.0 ± 1.0 (n = 16)	Normal
Sxl^{f7AI}	4.1 ± 0.9 (n = 16)	Normal
Sxl^{fODH}	7.1 ± 1.1 (n = 16)	Normal
Proximal deletions		
Sxl^{fRJ2}	13.5 ± 1.4 (n = 14)	Normal
Sxl^{fRL2}	13.7 ± 1.9 (n = 16)	Normal
Sxl^{fRC1}	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: *y cm* Sxl^{x}*/Binsinscy* × *y cv* Sxl^{fs1} *v f*/Y. 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

Differentiation of germ cells homozygous for Sxl^{fPb} derivatives

Genotype of homozygous clones	No. of fertile females with homozygous clones	No. of females tested
Sxl^{fPb}	14	292
l-(1)jnR1	18	586
Class A derivatives		
Sxl^{f7BO}	0	182
Sxl^{f7C2}	0	271
Class B derivatives		
Sxl^{f7AI}	0	417
Sxl^{f7AV}	0	789
Sxl^{fODH}	0	397
Proximal deletions		
Sxl^{fRC1}	0	495
Sxl^{fRL2}	0	471

Germline clones were induced by mitotic recombination in females heterozygous for ovo^{D1} (PERRIMON and GANS 1983). Larvae were irradiated at 32 to 48 hr after oviposition. After eclosion, adult females of the genotype *y cm Sxl*/ovo^{D1} *v* were collected as virgins and mated to balancer males (either *FM7* or *Binsinscy*) 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^{+} *Sxl* will produce functional eggs. These clones are produced by a mitotic recombination event proximal to both *Sxl* and ovo^{D1}. Distal mitotic recombination occuring between the two mutations will also generate a clone which will produce eggs due to the loss of ovo^{D1}. However, these clones are still heterozygous for *Sxl*. Distal clones were distinguished from proximal clones by the presence of y^{+} Sxl^{+} males among the resulting progeny.

ability to direct female differentiation, Sxl^{fRL2} appears to be equivalent to a null allele.

Germline function: *Sxl* germline function was initially assayed by complementation with Sxl^{fs1}, 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^{fs1} 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^{fs1} (Table 3).

The complementation between Sxl^{fs1} and a representative sample of the deletion derivatives is presented in Table 3. Both the class B rearrangements (Sxl^{f7A1}, Sxl^{f7C2}, Sxl^{fODH}) and the three proximal deletions partially complemented Sxl^{fs1}: Heteroallelic females were semifertile, 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^{fs1} 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^{f7BO} and Sxl^{f7C2}) did not complement Sxl^{fs1}. Ovaries of these heteroallelic females contained only cystic egg chambers identical to those of Sxl^{fs1}.

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^{+} 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^{fPb} 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)jnR1*, 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.

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, Sxl^{f7B0}, 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 Sxl^{fs1}, 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 Sxl^{fs1}. 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

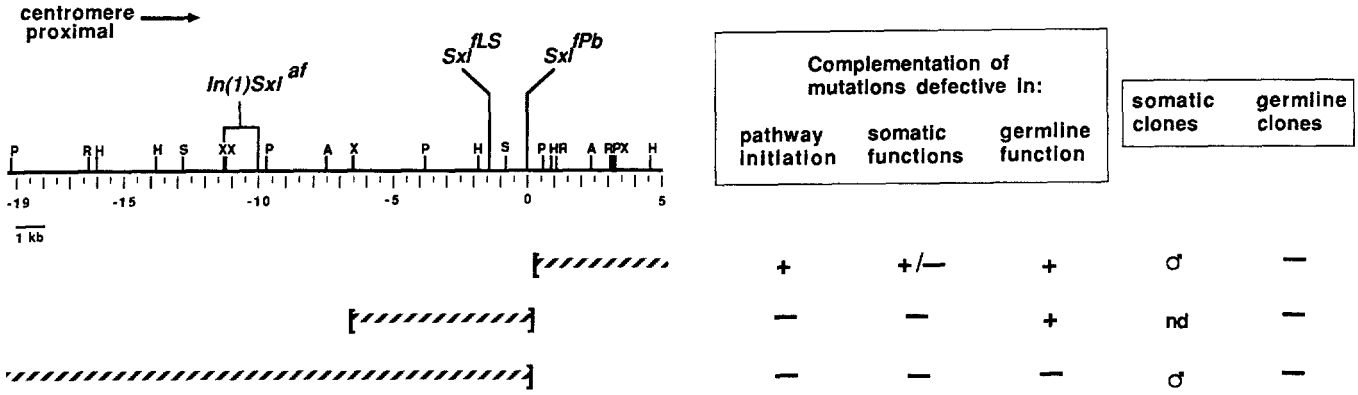

FIGURE 8.—Summary of the structural and functional analysis of deletions associated with a *Sxl* mutant phenotype. DNA sequences defined by the three sets of deletions are indicated by *slashed bars*.

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^{f9}, 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^{2593} indicates that they also retain some of the later functions required for maintenance and expression of the female specific pathway. Sxl^{2593} is a temperature sensitive mutation that reduces but does not eliminate *Sxl*'s somatic functions (MARSHALL and WHITTLE 1978; CLINE 1984). Sxl^{2593} 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^{fs1} 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

BAKER, B. S. AND J. M. BELOTE, 1983 Sex determination and dosage compensation in *Drosophila melanogaster*. Annu. Rev. Genet. **17:** 345–393.

CLINE, T. W., 1978 Two closely linked mutations in *Drosophila melanogaster* that are lethal to opposite sexes and interact with *daughterless*. Genetics **90:** 683–698.

CLINE, T. W., 1983 The interaction between *daughterless* and *Sex-lethal* in triploids: a lethal sex transforming maternal effect linking sex determination and dosage compensation in Drosophila. Dev. Biol. **95:** 260–274.

CLINE, T. W., 1984 Autoregulatory functioning of a Drosophila gene product that establishes and maintains the sexually determined state. Genetics **107:** 231–277.

CLINE, T. W., 1985 Primary events in the determination of sex in *Drosophila melanogaster*. pp. 301–327. In: *Origin and Evolution of Sex*, Edited by H. O. HALVORSON and A. MONROY. Alan R. Liss, New York.

CLINE, T. W., 1986 A female specific lethal lesion in an X-linked positive regulator of the Drosophila sex determination gene, *Sex-lethal*. Genetics **113:** 641–663. Corrigendum **114:** 345.

JOHNSON, T. K. AND B. H. JUDD, 1979 Analysis of the *cut* locus of *Drosophila melanogaster*. Genetics **92:** 485–502.

LEFEVRE, G., JR., 1981 The distribution of randomly recovered X-ray induced sex-linked genetic effects in *Drosophila melanogaster*. Genetics **99:** 461–480.

LINDSLEY, D. AND E. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LUCCHESI, J. C., 1983 The relationship between gene dosage, gene expression and sex in Drosophila. Dev. Genet. **3:** 275–282.

LUCCHESI, J. C. AND T. SKRIPSKY, 1981 The link between dosage compensation and sex differentiation in *Drosophila melanogaster*. Chromosoma **82:** 217–227.

MAINE, E. M., H. K. SALZ, T. W. CLINE AND P. SCHEDL, 1985a The *Sex-lethal* gene of Drosophila: DNA alterations associated with sex-specific lethal mutations. Cell **43:** 521–529.

MAINE, E. M., H. K. SALZ, P. SCHEDL AND T. W. CLINE, 1985b *Sex-lethal*, a link between sex determination and sexual differentiation in *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. **50:** 595–604.

MARSHALL, T. AND J. R. S. WHITTLE, 1978 Genetic analysis of the mutation *Female-lethal* in *Drosophila melanogaster*. Genet. Res. **32:** 103–111.

MORATA, G. AND P. RIPOLL, 1975 *Minutes*: mutants of Drosophila autonomously affecting cell division rate. Dev. Biol. **42:** 211–221.

NICKLAS, J. A. AND T. W. CLINE, 1983 Vital genes that flank *Sex-lethal*, an X-linked sex determining gene of *Drosophila melanogaster*. Genetics **103:** 617–631.

NOTHIGER, R. AND M. STEINMANN-ZWICKY, 1985 Sex determination in Drosophila. Trends Genet. **1:** 209–215.

O'HARE, K. AND G. M. RUBIN, 1983 Structures of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell **34:** 25–35.

PERRIMON, N. AND M. GANS, 1983 Clonal analysis of the tissue specificity of recessive female-sterile mutations of *Drosophila melanogaster* using a dominant female-sterile mutation *Fs(1)*K1237. Dev. Biol. **100:** 365–373.

PERRIMON, N., D. MOHLER, L. ENGSTROM AND A. P. MAHOWALD, 1986 X-linked female-sterile loci in *Drosophila melanogaster*. Genetics **113:** 695–712.

SANCHEZ, L., AND R. NOTHIGER, 1982 Clonal analysis of *Sex-lethal*, a gene needed for female sexual development in *Drosophila melanogaster*. Wilhelm Roux Arch. Dev. Biol. **191:** 211–214.

SCHUPBACH, T., 1985 Normal female germ cell differentiation requires the female *X* chromosome to autosome ratio and expression of *Sex-lethal* in *Drosophila melanogaster*. Genetics **109:** 529–548.

TSUBOTA, S. AND P. SCHEDL, 1986 Hybrid dysgenesis-induced revertants of insertions at the 5′ end of the *rudimentary* gene in *Drosophila melanogaster*: transposon-induced control mutations. Genetics **114:** 165–182.

VOELKER, R. A., A. L. GREENLEAF, H. GYURKOVICS, G. B. WISELY, S. HUANG AND L. L. SEARLES, 1984 Frequent imprecise excisions among reversions of a *P* element caused lethal mutations in Drosphila. Genetics **107:** 279–294.

Communicating editor: A. SPRADLING