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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-lethal* (*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-lethal*, *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 female-specific transcripts are found in embryos with an X:A ratio of 1. Two X-chromosomal elements of the X:A ratio, *sisterless-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 Sxl^{M1}, which constitutively expresses functions of the gene *Sex-lethal*, can become oogenic even when developing in a host testis (Steinmann-Zwicky et al., 1989). Sxl^{M1} therefore provides XX germ cells with an autonomous information for femaleness 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 Sxl^{M4} 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*^{301} kept at 18°C. Adult host flies were crossed to test partners. Sterile flies were dissected and their gonads were analysed with a microsope. 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*$^{10\text{-}1}$ *f*^{36a}/*FM6*/*y*^{2} *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*^{L} *Rsp*^{s} *B*^{S}/*Y; E(SD)Rsp*^{i} *bw/SD-ARM VO17, lt* 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*^{M1}/*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*$^{10\text{-}1}$ 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*$^{10\text{-}1}$. I transplanted pole cells from progeny of *sc*$^{10\text{-}1}$/*FM6* females crossed to *sc*$^{10\text{-}1}$/*Y* males carrying a *sc*$^{+}$ duplication on their Y chromosome. From here on, the chromosome carrying *sc*$^{10\text{-}1}$ 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 spermatogenic 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 1. The *sis-b* function is not required in the germ line

Number of injected host embryos	Number of adult host flies	Number of host flies containing XX donor germ cells: total	FM6 / sis-b	sis-b / sis-b	Number of host flies containing XY donor germ cells: total	FM6 / Y	sis-b / Y	Number of flies with empty gonads	Died
975	74 ♀	22	7	11	23			27	2
	59 ♂	9			24	4	16	26	

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

Table 2. XY germ cells carrying Sxl^{M4} do not become oogenic

Number of injected host embryos	Number of adult host flies	Number of host flies containing XY donor germ cells: total	FM7 Y	Sxl^{M4} Y	Number of host flies containing donor germ cells with 2 X chromosomes	Number of flies with empty gonads	Died
417	20 ♂	15	7	8	1	3	1
	17 ♀	9			1	6	1

XY animals carrying Sxl^{M1} 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 Skripsky, 1981). In some cases, adult tissue shows female-specific traits (Cline, 1979). XY germ cells carrying Sxl^{M1} 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.

Sxl^{M1} is still at least partially regulated by elements of the X:A ratio, as it is possible to make a stock in which females are *liz* Sxl^{M1}/*liz* Sxl^{M1} and males are *liz* Sxl^{M1}/*Y* (Steinmann-Zwicky, 1988). The observation that one Sxl^{M1} allele cannot fully rescue females mutant for *liz* or sc^{3-1} in the absence of Sxl^{+} or a second Sxl^{M1} allele, also suggests that Sxl^{M1} does not express *Sxl* functions quite constitutively (Steinmann-Zwicky, 1988; Torres and Sánchez, 1989).

Sxl^{M4} seems to depend on factors less than Sxl^{M1}. XY animals carrying Sxl^{M4} die before hatching. Males of genotype *liz* Sxl^{M4}/*Y* also die (Salz, 1992). Since the chromosome carrying Sxl^{M4} carries no lethal mutation (see below), it can be concluded that these animals are not rescued by introducing a *liz* mutation. They therefore seem to express *Sxl* functions independently of the X:A ratio and *liz*. Therefore, I chose to test whether XY germ cells carrying Sxl^{M4} can become oogenic.

Pole cells were taken from embryos of genotype Sxl^{M4}/*Y* or *FM7/Y* (see Materials and Methods) and transplanted into XX or XY embryos that had no germ cells of their own. Table 2 shows the results of this experiment. Of 20 surviving males, 15 were fertile. Among these, 7 had integrated *FM7/Y* cells and 8 had germ cells of genotype Sxl^{M4}/*Y*. One male differentiated only spermatocytes and could therefore have integrated XX cells, 3 males had empty testes and one animal died before it could be tested. 9 host females had ovaries filled with spermatocytes, which shows that they had integrated XY germ cells, 6 had empty gonads and one died during the tests. One female produced eggs and was fertile, but her progeny displayed that she had integrated germ cells carrying both chromosomes Sxl^{M4} and *FM7*. The donor embryo must therefore have arisen by maternal non-disjunction and it must have had two X chromosomes and one Y chromosome.

The results show that XY germ cells carrying Sxl^{M4} do not become oogenic even when developing in host ovaries. The nine females containing spermatocytes had probably integrated germ cells of genotype either Sxl^{M4}/*Y* or *FM7/Y*. The results obtained with males show that germ cells from Sxl^{M4}/*Y* embryos were transplanted equally often as germ cells from *FM7/Y* embryos. As I did not observe two different classes of host females with spermatocytes, there seems to be no difference in developmental performance between Sxl^{M4}/*Y* and *FM7/Y* germ cells.

To test the genotype of germ cells of fertile males, I crossed them to several females carrying various markers. One type of female was of genotype Sxl^{M4}/*FM7*. When males carrying Sxl^{M4}/*Y* germ cells were crossed to these females, female progeny arose that were homozygous for the Sxl^{M4} chromosome. Although these were less viable than their Sxl^{M4}/*FM7* sisters (on average I counted about 20 % of the expected Sxl^{M4}/Sxl^{M4} females), they showed that the chromosome carrying Sxl^{M4} has acquired a mutation affecting the eye similar to *lz*, but no lethal mutation, which means that males carrying this chromosome die because of Sxl^{M4}. This result is important for any analysis that tests the viability of animals carrying the Sxl^{M4} chromosome.

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 Sxl^{M1} does not feminize XY germ cells (Cline, 1983; Steinmann-Zwicky et al., 1989). In this paper, I show that even Sxl^{M4} 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 Sxl^{M4} 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 inductive signal, it follows that the X:A ratio must confer femaleness on them by activating *Sxl* (Fig. 1A). If XX germ cells are male in the absence of an inductive signal, a female X:A ratio is necessary but not sufficient to drive germ cells into oogenesis, either because elements of the X:A ratio provide factors that help to activate *Sxl* together with the inductive signal (Fig. 1B), or because the X:A signal controls other genes that are required for oogenesis in parallel to *Sxl* activity (Fig. 1C). This last possibility was largely ignored in previous publications. If *Sxl* expression is not sufficient to drive XY germ cells into oogenesis, as suggested by the finding that Sxl^{M4} does not feminize XY germ cells, this possibility becomes the one that is most likely. However, unless it can be shown that a functional *Sxl* product is present in Sxl^{M4}/Y germ cells whenever this product is required for germ cells to enter and maintain the female pathway, we cannot be sure that this is the right interpretation.

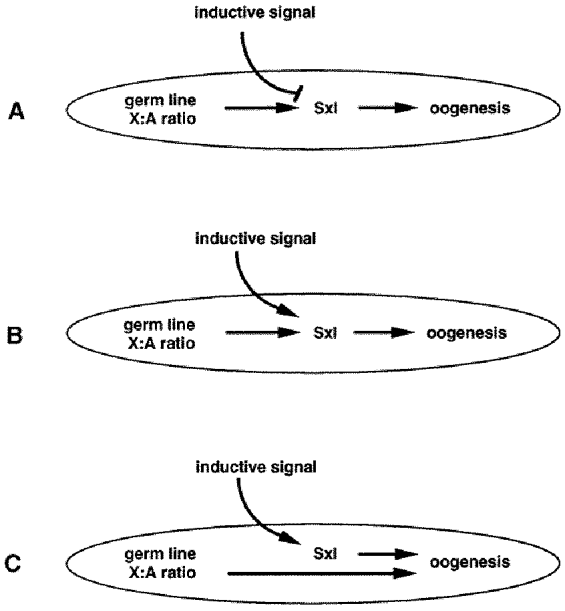

Fig. 1. Three models show how the germ line X:A ratio and a somatic inductive signal could determine the sex of germ cells (circled). (A) The X:A ratio activates *Sxl*, but a male-specific signal can repress this gene when XX cells develop in a male environment. (B) The X:A ratio and a female-specific 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*.

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