Sex determination in *Drosophila: sis-b, a major numerator element of the X:A ratio in the soma, does not contribute to the X:A ratio in the germ line*

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**SUMMARY**

In soma and germ cells of *Drosophila*, the X:A ratio builds a primary signal for sex determination, and in both tissues *Sex-let al* (Sxl) function is required for cells to enter the female pathway.

In somatic cells of XX animals, the products of X-chromosomal elements of the X:A ratio activate Sxl. Here I show that *sisterless-b (sis-b)*, which is the X-chromosomal element of the somatic X:A ratio that has best been analysed, is not required for oogenesis. I also present evidence that Sxl function might not be sufficient to direct germ cells into the female pathway. These results show that the elements forming the X:A ratio in the germ line are different from the elements forming the X:A ratio in the soma and they suggest that, in the germ line, Sxl might not be regulated by the X:A ratio.

Key words: germ cells, *Sex-let al*, *scute*, *sisterless-b*

**INTRODUCTION**

The sex of *Drosophila* germ cells is determined by a mechanism that is different from that acting in somatic cells (reviewed in Pauli and Mahowald, 1990; Steinmann-Zwicky, 1992a,b). XX cells enter the male pathway when developing in a male host animal. This shows that their sex is determined by induction. XY and XO cells, in contrast, form spermatocytes even when developing in a host ovary. They have an autonomous information for maleness and they do not respond to inductive signals (Steinmann-Zwicky et al., 1989). The sex of germ cells is thus determined by cell-autonomous and inductive signals. The sex of somatic cells, however, is determined solely by a cell-autonomous signal called the X:A ratio, which arises from relating the number of X chromosomes to the number of sets of autosomes (reviewed in Baker, 1989; Steinmann-Zwicky et al., 1990; Belote, 1992).

Both somatic tissue and germ cells require Sxl activity to enter the female pathway (Cline, 1978; Sánchez and Nöthiger, 1982; Schüpbach, 1985; Steinmann-Zwicky et al., 1989). In the soma, Sxl is regulated at the level of transcription (Torres and Sánchez, 1991; Keyes et al., 1992) and alternative splicing (Bell et al., 1988). Early femalespecific transcripts are found in embryos with an X:A ratio of 1. Two X-chromosomal elements of the X:A ratio, *sis-terless-a (sis-a)* and *sisterless-b (sis-b)* induce these early Sxl products together with the maternally provided transcription factor *daughterless (da)* and maybe other gene products. Later, the products of *fl(2)d, liz* (also called *fs(1)1621* and *snf*) and Sxl itself are required for maintaining Sxl active, probably for female-specific splicing of the Sxl pre-mRNA. XX animals that lack Sxl activity, or XX animals that lack *sis-a* or *sis-b*, die because both X chromosomes are transcribed at a high level, which is typical of the single X chromosome of males (Lucchesi and Skripsky, 1981; Cline, 1988; Steinmann-Zwicky, 1988; Granadino et al., 1990; Bell et al., 1991; Torres and Sánchez, 1991; Keyes et al., 1992; reviewed in Belote, 1992).

In germ cells, the products of *fl(2)d* and *liz* are also required for Sxl activity (Steinmann-Zwicky, 1988; Granadino et al., 1992; Salz, 1992). Little, however, is known about other genes regulating Sxl in the germ line. XX germ cells carrying the mutation SxlM1, which constitutively expresses functions of the gene *Sex-let al*, can become oogenic even when developing in a host testis (Steinmann-Zwicky et al., 1989). SxlM1 therefore provides XX germ cells with an autonomous information for female-ness and renders them insensitive to induction. This shows that the somatic inductive signal that determines the sex of XX germ cells exerts its action by regulating the gene Sxl.

Due to analogies to the situation in the soma, the cell-autonomous signal that renders XX germ cells sensitive to induction, while leaving XY and XO cells insensitive has been called ‘X:A ratio’ (reviewed in Steinmann-Zwicky, 1992a,b). Here I tested whether one of the elements forming the X:A ratio in somatic cells also participates in building the X:A ratio in germ cells. For this, I transplanted XX germ cells lacking *sis-b* function into host females. Such germ cells formed functional eggs, which shows that *sis-b* is not required for oogenesis. To test whether Sxl expression is sufficient to drive XY cells into the female pathway, I transplanted XY cells carrying the constitutive mutation SxlM4 into host animals of either sex. XY germ cells car-
rying this mutation did not become oogenic even when developing in ovaries.

MATERIALS AND METHODS

Pole cell transplantations

Pole cells were transplanted as described in Van Deusen (1976) and Steinmann-Zwicky et al. (1989). Agametic host embryos without germ cells were derived from mothers homozygous for osk^S01 kept at 18°C. Adult host flies were crossed to test partners. Sterile flies were dissected and their gonads were analysed with a microscope. Criteria used to identify the sex of germ cells are listed in Steinmann-Zwicky et al. (1989).

Stocks and alleles

The stock used to obtain XX embryos lacking sis-b function was: sc^{10-1}\^f^{10} FM6^{y} Y 67 g. To test the genotype of transplanted germ cells, adult host flies were individually crossed to y w f partners. Between 50 and 100 progeny from each fertile fly were scored.

To obtain donor XY embryos carrying Sxl^{M4} females of genotype cm Sxl^{M4}/FM7 were crossed to T(X;Y)22-3, y v f Y^{K} Rsp^{a} B^{9} Y^{l}; E(SD)Rsp^{a} bw/SD-ARM VO17 It males. These males carry mutations causing a segregation distortion so that they only transmit their Y chromosome (Walker et al., 1989).

To test the genotype of transplanted germ cells, I crossed each host male to three different types of females: (a) cm Sxl^{M4}/FM7, (b) y cm Sxl^{M4}/FM6, (c) cn bw. Host females were crossed to males of genotype cn bw. Mutations and balancer chromosomes are described in Lindsley and Zimm (1992).

RESULTS

The sis-b function is not required in the germ line

The X-chromosomal element of the X:A ratio that has best been analysed is sis-b (Cline, 1988; Torres and Sánchez, 1989; Erickson and Cline, 1991). The sis-b function is provided by one of the transcripts of the achaete-scute complex (AS-C), T4. The allele sc^{10-1} lacks all sis-b activity (Torres and Sánchez, 1989) since it contains a point mutation that places a stop codon within T4 (Villares and Cabrera, 1987). To test whether sis-b activity is required in the germ line, I investigated the developmental capacities of XX germ cells homozygous for sc^{10-1}. I transplanted pole cells from progeny of sc^{10-1}/FM6 females crossed to sc^{10-1}/Y males carrying a se^{+} duplication on their Y chromosome. From here on, the chromosome carrying sc^{10-1} will be called sis-b.

Table 1 shows the results of this experiment. 22 host females formed eggs and had therefore integrated XX germ cells. 4 of them did not lay their eggs, such that the genotype of these could not be identified. 7 females had progeny some of which carried the balancer chromosome FM6, showing that they had integrated germ cells of genotype sis-b/FM6. 11 fertile females, however, transmitted only the chromosome carrying sis-b to their progeny. These females had integrated germ cells that were homozygous for sis-b. The results show that germ cells do not require the sis-b function to enter or to complete oogenesis. Of the remaining females that had no progeny, 23 contained spermatocytes and had therefore integrated XY germ cells, 27 had empty ovaries, and 2 died during the test crosses.

24 host males produced sperm showing that they had integrated XY germ cells. 4 had no progeny, 4 transmitted the chromosome FM6 and 16 transmitted the chromosome carrying sis-b. 9 sterile males had spermatocytes in their testes and had therefore integrated XX cells. In one case, these displayed the crystals that are specifically formed by spermatogonial germ cells lacking a Y chromosome (Hardy et al., 1984; Livak, 1984; Steinmann-Zwicky et al., 1989). 26 males had empty gonads.

The fertile female and male hosts had integrated germ cells homozygous or hemizygous for sis-b more often than germ cells heterozygous or hemizygous for FM6. In the case of male hosts, this is especially striking. Either heterozygous females must transmit their sis-b chromosome more often than their FM6 chromosome, or XY embryos carrying FM6 might be selected against in the transplantation experiments, either because they develop at a different speed than their sis-b carrying brothers, or because many of them die before blastoderm. No biased transmission of chromosomes was observed in other transplantation experiments involving FM6 (Steinmann-Zwicky et al., 1989). When counting progeny from the experimental cross that were allowed to survive to adulthood, a small excess of males carrying the sis-b chromosome was observed. In one experiment, I counted 84 sis-b/Y males, 54 FM6/Y males and 70 sis-b/FM6 females.

XY germ cells carrying Sxl^{M4} are spermatogenic in host gonads

Sxl^{M1} and Sxl^{M4} are mutations that express female-specific Sxl functions even in the absence of factors normally required for Sxl expression. XX animals lacking da or liz product are rescued by both alleles (Cline, 1978; Maine et al., 1985; Steinmann-Zwicky, 1988; Salz, 1992). Since XX germ cells that lack liz function are also rescued (Steinmann-Zwicky 1988; Salz, 1992), we know that XX germ cells carrying Sxl^{M1} or Sxl^{M4} express Sxl functions without the requirement of liz.

<table>
<thead>
<tr>
<th>Number of injected host embryos</th>
<th>Number of adult host flies containing XX donor germ cells</th>
<th>Number of host flies containing XY donor germ cells</th>
<th>Number of host flies with empty gonads</th>
<th>Died</th>
</tr>
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<tbody>
<tr>
<td>975</td>
<td>74 F</td>
<td>22</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>59 F</td>
<td>9</td>
<td>7</td>
<td>4</td>
<td>16</td>
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</table>

Fertile host flies disclosed with their progeny the genotype of their germ cells.

Table 1. The sis-b function is not required in the germ line
XY animals carrying SxlM1 show no Sxl expression in early embryogenesis (Gergen, 1987), but they die as larvae and their X chromosome is only half as wide as that of control larvae, which probably reflects its hypoactivity (Lucchesi and Skrisky, 1981). In some cases, adult tissue shows female-specific traits (Cline, 1979). XY germ cells carrying SxlM1 are spermatogenic (Cline, 1983; Steinmann-Zwicky et al., 1989). This either means that Sxl is not expressed in these germ cells or that expressing Sxl is not sufficient for XY germ cells to become oogenic.

SxlM4 is still at least partially regulated by elements of the X:A ratio, as it is possible to make a stock in which introducing a females are

<table>
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<tr>
<th>Number of injected host embryos</th>
<th>Number of host flies containing XY donor germ cells</th>
<th>Number of host flies containing donor germ cells with 2 X chromosomes</th>
<th>Number of flies with empty gonads</th>
<th>Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>417</td>
<td>20 ♂</td>
<td>15 7 8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>17 ♀</td>
<td>9</td>
<td></td>
<td>1</td>
<td>6</td>
</tr>
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DISCUSSION

Three X-chromosomal elements, sis-a, sis-b and runt, are known to regulate the expression of Sxl in somatic cells in a dose-dependent manner (Cline, 1988; Torres and Sánchez, 1989, 1992; Duffy and Gergen, 1991). They are therefore called numerator elements of the X:A ratio. The products of sis-a and sis-b are probably transcriptional activators that control the expression of Sxl (Torres and Sánchez, 1991; Keyes et al., 1992). For the third element, the segmentation gene, runt, the situation is different. XX embryos that lack runt form no Sxl product in the middle region where runt is normally expressed. They, however, express Sxl at both terminal regions, anterior and posterior (Duffy and Gergen, 1991; Torres and Sánchez, 1992). This shows that runt product is required in some regions of the embryo but not in others to activate Sxl. The runt function might therefore participate indirectly in the regulation of Sxl, maybe by repressing a segmentation gene whose product, when abnormally expressed, could interfere with proper activation of Sxl.

Because of their direct involvement in the activation of Sxl, the elements sis-a and sis-b seem better suited than runt to test whether genes that regulate Sxl in the soma, also regulate Sxl in the germ line. The only sis-a mutation available is known to be a hypomorphic allele, and homozygous sis-a females can occasionally survive. Germ cells that became homozygous for sis-a as a consequence of mitotic recombination induced after 48 hours of development were oogenic (Cline, 1986). This could mean that sis-a function is required early for oogenesis, but not after 48 h, for example because the state of activity of Sxl is already irreversibly fixed at that time, which is the case in somatic cells.
(Sánchez and Nöthiger, 1983). Alternatively, this could mean that this hypomorphic allele of sis-a provides enough gene function for oogenesis. The third possibility is that sis-a function is not required at all in the female germ line. Transplanting germ cells would not enable us to distinguish between the latter two alternatives.

I therefore decided to test whether sis-b, for which a null allele is available, is required for oogenesis. My results show that XX germ cells lacking sis-b produce functional eggs when allowed to develop in a host female. Thus, germ cells, unlike somatic cells, do not require sis-b product to enter the female pathway. XX flies lacking sis-b die as embryos because they cannot activate their Sxl gene (Cline, 1988; Torres and Sánchez, 1989, 1991). From previous work, we know that oogenic germ cells require Sxl (Schüpbach, 1985; Steinmann-Zwicky et al., 1989). Thus, since XX germ cells lacking sis-b become oogenic, they must express Sxl without requiring sis-b. The transcription factor da had previously been shown not to be required for oogenesis (Cronmiller and Cline, 1987). This already suggested that, in germ cells, Sxl is activated by a mechanism that is different from that acting in the soma. In somatic cells, the products of da and sis-b, which are both helix-loop-helix (HLH) proteins, associate and the heterodimer probably activates Sxl (Dambly-Chaudière et al., 1988; Murre et al., 1989; Van Daren et al., 1991). The autosomal gene da, however, is not a numerator element of the X:A ratio. The da product is provided maternally to all eggs and plays no discriminative role in the process of activating Sxl in females, but not in males. The finding that da is not required in the germ line does not exclude that sis-b activates Sxl in the germ line without the help of da product. My results show that this is not so.

Although the X:A ratio provides a sex-determining signal in the germ cells, the elements forming this signal are different from those forming the X:A ratio in somatic cells. The target of the somatic X:A signal is Sxl. We can now ask whether the target of the germ line X:A signal is also Sxl. If both the inductive signal that determines the sex of XX germ cells and the autonomous germ line X:A signal that makes germ cells responsive to induction regulate the gene Sxl, expressing Sxl should be sufficient to direct XY germ cells into oogenesis. We already knew that SxlM4 does not feminize XY germ cells (Cline, 1983; Steinmann-Zwicky et al., 1989). In this paper, I show that even SxlM4 which is known to constitutively express Sxl functions in somatic XX and XY cells and in germ cells carrying two X chromosomes, does not drive XY germ cells into oogenesis, not even when developing in an ovary. This leaves us with two alternatives. Either SxlM4 does not express Sxl functions in XY germ cells, or Sxl expression is not sufficient for germ cells to enter the female pathway.

The sex of XX germ cells is determined by an inductive signal that emanates from somatic cells. This signal regulates Sxl by either of two mechanisms. (1) XX germ cells might enter the male pathway unless they are feminized by an inductive signal that leads to the activation of Sxl. (2) XX germ cells might enter the female pathway unless they are masculinized by an inductive signal that leads to the repression of Sxl.

If XX germ cells are female in the absence of an induc-

Fig. 1. Three models show how the germ line X:A ratio and a somatic inductive signal could determine the sex of germ cells (circled). (A) The X:A ratio activates Sxl, but a male-specific signal can repress this gene when XX cells develop in a male environment. (B) The X:A ratio and a female-specific inductive signal activate Sxl together when XX germ cells develop in a female environment. (C) A female-specific inductive signal activates Sxl when germ cells develop in a female environment. The X:A ratio controls the expression of other genes necessary for germ cells to enter the male or the female pathway. Germ cells become oogenic only when female-specific genes are expressed in parallel to Sxl.

I thank Rolf Nöthiger for critically reading the manuscript, Eva Niederer for excellent technical assistance and Tatjana Kabat for patient typing. The project was supported by a grant of the Swiss National Science Foundation and by the Kanton Zurich.

REFERENCES


