Sex determination in the germ line of *Drosophila melanogaster*: activation of the gene *Sex-lethal*

Begoña Granadino¹, Pedro Santamaria² and Lucas Sánchez¹

¹Centro de Investigaciones Biológicas, Velázquez 144, 28006 Madrid, Spain
²Centre de Génétique Moleculaire du CNRS, 91198 Gif-sur-Yvette Cedex, France

**SUMMARY**

The germ line exhibits sexual dimorphism as do the somatic tissues. Cells with the 2X;2A chromosome constitution will follow the oogenic pathway and X;2A cells will develop into sperm. In both somatic and germ-line tissues, the sexual pathway chosen by the cells depends on the sex of the cells is autonomously determined by the X:A signal while, in the germ line, the sex is determined by cell autonomous (the X:A signal) and somatic inductive signals. Three X-linked genes have been identified, *scute (sc)*, *sisterless-a (sis-a)* and *runt (run)*, that determine the initial functional state of *Sxl* in the soma. Using pole cell transplantation, we have tested whether these genes are also needed to activate *Sxl* in the germ line. We found that germ cells simultaneously heterozygous for *sc, sis-a, run* and a deficiency for *Sxl* transplanted into wild-type female hosts develop into functional oocytes. We conclude that the genes *sc, sis-a and run* needed to activate *Sxl* in the soma seem not to be required to activate this gene in the germ line; therefore, the X:A signal would be made up by different genes in somatic and germ-line tissues. The *Sxl¹⁷M¹/Sxl¹⁹* females do not have developed ovaries. We have shown that germ cells of this genotype transplanted into wild-type female hosts produce functional oocytes. We conclude that the somatic component of the gonads in *Sxl¹⁷M¹/Sxl¹⁹* females is affected, and consequently germ cells do not develop. This result supports the existence of a somatic positive feminizing signal for germ-line development. The activation of *Sxl* in the germ line would be controlled by cell-autonomous genetic factors (the X:A signal) and a positive feminizing function from the female gonadal soma.

Key words: *Drosophila*, germ line, sex determination, *Sex-lethal*

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**INTRODUCTION**

In *Drosophila melanogaster*, sex determination is under the control of the gene *Sex-lethal (Sxl)*. The functional state of *Sxl* is determined by the ratio of the number of X chromosomes to autosomal sets (X:A); in 2X;2A flies *Sxl* will be ON, while in X;2A flies *Sxl* will be OFF (Cline, 1978). Activation of *Sxl* also requires the maternal *daughterless (da)* product (Cline, 1978). At the time that this X:A signal activates *Sxl* in somatic cells, around blastoderm stage (Sánchez and Nöthiger, 1983; Bachiller and Sánchez, 1991), germ cells do not show *Sxl* expression (Bopp et al., 1991). Two elements of this X:A signal have been identified: *sisterless-a (sis-a)* (Cline, 1986) and a region of the *Achaete-scute* complex that has been named *sisterless-b (sis-b)* (Cline, 1988) and which corresponds to the gene *scute (sc)* (Torres and Sánchez, 1989, 1991; Parkhurst et al., 1990; Erickson and Cline, 1991). Recently, it has been shown that the segmentation gene *runt (run)* is also required for *Sxl* activation in females (Duffy and Gergen, 1991; Torres and Sánchez, 1992). Moreover, it has been shown that the neurogenic gene *deadpan (dpm)* acts early in development as a denominator element of the X:A signal, and that the maternal product of another neurogenic gene, *extra-macrhoaetae (emc)*, acts as a negative regulator of *Sxl* (Younger-Shepherd et al., 1992).

The germ line exhibits sexual dimorphism as does somatic tissue. Cells with the 2X;2A chromosomal constitution will follow the oogenic pathway and X;2A cells will develop into sperm. However, the genetic control of the sexual development of the germ line differs from that of the soma. Cell-autonomous and somatic inductive signals determine the sex of the germ line, by regulating the gene *Sxl* (Steinmann-Zwicky et al., 1989; Nöthiger et al., 1989), whose activity is required for normal female germ cell development (Cline, 1983; Schüpbach, 1985; Steinmann-Zwicky et al., 1989; Nöthiger et al., 1989). The existence of two SXL proteins specifically associated with the development of the female-germ line has been reported (Salz et al., 1989). Female germ cells mutant for the X-linked gene *sans-fille (sfn)* (liz in Steinmann-Zwicky’s terminology, 1988) enter the spermatogenic pathway, while mutant male germ cells are not affected (Oliver et al., 1988; 1990; Steinmann-Zwicky, 1988). These mutant females lack the two germ-line-specific SXL proteins (Salz, 1992), leading to the conclusion that *sfn* is needed to activate *Sxl* in the female germ...
line. However, snf, itself, seems not to behave as an element of the X:A signal operating in this tissue, since females heterozygous for a deficiency for snf are fertile.

The first goal of the experiments reported here was to determine whether or not the genes involved in the activation of Sxl in the soma (sc, sis-a and run) are also involved in the activation of Sxl in the germ line.

In the analysis of different Sxl mutant alleles, it was found that females homozygous for Sxl<sup>7M1</sup> develop as sterile males (Cline, 1984) and that females homozygous for Sxl<sup>f7M1</sup> have reduced viability (Granadino et al., 1991). Nevertheless, germ cells homozygous for Sxl<sup>7M1</sup> (Cline, 1984; Schüpbach, 1985), or Sxl<sup>f7M1</sup> (Granadino et al., 1991) give rise to functional oocytes when they are in a wild-type soma. However, females of genotype Sxl<sup>7M1/Sxl<sup>f7M1</sup></genotype have a reduced viability, and those that survive do not have well-developed ovaries. The second experiment reported here was aimed at establishing whether the genotype Sxl<sup>7M1/Sxl<sup>f7M1</sup></genotype is affecting the development of the gonadal soma or the development of the germ line.

**MATERIALS AND METHODS**

Flies were cultured on standard food at 25°C unless otherwise stated. For full description of markers and chromosomes used, see Lindsley and Zimm (1992). Pole cells were transplanted according to Santamaría’s (1986) procedure. The transplantation of pole cells was carried out at 18°C, and the injected embryos were kept at this temperature until the larvae hatched. These were then transferred to vials with Drosophila food and cultured at 25°C for the rest of their development. The adult hosts were individually test crossed to determine the genotype of the donor germ cells.

**RESULTS**

**Transplantation of pole cells heterozygous for Sxl, sc, sis-a and run**

Both sc<sup>-Xxl-sis-a<sup>run<sup>+</sup></genotype +sc<sup>+</sup>Xxl<sup>+</sup>sis-a<sup>+</sup>run<sup>+</sup> and sc<sup>-Xxl-sis-a<sup>run<sup>+</sup></genotype +sc<sup>+</sup>Xxl<sup>+</sup>sis-a<sup>run<sup>+</sup></genotype females die as embryos, due to a failure to activate Sxl, as a consequence of an alteration in the X:A signal (Cline, 1988; Torres and Sánchez, 1989, 1991, 1992; Parkhurst et al., 1990; Duffy and Gegen, 1991). We transplanted the pole cells of these female embryos into wild-type female embryos lacking their own pole cells to check their capability of giving rise to functional oocytes (for details of crosses and experimental procedure see Footnote to Table 1). The purpose of introducing Sxl<sup>-</sup> (a deficiency for Sxl) was to sensitize the cell’s response to an altered signal for Sxl activation. The Sxl<sup>-</sup>/Sxl<sup>+</sup> constitution does not, by itself, prevent the development of pole cells, because females of this genotype are fully fertile.

We injected 1307 embryos from which we obtained 312 adults: 168 females and 144 males. Among the males, 20 hosts were fertile. 19 of these males received pole cells of genotype FM6/2<sup>y</sup>Y<sup>67g</sup>sc<sup>+</sup>. The remaining male contained motile sperm but we could not ascertain the genotype of the donor germ cells because he did not give rise to progeny. Presumably, this male received FM6/2<sup>y</sup>Y<sup>67g</sup>sc<sup>+</sup> pole cells, since pole cells of genotype Df(1)run<sup>1112</sup> are not able to develop into functional gametes (Lindsley and Zimm, 1992).

Table 1 presents the results of the female hosts. Among the 168 females, 14 hosts were fertile. One female laid a small number of eggs that did not develop, so that we could not determine the genotype of the pole cells that she received. Of the remaining 13 females, 4 females received pole cells of genotype sc<sup>-Xxl-sis-a<sup>run<sup>+</sup></genotype +sc<sup>+</sup>Xxl<sup>+</sup>sis-a<sup>+</sup>run<sup>+</sup> and 9 females received pole cells of genotype sc<sup>-Xxl-sis-a<sup>run<sup>+</sup></genotype +sc<sup>+</sup>Xxl<sup>+</sup>sis-a<sup>run<sup>+</sup></genotype.

We conclude that the genes sc, sis-a and run seem not to be required to activate Sxl in the female germ line. Thus, the X:A signal in both somatic and germ-line tissues would be assessed by different genes.

**Transplantation of Sxl<sup>7M1/Sxl<sup>f7M1</sup></genotype pole cells**

As mentioned in the Introduction, females of genotype Sxl<sup>7M1/Sxl<sup>f7M1</sup></genotype do not have well-developed ovaries (Granadino et al., 1991). We have transplanted pole cells of this genotype into wild-type embryos lacking their own pole cells to check their capability of giving rise to functional oocytes (for details of crosses and experimental procedure see Footnote to Table 2). We injected 1127 embryos from which we obtained 330 adults: 190 females and 140 males. Among these males, 19 hosts were fertile: 8 males received FM7/Y pole cells and 11 males received Sxl<sup>7M1/Y</sup> pole cells.

Table 2 shows the results of the female hosts. Among the 190 females, 22 hosts were fertile: 12 females received Sxl<sup>f7M1</sup>/+ pole cells and 10 females received Sxl<sup>7M1/Sxl<sup>f7M1</sup></genotype.

We conclude that germ cells of genotype Sxl<sup>7M1/Sxl<sup>f7M1</sup></genotype...
Table 2. Transplantation of Sxl<sup>7M1</sup>/Sxl<sup>f</sup> pole cells

<table>
<thead>
<tr>
<th>Number of injected embryos</th>
<th>Number of female adult hosts</th>
<th>Total number of fertile females grouped according to the genotype of the implanted cells</th>
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<td>Number of fertile females</td>
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<td>Sxl&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>1127</td>
<td>190</td>
<td>22</td>
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To generate the host embryos we carried out the cross described in the Footnote to Table 1. The donor embryos come from the cross of cm Sxl<sup>7M1</sup>cf<sup>+/Sxl</sup> females with y Sxl<sup>+/cf</sup> males. The adult hosts were test-mated with Oregon-R flies. Female hosts that received cm Sxl<sup>7M1</sup>cf<sup>/y</sup> Sxl<sup>f</sup>-cf<sup>+</sup> pole cells were distinguished from the females that received FM7<sup>y</sup> Sxl<sup>f</sup>-cf<sup>+</sup> pole cells because the latter females gave rise to progeny with Bar phenotype due to the FM7 chromosome.

The Sxl<sup>7M1</sup>/Sxl<sup>f</sup> constitution specifically affects the gonadal soma

Adult females of genotype Sxl<sup>7M1</sup>/Sxl<sup>f</sup> do not have well-developed ovaries (Granadino et al., 1991). As both somatic and germ cells are necessary for the production of eggs, female-sterile mutations could affect either one or both of these cell lineages. In this report, we have shown that Sxl<sup>7M1</sup>/Sxl<sup>f</sup> germ cells transplanted into wild-type female hosts develop into functional oocytes. We conclude that the Sxl<sup>7M1</sup>/Sxl<sup>f</sup> females have affected the somatic component of the gonads, and consequently their germ cells do not develop. We cannot attribute the ovarian phenotype of these females to the death of their germ cells, since these were observed at the blastoderm stage and in the larval ovaries of the Sxl<sup>7M1</sup>/Sxl<sup>f</sup> females (Granadino et al., 1991). We cannot discard the possibility that the germ cells die later in development as a consequence of an alteration in the gonadal soma. Also females homozygous for loss-of-function mutations at the gene ovo do not have well-developed ovaries, as a consequence of the death of their germ cells (Oliver et al., 1987). The ovo mutations autonomously affect the development of the germ cells (Perrimon and Gans, 1983). Therefore, the ovarian phenotype in both Sxl<sup>7M1</sup>/Sxl<sup>f</sup> and ovo<sup>ovo</sup> females (none of these mutations affect the development of male germ cells) is the result of a different cause. It has been suggested that the gene ovo may be responsible for the reception, or the interpretation, of the inductive signal from the gonadal soma (Oliver et al., 1990). In this context, our results support the idea that the Sxl<sup>7M1</sup>/Sxl<sup>f</sup> females lack a positive feminizing signal from the female gonadal soma needed for XX germ cells to enter oogenesis.

The absence of this somatic signal in females Sxl<sup>7M1</sup>/Sxl<sup>f</sup> could be due to a failure of the gonadal somatic cells to produce it, or to the death of these gonadal cells, as a consequence of having perturbed its dosage compensation process. Although both possibilities can formally be posed, the first one seems more plausible for the following reason. The Sxl<sup>7M1</sup>/Sxl<sup>f</sup> constitution affects female viability; only 10% of them survive (Granadino et al., 1991). These ‘escapers’ show a wild-type female phenotype with the only occasional presence of a small male spot in the fifth or sixth tergites. Thus, these ‘escapers’ are those that express high levels of Sxl<sup>+<sup> activity to allow their survival and their development as females.

Activation of Sxl in the germ line

In the soma, the state of activity of Sxl is autonomously determined by the X:A signal (Sánchez and Nöthiger, 1983; Bachiller and Sánchez, 1991). Previous experiments demonstrated that, in the germ line, cell-autonomous and somatic inductive signals determine the functional state of Sxl (Steinmann-Zwicky et al., 1989; Nöthiger et al., 1989). Two alternative hypothesis have been put forward to explain the somatic effect on the sexual phenotype of the germ cells; either XX germ cells enter spermatogenesis unless they receive a feminizing signal from the female gonadal soma, or they enter oogenesis unless a masculinization signal from the male gonadal soma directs them into spermatogenesis (Steinmann-Zwicky et al., 1989; Nöthiger et al., 1989). We

The X:A signal differs in the soma and the germ line

Three X-linked genes have been identified whose function is required for the initial activation of Sxl in somatic cells: sc, sis-a and run (Cline, 1986; 1988; Torres and Sánchez, 1989; 1991; 1992; Parkhurst et al., 1990; Erickson and Cline, 1991; Duffy and Gergen, 1991). In this report, we questioned the necessity of these three genes to activate Sxl in the germ line. We analyzed the development of germ cells affected by mutations at the genes sc, sis-a and run. Simultaneous heterozygosity for sc, sis-a and run and a deficiency for Sxl is a severe constitution (even more severe than, for example, homozygosity for sc<sup>−</sup> alone), which causes somatic cell lethality as a consequence of a failure to activate Sxl (Torres and Sánchez, 1991). We found that germ cells heterozygous for sc, sis-a, run and a deficiency for Sxl transplanted into female hosts develop into functional oocytes. We conclude that the genes sc, sis-a and run needed to activate Sxl in the soma seem not be needed for the activation of this gene in the germ line.

Loss-of-function mutations at da and sc display female-specific dominant synergism, each enhancing the other’s sex-specific lethal effect (Cline, 1988; Torres and Sánchez, 1989). These genes encode helix-loop-helix (HLH) proteins (Villares and Cabrera, 1987; Caudy et al., 1988), capable of forming heterodimers (Murre et al., 1989a,b) and interacting with DNA (Murre et al., 1989b; Benzerza et al., 1990; Sun and Baltimore, 1991). Thus the SC and DA products might interact for the activation of Sxl in the soma. The exemption of the gene sc for the activation of Sxl in the germ line would agree with the fact that da is not needed either for the activation of Sxl in the germ line (Crommiller and Cline, 1987). Furthermore, Cline (1986) reported that clones of germ cells homozygous for sis-a develop into functional oocytes. All of these results indicate that the X:A signal in both somatic and germ-line tissues would be made up by different genes, and consequently the activation of Sxl in both tissues occurs differently.

develop into functional oocytes when they are in female wild-type ovaries.

DISCUSSION

The X:A signal differs in the soma and the germ line

The absence of this somatic signal in females Sxl<sup>7M1</sup>/Sxl<sup>f</sup> activity to allow their survival and their development as females.
have presented evidence for the existence of a positive feminizing somatic function in the development of the germ line. This would support the idea that the state of activity of Sxl is controlled by both cell-autonomous (the X:A signal) and a positive feminizing function from the female gonadal soma, rather than the existence of a testicular somatic signal repressing the function of Sxl in males.

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REFERENCES


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Note added in proof

It has recently been reported that XX germ cells mutant for *scut6-1* give rise to functional oocytes when transplanted into wild-type female hosts.