Genetic Characterization of small ovaries, a Gene Required in the Soma for the Development of the Drosophila Ovary and the Female Germline

Sigrid Wayne, Kristen Liggett, Janette Pettus and Rod N. Nagoshi

Department of Biological Sciences, The University of Iowa, Iowa City, Iowa 52242

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ABSTRACT

The small ovary gene (sov) is required for the development of the Drosophila ovary. Six EMS-induced recessive alleles have been identified. Hypomorphic alleles are female sterile and have no effect on male fertility, whereas more severe mutations result in lethality. The female-sterile alleles produce a range of mutant phenotypes that affect the differentiation of both somatic and germline tissues. These mutations generally produce small ovaries that contain few egg cysts and disorganized ovarioles, and in the most extreme case no ovarian tissue is present. The mutant egg cysts that develop have aberrant morphology, including abnormal numbers of nurse cells and patches of necrotic cells. We demonstrate that sov gene expression is not required in the germline for the development of functional egg cysts. This indicates that the sov function is somatic dependent. We present evidence using loss-of-function and constitutive forms of the somatic sex regulatory genes that sov activity is essential for the development of the somatic ovary regardless of the chromosomal sex of the fly. In addition, the genetic mapping of the sov locus is presented, including the characterization of two lethal sov alleles and complementation mapping with existing rearrangements.

The developing Drosophila egg is a mosaic of somatic and germline cells whose coordinate differentiation is essential for normal oogenesis. An active interaction between the germline and soma controls the deposition of yolk protein into the egg, the production of the chorion egg "shell" (Mahowald and Kambysellis 1980) and dorsal-ventral patterning in the egg chamber and embryo (Wieschaus 1979; Schüpbach 1987; Stevens et al. 1990). In addition, the proliferation and differentiation of both male and female germ cells are influenced by the sexual identity of the somatic gonad. Pole cell transplantation studies demonstrate that functional gametes are only produced when X/X germ cells develop in ovaries and X/Y germ cells in testes (van Deusen 1976; Marsh and Wieschaus 1978; Schüpbach 1982; Steinmann-Zwicky et al. 1989). Therefore, it appears that the genotype of the germline is not sufficient to support spermatogenesis or oogenesis in somatic tissue of the inappropriate sex. In fact, sex-specific somatic signals can induce germ cells to undergo a differentiation pathway that is contrary to what would be expected from their X:A ratio. When X/X germ cells were transplanted into testes, they attempted to undergo what appeared by morphological criteria to be the initial stages of spermatogenic differentiation (Steinmann-Zwicky et al. 1989). Apparently the male soma can "impose" its sexual identity on the X/X germline.

The germline is in physical proximity to the somatic gonad beginning at early stages in embryogenesis. The pole cells migrate during germ band extension to the mesodermal precursors of the gonad. These somatic cells can differentiate into either ovary or testes depending on the action of the somatic sex regulatory genes. Sexual dimorphism in the gonads become visible during the early larval stages both in terms of gonad size and germ cell morphology. This sexual differentiation is dependent on sex-specific interactions between the soma and germline that occur during this period (Steinmann-Zwicky 1994).

Despite this close interaction between the soma and germline, the sexual differentiation of these tissues is regulated by two distinct sets of genes. Somatic sex determination depends on the interpretation of the X:A ratio by the Sex-lethal (Sxl) gene (reviewed in Parkhurst and Meneely 1994). A female X:A ratio of 1:1 (X/X) activates the Sxl RNA splicing activity that causes the transformer (tra) gene to produce a female-specific product. The tra function acts with the product of another unlinked gene, transformer-2 (tra-2), to control the sex-specific expression of the functionally dimorphic doublesex (dsx) gene. Mutations in any of these genes can alter the somatic sexual differentiation of the fly. For example, loss-of-function mutations in tra cause X/X flies to develop as males with fully developed male somatic structures (Sturtevant 1945; Brown and King 1961). However, these sexually altered flies are sterile with only rudimentary germline development, indicating that the germline is not similarly sexually
transformed (Brown and King 1961; Nöthiger et al. 1989). Furthermore, pole cell transplantation experiments demonstrate that X/X germ cells mutant for tra, tra-2 or dsx can develop normally if placed in a female somatic environment (Marsh and Wieschaus 1978; Schüpbach 1985). These results suggest the existence of a separate genetic pathway to regulate sexual differentiation of the germine. Several genes have been implicated in this process, including otu, ovo, Sxl and sans file (snf), based on morphological and molecular observations that suggest mutations in these genes can cause X/X germ cells to take on some male characteristics. However, this presumed sexual transformation is incomplete and subject to other interpretations (Bae et al. 1994).

Later in development, the differentiation of the egg cysts becomes dependent on interactions that occur between the germine and the somatically derived follicle cells. By the late larval and pupal stages, the female germine has begun the process of differentiating into egg chambers. Initially, germine stem cells divide asymmetrically to produce a daughter stem cell and a cystoblast. The cystoblast undergoes four mitotic divisions, each characterized by incomplete cytokinesis, to form a 16-cell syncytium. One of these cells becomes the oocyte, whereas the other 15 differentiate into nurse cells that provide much of the material for the maturation of the oocyte. As the 16-cell syncytium develops, it becomes surrounded by follicle cells and together these form the egg chamber.

Follicle cells have specialized behaviors and functions that are essential for the development of the egg. For example, the delamination of follicle cells at the anterior end of the cyst and their subsequent differentiation into stalk cells act to separate individual egg chambers to form the egg chamber. The morphology of the micropyle (Montell et al. 1992). Mutations that disrupt the differentiation of specific follicle cells can have dramatic effects on the morphology of the ovary and the viability of the female germine. For example, the neurogenic Notch and Delta genes are also required in the ovaries for the establishment of follicle cell fate and oocyte polarity. Mutations in these genes result in fused and disorganized egg chambers and are often associated with necrotic germ cells (Ruohola et al. 1991).

These results indicate that the development of the female germine is dependent on the sexual state of the soma, as controlled by the somatic sex determination genes, as well as on the genes that control the differentiation of the follicle cells. How the female germine is affected by these somatic factors is not well understood. In this manuscript we examined the soma–germine interaction by characterizing a gene, small ovaries (sov), that is required both for the formation of the somatic ovary and for the development of the female germine. We demonstrate that the expression of the sov gene is required in the soma for both the development of the somatic ovary and the normal differentiation of the female germine. This ovary-specific sov function is dependent on regulation by the somatic sex determination genes. We suggest that sov serves to mediate at least a subset of the interactions that occur between the somatic and germine tissues.

**MATERIALS AND METHODS**

**Fly strains:** The sov^1--3^ alleles were isolate in an EMS mutagenic screen designed to identify sex-specific sterility (Mohler 1977). Descriptions of other mutations and balance chromosomes used in this study are found in Lindley and Zimm (1992). Flies were raised on a standard cornmeal, molasses, yeast, agar media containing propionic acid as a mold inhibitor and supplemented with live yeast.

EMS mutagenesis used to isolate lethal sov alleles: sov^M1.159^ and sov^M1.157^ were isolated in an F2 screen for EMS-induced X-linked lethal and sterile mutations. W^1118^/Y males were fed 25 mM EMS in 5% sucrose for 24 hr using standard protocols (Ashburner 1989). The mutagenized (w^1118^/Y) males were mated enmasse to FMO/CIB females and the w^1118^/FMO and W^1118^/CIB female progeny were individually mated to FMO/Y males. The progeny from each pair mating was examined for the presence of an X-linked lethal by the absence of B^+^ (W^1118^/Y) males. Pair matings with viable mutagenized X chromosomes were tested for the presence of female-sterile lesions. In these cases the mutagenized chromosomes were made homozygous and the resulting females were tested for fertility. From 1821 mutagenized chromosomes we obtained 756 (41.5%) X-linked lethals and 66 (3.6%) X-linked female steriles. We tested each mutation against sov-- for female fertility.

Two of the lethal lines were shown to be allelic to sov--.

**Morphological analyses of gonads:** The morphology of the mutant gonads were examined by either Feulgen or DAPI staining, both of which specifically label nuclei. Fly cultures were kept under uncrowded conditions at 25°C. Female flies of the appropriate genotypes were aged 2-3 days after eclosion at 25°C. The ovaries were dissected in phosphate-buffered saline (PBS, 130 mM NaCl, 7 mM Na_{2}HPO_{4}, 2H_{2}O, 3 mM NaH_{2}PO_{4}, 2H_{2}O) and then stained by Feulgen reaction using a modification of the procedure described in Galigher and Kosloff (1971). Ovaries were hand dissected and fixed in Carnoy’s solution (1:4 acetic acid:ethanol) for 2-3 min. After fixation, the ovaries were incubated in 5 N HCl for 3-4 min. This was followed by incubation in Feulgen reagent until the nuclei were appropriately stained. Staining was stopped by a 5-min incubation in dilute sulfuric acid. The ovaries were dehydrated by a series of washes in 10%, 30%, 50%, 70%, 90%, 100% ethanol. The stained ovaries were cleared in xylene and mounted in permount. Specimens can be visualized under visible light or fluorescence using a green excitation filter.

For DAPI staining, adult gonads were dissected in PBS and
then incubated in 50% fixative:50% heptane in a covered depression slide with agitation from a rotary shaker for 3 min. The tissue was rinsed three times in in PBS + 0.1% Triton and incubated in DAPI solution (0.5 μg/ml in 180 mM Tris-HCl, pH 7.5) for 1 hr to fluorescently stain nuclei. The preparation was washed for 20 min five times with PBS. The tissue was mounted in 50% glycerol in PBS. The stock solutions used for this procedure were as follows: solution B, 1.4 g/l Na₂HPO₄, 0.1 g/l KH₂PO₄, take to pH 7 with NaOH, and solution C, 6.75 g/l NaCl, 6.65 g/l KCl, 0.66 g/l MgSO₄, 7H₂O, 0.54 g/l MgCl₂·6H₂O, 0.33 g/l CaCl₂·2H₂O in 3.7% formaldehyde. The fixative was made up by mixing nine parts solution C with 10 parts solution B. DAPI-stained specimens were observed under fluorescence using a UV excitation filter.

Germline clonal analysis: Germline clones were produced by the well-established dominant female-sterile procedure (PERRIMON and GANS 1983). The dominant female-sterile allele, sos⁻²⁻ (or Fs(1)K1237), blocks oogenesis when present in one copy in the germline stem cells. The progeny from the mating of ysov⁻²⁻v/Y females to sos⁺/Y males were irradiated with 1000 rads from a ²⁵²Cf gamma source at 44–52 hr postoviposition to induce mitotic recombination in the germline. Clones induced by this method often occupy several ovarioles (WIESCHAUS and SZBAD 1979). Irradiated females of the genotype ysov⁻²⁻v/f/Y females were tested for fertility by matings with ycv⁻¹/f/Y males. Clones resulting from recombination events proximal to f (and therefore sos and sos⁺ as well) must be ycv⁻¹ sos⁻²⁻, producing eggs of the genotype ysov⁻²⁻v f/Y. These proximal clones were identified by the production of progeny that were yellow, crossveinless, vermilion and forked when crossed to ycv⁻¹/f/Y males. Confirmation that the recombination chromosomes were sos⁻²⁻ came by complementation testing against sos²⁻.

RESULTS

sos mutations affect both somatic ovary and germline development: A normally developing ovary consists of egg cysts organized in linear arrays called ovarioles. Egg chambers of different developmental stages are found in each ovariole (ovl), with the least mature cysts located apically and the mature yolky oocytes (y) arranged near the oviduct (Figure 1A). sos mutations affect both the somatic and germline components of the ovary, affecting the organization of the gonad as well as the differentiation and viability of the germ cells. Three EMS-induced female-sterile sos alleles had been previously identified (MOHLER and CARROLL 1984) and were used in the characterization of the sos mutant phenotype. Because the sos mutations result in a range of ovarian phenotypes, we could not unambiguously determine the relative severity of the different alleles, although mutant combinations with sos⁺ generally produce the most severely affected ovaries (Table 1).

The most common mutant phenotype obtained is the disruption of ovariole structure that result in ovaries (ovl) containing a haphazard arrangement of the cysts (Figure 1B; Table 1). In many cases, ovarioles do not appear to form at all, producing large sacs filled with irregularly shaped cysts containing a mix of yolk globules, cells with large nuclei that resemble nurse cells and cells with pycnotic nuclei that appear necrotic (Figure 1C). The mutant ovaries are often associated with unencysted polyplloid cells located in the oviduct. These are morphologically similar to nurse cells, perhaps resulting from aberrant cyst formation or the degeneration of follicle cells. In the most severely affected ovaries, the oviducts (ovd) end in small nubs (nb) that are absent germ cells and somatic ovarian tissue (Figure 1D). These could result from necrosis, reduced proliferation of the ovarian tissue or from a failure of the oviduct to join with the female gonad during development. In most cases, we observed that the nub phenotype occurs when the ovary lobe fails to develop, because we usually cannot find a detached gonad elsewhere in the abdomen. Occasionally, however, a failure of the gonad and oviduct to attach must occur as free-floating clusters of egg chambers are sometimes found.

In addition to these affects on the somatic structure of the ovary, sos mutations also affect the viability, proliferation and differentiation of the germline. sos mutant ovaries often contain egg cysts that contain fewer than the normal 15 nurse cells. These could result from cell death as the cysts often contain condensed, irregularly shaped nuclei that are associated with degenerating nurse cells (KING 1970). However, some hyponumerary cysts appear to be undergoing advanced stages of oogenesis without evidence of necrosis (Figure 1E). This suggests that the sos mutations can affect the number of mitotic divisions that individual cystoblasts undergo, without affecting their capacity to differentiate in a female-specific manner. Hypronumerary cysts are also found that can contain more than the normal 15 nurse cells (Figure 1F). To test whether these cysts resulted from the fusion of egg chambers or increased germ cell proliferation, we examined the number of ring canals that form in the hypronumerary cysts. In normal oogenesis the cystoblast undergoes four mitotic rounds associated with incomplete cytokinesis to produce a 16 cystocyte syncytium. The cytoplasmic bridges connecting the cystocytes are associated with a ring canal that can be visualized using phalloidin, an actin-specific fluorescent stain. Therefore, the number of ring canals indicates the number of cell divisions that each cystocyte has undergone, with a maximum of four divisions in normal oogenesis. In sos mutant cysts, we found no germ cells that contained more than four ring canals, and most had only one (data not shown). We therefore believe that most hypronumerary cysts occur by the fusion of one or more egg chambers containing cysts undergoing the normal number of mitotic divisions. However, we cannot preclude the possibility of some aberrant proliferation, particularly if it occurs with complete cytokinesis and no ring canal formation.

sos mutations can also result in abnormal germ cell differentiation. Cysts often form that are phenotypically similar to the ovarian tumor egg chambers (tc) that
FIGURE 1.—The morphologies of wild-type and sav mutant ovaries. A wild-type ovary (A) is compared with the range of mutant ovarian phenotypes that result from the genotype sav¹ l(1) EA42/y co sav² (B–F). The mutant phenotypes are similar to those obtained from other sav allele combinations. Ovaries are stained with the nucleic acid-specific Feulgen reagent and photographed under bright field optics. Nuclei stain dark. B, C and E were photographed under twofold higher magnification than A, D and F. (A) Wild-type ovary with ovarioles and yolky egg cysts. (B) Mutant ovary with some ovariole structure and aberrant egg chambers. (C) Disorganized ovary with no ovarioles. (D) Oviduct with one small lobe containing a tumorous egg cyst and a "nub." (E) Mutant cyst containing eight nurse cells (normally have 15 nurse cells). (F) Mutant cyst containing ≥30 nurse cells. ec, egg chamber; H, hypernumerary cysts; h, hyponumerary nurse cell cysts; nb, nub; nc, nurse cell; ovd, oviduct; ovl, ovariole; tc, tumorous cyst; y, yolky egg chamber.
A Somatic-Dependent Ovarian Gene

**TABLE 1**

Comparison of *sod* mutant ovarian phenotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ovary lobes with ovarioles&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Disorganized ovary, no ovarioles&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No ovary lobes (nubs)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^+$</td>
<td>1.00 (20/20)</td>
<td>0.00 (0/20)</td>
<td>0.00 (0/20)</td>
</tr>
<tr>
<td>$^{+}/^{+}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{sod}/^{sod}$</td>
<td>0.19 (5/26)</td>
<td>0.54 (14/26)</td>
<td>0.27 (7/26)</td>
</tr>
<tr>
<td>$^{sod}/^{sod}$</td>
<td>0.40 (34/84)</td>
<td>0.49 (41/84)</td>
<td>0.11 (9/84)</td>
</tr>
<tr>
<td>$^{sod}/^{sod}$</td>
<td>0.02 (2/92)</td>
<td>0.28 (26/92)</td>
<td>0.70 (64/92)</td>
</tr>
</tbody>
</table>

Parental cross: *sod<sup>+</sup>/Balancer (FM6 or FM9) × *sod<sup>+</sup>/Y.

<sup>a</sup>Ovary has ovarioles but defective egg chambers.

<sup>b</sup>Ovary is disorganized, contains germ cells but no discernable ovarioles.

<sup>c</sup>No somatic or germline gonadal tissue present, oviducts end in nubs.

<sup>d</sup>Because *l(1)EA42* is a recessive lethal, homozgyous and hemizgyous *l(1)EA42* flies were obtained in combination with the duplication *Dp(1,3)snf<sup>154</sup>*. Parental cross: *l(1)EA42/FM6 × l(1)EA42/Y; Dp(1,3)snf<sup>154</sup>/TM6*. Full genotypes: *sod<sup>+</sup> = y <i>c</i> <i>v</i> sod<sup>+</sup> <i>v</i> /<i>v</i>; *sod<sup>+</sup> <i>v</i> = y <i>c</i> sod<sup>+</sup> <i>v</i> /<i>v</i>; *sod<sup>+</sup> = y <i>c</i> sod<sup>+</sup> <i>v</i> /<i>v</i>; *EA42 = l(1)EA42; sod<sup>+/<sup>154</sup></i> = <i>y</i><sup>118</sup>sod<sup>+/<sup>154</sup></i>; sod<sup>+/<sup>154</sup></i> = <i>y</i><sup>118</sup>sod<sup>+/<sup>154</sup></i>.

**TABLE 2**

Effect of *sod* mutations on male sterility

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% fertile males</th>
<th>Average no. progeny/male&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{yw}/^{yw}$</td>
<td>87 (20/23)</td>
<td>76.8 ± 6.7</td>
</tr>
<tr>
<td>$^{yw}/^{yw}$</td>
<td>100 (32/32)</td>
<td>90.4 ± 3.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± SE.

develop from some female-sterile mutations, including Sex-lethal and ovarian tumor (King 1979; King and Riley 1982; Perrimon et al. 1986; Salz et al. 1987) (Figure 1D). The tumorous cysts are filled with hundreds to thousands of small germ cells that fail to undergo female differentiation. The mutant egg cysts also frequently contain irregularly shaped pycnotic nurselike cells that may represent abnormal development or cell death (Figure 1, B and C).

In contrast to the ovary, testis morphology is not affected by any combination of the three *sod* alleles. *sod<sup>-</sup>* males are fertile and contain mature testes that have wild-type pigmentation and coiling. No morphological aberrations in spermatogenesis can be discerned. To examine more subtle effects on spermatogenesis, males mutant for *sod<sup>+</sup>* were individually crossed to *sod<sup>-</sup>* females to test for fertility and fecundity. We found no increase in male sterility nor was the number of progeny produced by each male deleteriously affected (Table 2). Therefore, it appears the female-sterile alleles of *sod* do not have a male function.

**Germline clonal analysis demonstrates that *sod* is somatic-line dependent:** Mutations in the *sod* gene affect the development of both somatic and germline ovarian cells. This could reflect a requirement in both tissues for *sod* expression. Alternatively, it is possible that *sod* expression is required only one tissue but is required in a cell nonautonomous manner in the other. To examine this question, we created mosaic females in which the somatic tissue carried a wild-type *sod* allele and the germ line was homozygous mutant. If the mosaic animals remain sterile, this would indicate that the requirement for *sod* activity in female germ cells is cell autonomous. Alternatively, the production of progeny would mean that *sod* expression in the somatic tissue is sufficient to support oogenesis even in the absence of germline *sod* function.

Germline clones were generated by the dominant female-sterile technique in first instar larvae (Perrimon and Gans 1983). Flies heterozygous for *sod<sup>-</sup>* and the dominant female sterile mutation, ovo<sup>Di</sup>, were irradiated to induce mitotic crossing over. In the absence of recombination, the adult females are sterile due to the presence of ovo<sup>Di</sup>. Mitotic crossing over proximal to both the dominant female-sterile mutation and *sod* will produce recombinant germline cells that are homozygous for *sod<sup>-</sup>* and wild-type for the *ovo* gene. If the
Alternatively, a cell autonomous germline requirement for expression of crossover event proximal to ment is controlled by the somatic sex regulatory on germ cell morphology. Therefore, the somatic expression of fertility despite the severe effects of derived from a.

Iv. development of the somatic ovary and the completion of oogenesis indicates that activity would preclude the induction of fertile clones. All progeny carried the allele and were visible, indicating they were derived from ovo+ sou− ovo− sou+ germ cells.

All progeny carried the allele, indicating they were derived from a sou+ ovo− ovo+ sou+ germ cell.

expression of sou is not required in the germline, then these cells should be able to produce functional eggs. Alternatively, a cell autonomous germline requirement for sou activity would preclude the induction of fertile sou− clones. As shown in Table 3, fertile clones were induced with the sou−, sou+ and sou+ alleles, indicating that sou function is not required in the female germline for fertility despite the severe effects of sou mutations on germ cell morphology. Therefore, the somatic expression of sou, at least from the time of clonal induction (first instar larvae), is sufficient to allow female germline development.

The requirement for sou activity in ovarian development is controlled by the somatic sex regulatory genes: The sex specificity of sou function for both the development of the somatic ovary and the completion of oogenesis indicates that sou is ultimately responding to the X:A ratio, the initial signal of sex determination. This can occur in one of two ways. Most aspects of the sexually dimorphic somatic differentiation of the gonad are regulated by the activities of the transformer (tra), transformer-2 (tra-2) and doublesex (d sx) genes (Baker and Ridge 1980; Belote and Baker 1982; Wieschaus and Nöthiger 1982). Although clonal analysis has demonstrated that tra, tra-2 and d sx activity are not required in the female germline for oogenesis (Marsh and Wieschaus 1978; Schöpbach 1982), these genes are similar to sou in that mutations in them cause necrosis and aberrant germ cell morphologies that are the indirect effect of disrupted female somatic development (McKeown et al. 1988; Steinmann-Zwicky et al. 1989; Steinmann-Zwicky 1994). These observations could be explained by a mechanism where the somatic sex regulatory genes determine the state of sou activity, which in turn is required for the formation of the somatic ovary and the support of X/X germ cell development.

Alternatively, sou may respond to the the X:A ratio by a pathway independent of that controlled by tra, tra-2 and d sx. At least one such pathway is known to exist for dosage compensation in males (Baker and Belote 1983; Lucchesi and Manning 1987). The male-specific lethal genes (msls) control the hypertranscription of the male X chromosome but appear to have no role in females (Belote and Lucchesi 1980; Kuroda et al. 1991). The functions of these genes are not affected by mutations in tra, tra-2 or d sx, indicating a separate mechanism for their sex-specific activity. The same may also be true for the regulation of sou.

One direct way to examine how sex-specific sou activity is regulated is by determining whether the loss of sou activity can alter the gonad mutant phenotypes associated with tra, tra-2 or d sx mutations. Loss of function mutations in tra or tra-2 and certain allele combinations of d sx result in chromosomally female (X/X) flies developing somatically as males (Fujihara et al. 1978; Baker and Ridge 1980). These X/X “pseudomales” produce malelike somatic testes (pseudotestes) that contain a degenerating and morphologically aberrant germ cell population (Figure 2A) (Brown and King 1961; Belote and Baker 1982). If sou is regulated by the somatic sex regulatory genes to promote ovarian development, then X/X flies transformed to a male identity by mutations in tra, tra-2 or d sx will not express the sou ovarian activity even if the sou− allele is present. Therefore, the presence of sou mutations in these pseudomales should have no effect on the pseudotestis phenotype. Alternatively, if sou responds to the the X:A ratio by a pathway independent of that controlled by tra, tra-2 or d sx, then the pseudotestis will still require sou function because of its X/X genotype. In this case, we would expect that sou mutations will disrupt the development of the X/X pseudotestis in a manner analogous to sou mutant X/X ovaries.

Our results indicate that the sou product is required in somatic cells undergoing ovarian development regardless of their X:A ratio. Typically, sou− pseudotestes are short with most of the germ cells located apically and in an undifferentiated and degenerating state (Figure 2A). sou− pseudotestes have a range of gonadal phenotypes that are indistinguishable from those seen in sou+ pseudomales (compare Figure 2A with B). This

<table>
<thead>
<tr>
<th>Genotype of</th>
<th>No.</th>
<th>Fertile mitotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>irradiated</td>
<td>irradiated</td>
<td>crossovers</td>
</tr>
<tr>
<td>female*</td>
<td>females</td>
<td>resulting in sou−</td>
</tr>
<tr>
<td>I.</td>
<td>406</td>
<td>12† (sou− ovo−)</td>
</tr>
<tr>
<td>II.</td>
<td>142</td>
<td>8† (sou− ovo−)</td>
</tr>
<tr>
<td>III.</td>
<td>142</td>
<td>8 (sou− ovo−)</td>
</tr>
<tr>
<td>IV.</td>
<td>554</td>
<td>16† (sou− ovo−)</td>
</tr>
<tr>
<td>V.</td>
<td>391</td>
<td>20† (sou− ovo−)</td>
</tr>
</tbody>
</table>

*First instar larvae were irradiated from the crosses of I, y F M X X A Y; II, y F M X X A Y; III, y F M X X A Y; IV, y F M X X A Y; V, A Y X X A Y.
†Progeny derived from crossovers were f−, indicating a crossover event proximal to ovo+ and sou resulting in ovo− sou− ovo− sou− germ cells.
‡All progeny carried the sou− allele and were f+, indicating they were derived from ovo+ sou− ovo− sou− germ cells.
§All progeny carried the allele, indicating they were derived from a sou− ovo+ sou+ ovo+ germ cell.

TABLE 3

Germline clonal analysis
A Somatic-Dependent Ovarian Gene

FIGURE 2.—The effect of sou mutations on the gonads of X/X pseudomales. (A) Pseudotestis from X/X flies transformed into a somatic male by a mutation in the tra-2 gene. Germ cells (gc) proliferate into the adult stage but are largely undifferentiated. These flies have two copies of sou+. (B) sou-/sou2, tra-2 pseudotestes are indistinguishable from sou+/sou+ pseudotestes. (C) sou2/sou+ pseudotestis from sibling of fly shown in B. Flies of this genotype typically give rise to large testes with multiple clusters of germ cells distributed throughout the lumen of the gonad. (D) An extreme mutant phenotype often seen in sou2/sou2 pseudotestes is gonads that are largely devoid of germ cells. Even in this extreme case, the somatic gonad still develops. (E) X/Y testis from a tudur- mother. The tudom mutation acts maternally to block germ cell development in both male and female progeny. Even in the absence of germ cells, the somatic gonad can still develop. The nuclei of the preparations were stained by Feulgen reaction. t, testis; gc, germ cells.

is true even with the most severely affected pseudotestes. In the absence of sou activity, many of the pseudotestes contain few germ cells, resulting in gonads that appear as elongated collapsed tubes (Figure 2D). We compared the morphology of these pseudotestes with those resulting from males derived from tudor- mothers. Mutations in tudor cause females to give rise to progeny that completely lack germ cells (Boswell and Mahowald 1985). X/Y testes that are somatically normal but lack a germline are virtually indistinguishable from the severe sou- X/X pseudotestes (Figure 2E). These results indicate that X/X mesodermal cells that give rise to the somatic gonad do not require sou activity when developing as testes.

Support for this conclusion comes from the complementary experiment in which X/Y flies were transformed into “pseudofemales” by the ectopic expression of the tra gene. A transgenic fly strain was obtained that carried a construct in which the female-specific tra cDNA was fused to the hsp83 promoter (from the laboratory of Dr. P. Schedl, Princeton University). At 25°, X/Y flies carrying one copy of this construct developed somatically as females. The ovaries of these X/Y pseudofemales generally produce egg cysts that contain hundreds of small undifferentiated cells (McKeown et al. 1988) (Figure 3A). This phenotype is very similar to the “ovarian tumor” cysts resulting from mutations in the olu gene. When X/Y pseudofemales were made sou-, the resulting gonads were severely deformed in a manner similar to sou- X/X ovaries (Figure 3B). They lacked ovarioles and often the oviducts ended in “nubs” (see Figure 1D). These observations demonstrate that the sex-specific requirement for sou in gonadal development is controlled by the somatic sex regulatory genes, tra, tra-2 and dxx.

In addition to the abnormal development of the somatic ovary, sou mutations also cause morphological aberrations and necrosis in X/X germ cells. We were interested in determining whether X/X germ cells developing in a male soma still required sou function. This was tested by examining the morphology of the germ cells produced in pseudotestes carrying different com-
Figure 3.—The effect of sov mutations on the gonads of X/Y pseudomales. (A) Pseudoovaries produced by the expression of the tra gene in an X/Y fly. The tra structural sequence was fused to the Drosophila hsp83 heat shock promoter. At 25°, X/Y flies carrying a single copy of the hsp-tra construct are transformed to phenotypic females. The pseudoovaries contain egg cysts filled with hundreds of small, undifferentiated cells. (B) X/Y pseudoovary mutant for sm2. These ovaries are small and rarely contain egg cysts, resembling severe sov−, X/X ovaries. The nuclei of the preparations were stained by Feulgen reaction. ov, ovary; tc, tumorous cyst.

Combinations of wild-type and sov− alleles. We classified the pseudotestes into three phenotypic categories. Group A is composed of agamic gonads in which few, if any, germ cells could be detected (Figure 2, D and E). Group B consists of gonads containing varying numbers of germ cells that appear either undifferentiated and degenerating or are arrested during early stages of spermatogenesis (Figure 2, A and B). Group C is represented by gonads carrying one or more polyploid cells that are morphologically similar to nurse cells (Figure 2C). These cells are often found in clusters separate from the less-differentiated germ cells and may represent an abortive attempt at oogenesis. We compared the pseudotestes phenotype of sov−/sov+ pseudomales with their sibling sov−/sov+ pseudomale gonads. Both genotypes were derived from the same parents and were grown simultaneously under identical culture conditions. Both in turn were compared with sov+ /sov+ pseudomales obtained in a separate set of crosses. Our results showed no consistent effect of different doses of

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Group A (agametic)</th>
<th>Group B (male or undifferentiated)</th>
<th>Group C (nurse-like cells)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ tra</td>
<td>0.07</td>
<td>0.89</td>
<td>0.04</td>
<td>82</td>
</tr>
<tr>
<td>+ tra⁻²⁻</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sov²⁻⁻</td>
<td>0.05</td>
<td>0.92</td>
<td>0.03</td>
<td>157</td>
</tr>
<tr>
<td>sov²⁻⁻</td>
<td>0.03</td>
<td>0.80</td>
<td>0.17</td>
<td>113</td>
</tr>
<tr>
<td>sov²⁻⁻</td>
<td>0.01</td>
<td>0.99</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>sov²⁻⁻</td>
<td>0.01</td>
<td>0.59</td>
<td>0.40</td>
<td>154</td>
</tr>
<tr>
<td>sov²⁻⁻</td>
<td>0.24</td>
<td>0.67</td>
<td>0.09</td>
<td>70</td>
</tr>
<tr>
<td>+ dsx²⁻⁻</td>
<td>0</td>
<td>0.78</td>
<td>0.22</td>
<td>54</td>
</tr>
<tr>
<td>sov²⁻⁻</td>
<td>0</td>
<td>0.80</td>
<td>0.20</td>
<td>50</td>
</tr>
<tr>
<td>sov²⁻⁻</td>
<td>0.10</td>
<td>0.57</td>
<td>0.33</td>
<td>40</td>
</tr>
</tbody>
</table>

Results are given as ratio (observed pseudotestes:total). Genotypes: sov²⁻⁻ = y ev sov²⁻⁻ f. tra⁻²⁻ = kar² ry² tra⁻²⁻ red. + = FM6.tra⁻²⁻ = cn² tra⁻²⁻ bw. dsx²⁻⁻ = dsx²⁻⁻ f.
ssov activity on the viability or differentiation of the X/ X germ cells developing in pseudotestes (Table 4). Although there was substantial variability in the distribution of the phenotypic classes, no convincing pattern emerged in pseudomales resulting from mutations in tra, tra-2 or dsx. Therefore, although X/X germ cells require somatic ssov activity when developing in ovaries, this is not the case when developing in a somatic testis. This indicates that the male soma can support the viability of the X/X germline independent of ssov function.

A curious result was seen in tra-2 mutant pseudotestes heterozygous for ssov (Figure 2C; Table 4). Forty percent of the gonads produced by this genotype contained clusters of nurrselike cells and similar high frequencies of this phenotype were consistently attained in multiple experiments with other tra-2 allele combinations (data not shown). In contrast, nurrselike cells were only rarely found in sibling ssov/ssov tra-2 mutant pseudomales or in ssov/+ ssov control pseudomales (Table 4). dsx-derived pseudomales also produced a substantial proportion of gonads (20–30%) that contained one or more clusters of nurrselike cells, although in this case the phenotype was independent of the dosage of ssov+. The reason for these consistent shifts toward female germline differentiation is not clear but may reflect the effects of genetic background or the degree of male transformation caused by the alleles used.

The cytological and genetic mapping of the ssov gene: Three female-sterile alleles of ssov, ssov1,2,3, were isolated from a single EMS mutagenic screen (Mohler 1977). Recombination mapping experiments localized the ssov mutations to recombinant map position 18.5 on the X chromosome, placing it in the vicinity of cytological region 6C (Mohler and Carroll 1984). The ssov mutations can be complemented by the deletions Df(1)N73, Df(1)ctJ6, In(1)cm-df and In(1)G4EL24IR (Figure 4). In addition, two duplications were tested for their ability to rescue ssov. Neither a duplication of 3F3–5E8 (Tp(1;2)rb71g) nor a duplication of 6D-7C (Dp(1;3)sn13a1) included the ssov gene. This places ssov in the 6B-6C region (Figure 4).

Our initial studies suggested that the ssov female-sterile mutations were allelic to a lethal mutation that mapped in the cytological interval between 6D1 and 6D7. We tested ssov alleles against an EMS-induced lethal allele of l(l)EA42. The lethal mutation was viable in all combinations with the ssov alleles but resulted in female sterility and the formation of rudimentary ovaries. This inability to complement ssov mutations indicates that the l(l)EA42 chromosome is mutant for ssov function. However, we found that the l(l)EA42 lethality is separable from the ssov mutant phenotype. The Dp(1;3)sn13a1 duplication does not carry the ssov gene, as shown by its inability to suppress the mutant phenotype of female-sterile ssov alleles. However, this duplication can rescue the l(l)EA42 lethality, although the surviving females are sterile and display an ovarian phenotype similar to severe ssov mutations (Table 1). These data suggest that the ssov and l(l)EA42 mutations may map to separate genes.

The ssov gene is associated with recessive lethality: A mutagenic screen was performed that was designed to isolate both sterile and lethal mutations on the X chromosome. Some 756 recessive lethals and 66 female-sterile EMS-induced mutations were individually tested for allelism with ssov. The lethals were identified by the absence of hemizygous males. It was not possible to test the homozygous condition to confirm that they are also lethal in females. Two of the lethal lines gave rise to sterile females when made heterozygous with ssov and
are designated 

\[ \text{sou}^{\text{ML.150}} \text{ and } \text{sou}^{\text{ML.185}} \]. We believe that the lethal phenotype associated with \( \text{sou}^{\text{ML.150}} \text{ and } \text{sou}^{\text{ML.185}} \) is due to the disruption of \( \text{sou} \) function. Recombination mapping of the lethality of both \( \text{sou}^{\text{ML.150}} \) and \( \text{sou}^{\text{ML.185}} \) place the mutations to within five map units of the \( \text{sou} \) gene (data not shown) and neither lethal allele are rescued by nearby duplications that do not contain the \( \text{sou} \) locus (\( \text{Trp}(1;2) \text{rb}^+71 \text{g} \text{ and } \text{Dp}(1;3) \text{sn}^{134/1} \)). In complementation tests with \( (1;1) \text{EA42} \), both \( \text{sou}^{\text{ML.150}} \) and \( \text{sou}^{\text{ML.185}} \) complement the nonsex-specific lethality of \( (1;1) \text{EA42} \) but not the female sterility. Females that are \( \text{sou}^{\text{ML.150}} / (1;1) \text{EA42} \) and \( \text{sou}^{\text{ML.185}} / (1;1) \text{EA42} \) are fully viable, but most fail to produce ovaries (\( >95\% \), Table 1). The complementation between \( (1;1) \text{EA42} \) and lethal alleles of \( \text{sou} \) support the contention that \( (1;1) \text{EA42} \) is closely linked but not associated with the \( \text{sou} \) gene. Therefore, we tentatively designate the \( \text{sou} \) mutation on the \( (1;1) \text{EA42} \) chromosome as \( \text{sou}^{-} \).

When the \( \text{sou} \) female-sterile alleles were made heterozygous for \( \text{sou}^{\text{ML.150}} \) or \( \text{sou}^{\text{ML.185}} \), the mutant ovarian phenotype became more severe. In \( \text{sou}^{-} \) homozygotes, \( >10\% \) of the females completely lack one or both ovarian lobes and \( \sim 50\% \) of the ovaries had a disorganized structure in which ovarioles were not detected (Table 1). When \( \text{sou}^{-} \) was made heterozygous with \( \text{sou}^{\text{ML.150}} \) or \( \text{sou}^{\text{ML.185}} \), there was a substantial increase in the frequency of females absent one or both ovary lobes. The increase in the severity of the average mutant phenotype is consistent with \( \text{sou}^{-} \) and \( \text{sou}^{\text{ML.185}} \) eliminating \( \text{sou} \) activity. From these results we propose that the \( \text{sou} \) lethal alleles represent null mutations in which \( \text{sou} \) activity is completely blocked, whereas the viable female-sterile alleles are hypomorphic lesions that either specifically disrupt an ovarian specific \( \text{sou} \) product or reduces \( \text{sou} \) function such that only ovarian development is affected.

**DISCUSSION**

**\( \text{sou} \) is a somatic function required for both somatic and germline ovarian development:** The \( \text{sou} \) gene is essential for the development of the somatic ovary as well as the female germline. Mutations in \( \text{sou} \) result in a dramatic decrease in the size of the ovary, in the most severe cases causing the complete absence of the female gonad in the adult fly. In the intermediate phenotypes, the mutant ovaries display varying degrees of disorganization consistent with aberrant somatic development, including the absence of ovarioles, the formation of fused egg cysts and misshapen yolk egg chambers. The effects of \( \text{sou} \) mutations on the morphology and development of the germline are equally severe. \( \text{sou} \) mutant egg cysts generally contain abnormal numbers of nurse cells and rarely develop a recognizable oocyte. Many cysts carry pyknotic nuclei that likely represent instances of nurse cell degeneration. Less frequently, \( \text{sou} \) mutations can result in the formation of “tumorous cysts” similar to that seen in ovaries mutant for certain alleles of the ovarian tumor gene. These egg chambers contain hundreds of small undifferentiated germ cells that fill the entire egg chamber. These studies demonstrate that \( \text{sou} \) is required for the development of the somatic organization of the ovary and can also influence the viability and differentiation of the \( X/X \) germline.

Despite the severe effects of \( \text{sou} \) mutations on oogenesis, mosaic studies indicate that germ cells that are made \( \text{sou}^{-} \) during the embryonic and early larval stages can develop into functional oocytes. This can result from either one of two mechanisms. The first possibility is that the germline requires \( \text{sou} \) activity during the embryonic period. By the time the \( \text{sou} \) germ line clones are made during the larval stages, the requirement for \( \text{sou} \) expression has passed. Alternatively, the \( \text{sou} \) gene may need to be expressed only in the somatic tissues, which, in a cell nonautonomous manner exerts an essential function on germline development. We believe that the latter interpretation is more likely because the morphological examination of \( \text{sou} \) mutant ovaries indicate that relatively late stages in oogenesis are affected. For example, aberrations in the number and morphology of nurse cells and the increase in nurse cell mortality suggest that the \( \text{sou} \) mutations affect nurse cell/oocyte differentiation and viability, processes that occur late in larval development. This suggests that \( \text{sou} \) function is required well after embryogenesis and past the time of clonal induction.

**\( \text{sou} \) is essential for organismal viability:** The deleterious effects of the female-sterile \( \text{sou} \) alleles appear to be completely sex specific. Examination of male fertility, fecundity and testis morphology failed to demonstrate any effect of these alleles on male gonadal development. However, we believe that \( \text{sou} \) is essential for the viability of the fly. In a mutagenic screen for \( X \)-linked lethals and steriles, the only two \( \text{sou} \) alleles isolated were associated with a recessive lethality. When the female-sterile \( \text{sou} \) alleles are made heterozygous with either lethal allele, the females are viable but their ovarian mutant phenotype becomes more severe than when homozygous. We therefore believe that the female-sterile alleles represent hypomorphic mutations in what is an essential gene for males and probably females as well. In this regard, \( \text{sou} \) appears similar to the neurogenic genes \( \text{Notch} \) and \( \text{Delta} \). Null mutations in either of these loci result in embryonic lethality due to disruptions in neurogenesis. Hypomorphic alleles of either \( \text{Notch} \) or \( \text{Delta} \) can cause female sterility associated with defects in the differentiation of the somatically derived follicle cells. Because the focus of this study is on the role of \( \text{sou} \) on ovarian development, a detailed study of the lethal phenotype will be presented elsewhere.

**The ovarian requirement for \( \text{sou} \) is regulated by the somatic sex differentiation genes:** In flies with two \( X \)...
chromosomes and two sets of autosomes, the \textit{tra} gene is expressed and acts with \textit{tra-2} to control the expression of the \textit{dsx} gene, which in turn regulates the differentiation of female-specific somatic structures. This includes the development of the female gonad from certain mesodermal cells that surround the embryonic germline. In the absence of \textit{tra} or \textit{tra-2} function, or with certain combinations of \textit{dsx} alleles, these same mesodermal cells take on a male identity and give rise to somatic testes. However, not all sexually dimorphic processes are controlled by the action of these somatic sex regulatory genes. For example, the twofold difference in \textit{X}-linked gene expression between male and female flies, that is, dosage compensation, is regulated by a different genetic pathway involving male-specific lethal genes (reviewed in Luccchesi and Manning 1987). Similarly, germline sexual differentiation, the choice between spermatogenesis and oogenesis, requires germline-specific genes that act independent of \textit{tra}, \textit{tra-2} and \textit{dsx} (reviewed in Parkhurst and Meneely 1994). Further complexity is demonstrated by the finding that the sex-specific development of certain male abdominal muscles depends on the activity state of \textit{tra} and \textit{tra-2} but not \textit{dsx} (Taylor 1992). These observations indicate that although each sex-specific process is ultimately controlled by the \textit{X}:\textit{A} ratio, they depend on different regulatory loci for the regulation of subsequent steps in sexual differentiation.

We were interested in determining the basis for the sex-specific requirement for \textit{sov} activity in gonad development. Using flies sexually transformed because of mutations in the somatic sex regulatory pathway, we found that both \textit{X}/\textit{Y} and \textit{X}/\textit{X} mesodermal cells require \textit{sov} activity for the development of ovaries but neither cell type requires \textit{sov} for testis development. This indicates that the \textit{sov} requirement in ovarian development can be separated from the \textit{X}:\textit{A} ratio by mutations in the somatic sex regulatory genes. Therefore, in the mesodermal precursors to the somatic gonads, the somatic sex regulatory genes \textit{tra}, \textit{tra-2} and \textit{dsx} must act before \textit{sov} to initiate sex-specific gonadal differentiation. The \textit{sov} gene then responds to the sexual state of soma such that only cells with a female identity require the ovary-specific \textit{sov} function. This sex- and ovary-specific regulation contrasts with the male, and presumably female, lethality associated with the absence of \textit{sov} activity. Therefore, it appears that the \textit{sov} viability and ovarian functions are controlled by different genetic pathways.

**\textbf{X}/\textbf{X} germ cells do not require \textit{sov} activity if developing in a male soma:** Our findings also provide some insight into the sex-specific interactions between the soma and germline that affect the viability and differentiation of the germ cells. In \textit{X}/\textit{X} flies, \textit{sov} mutations result in aberrant differentiation of the female germline and an increase in germ cell necrosis. Because \textit{sov} function is soma dependent, this effect on the germline must reflect some undefined interaction between the somatic cells and germline that is essential for oogenesis. In the absence of sufficient \textit{sov} activity in the soma, this interaction is disrupted and germline development is arrested. However, we find that if the \textit{X}/\textit{X} soma is transformed to a male differentiated state, then \textit{X}/\textit{X} germ cell proliferation can occur and is not affected by reductions in \textit{sov} activity. This suggests that the pseudo-ovaries can support \textit{X}/\textit{X} germ cell viability and proliferation by a mechanism different than that which occurs in a female differentiated somatic gonad. A similar effect occurs in the reciprocal experiment. When \textit{X}/\textit{Y} flies are transformed to a somatic female differentiated state, the pseudo-ovaries are capable of supporting substantial proliferation of the \textit{X}/\textit{Y} germ cells (Figure 3A). In the absence of \textit{sov} activity however, the pseudo-ovaries contain few germ cells (Figure 3B). This indicates that ovarian somatic tissue, even if \textit{X}/\textit{Y}, cannot support the proliferation or viability of either \textit{X}/\textit{Y} or \textit{X}/\textit{X} germ cells in the absence of \textit{sov} activity.

In conclusion, sex determination and differentiation in Drosophila are initiated by the interpretation of the \textit{X}:\textit{A} ratio and is subsequently controlled by a hierarchy of regulatory genes that have progressively greater specificity in their actions. The \textit{X}:\textit{A} gene is required systemically for all aspects of sexually dimorphic characteristics, whereas the \textit{tra}, \textit{tra-2} and \textit{dsx} genes are limited to controlling sexual differentiation in the somatic tissue. The sex-specific instructions imposed by \textit{tra}, \textit{tra-2} and \textit{dsx} must in turn be read by a set of genes that have more defined roles in the differentiation of specific sexually dimorphic tissues. We believe that \textit{sov} represents one of these genes. We propose that \textit{sov} participates in the development of the somatic ovary in response to the sexually differentiated state of the gonadal precursors. This function of \textit{sov} has apparently been appropriated for other developmental pathways as well because \textit{sov} is also essential for both male and female viability. Both the ovarian and viability functions of the \textit{sov} gene are under investigation.

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**LITERATURE CITED**
