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Abstract

In Drosophila, the sex of germ cells is determined by cell-autonomous and inductive signals. XY germ cells autonomously enter spermatogenesis when developing in a female host. In contrast, XX germ cells non-autonomously become spermatogenic when developing in a male host. In first instar larvae with two X chromosomes, XX germ cells enter the female or the male pathway depending on the presence or absence of transformer (tra) activity in the surrounding soma. In somatic cells, the product of tra regulates the expression of the gene double sex (dsx) which can form a male-specific or a female-specific product. In dsx mutant larvae, XX and XY germ cells develop abnormally, with a seemingly intersexual phenotype. This indicates that female-specific somatic dsx products feminize XX germ cells, and male-specific somatic dsx products masculinize XX and XY germ cells. The results show that tra and dsx control early inductive signals that determine the sex of XX germ cells and that somatic signals also affect the development of XY germ cells. XX germ cells that develop in pseudomales lacking the sex-determining function of Sxl are spermatogenic. If, however, female-specific tra functions are expressed in these animals, XX germ cells become oogenic. Furthermore, transplanted XX germ cells can become oogenic and form eggs in XY animals that express the female-specific function of tra. Therefore, TRA product present in somatic cells of XY animals or in animals lacking the sex-determining function of Sxl, is sufficient to support developing XX germ cells through oogenesis.

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Sex determination of the *Drosophila* germ line: *tra* and *dsx* control somatic inductive signals

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SUMMARY

In *Drosophila*, the sex of germ cells is determined by cell-autonomous and inductive signals. XY germ cells autonomously enter spermatogenesis when developing in a female host. In contrast, XX germ cells non-autonomously become spermatogenic when developing in a male host. In first instar larvae with two X chromosomes, XX germ cells enter the female or the male pathway depending on the presence or absence of *transformer* (*tra*) activity in the surrounding soma. In somatic cells, the product of *tra* regulates the expression of the gene *double sex* (*dsx*) which can form a male-specific or a female-specific product. In *dsx* mutant larvae, XX and XY germ cells develop abnormally, with a seemingly intersexual phenotype. This indicates that female-specific somatic *dsx* products feminize XX germ cells, and male-specific somatic *dsx* products masculinize XX and XY germ cells. The results show that *tra* and *dsx* control early inductive signals that determine the sex of XX germ cells and that somatic signals also affect the development of XY germ cells.

XX germ cells that develop in pseudomales lacking the sex-determining function of *Sxl* are spermatogenic. If, however, female-specific *tra* functions are expressed in these animals, XX germ cells become oogenic. Furthermore, transplanted XX germ cells can become oogenic and form eggs in XY animals that express the female-specific function of *tra*. Therefore, TRA product present in somatic cells of XY animals or in animals lacking the sex-determining function of *Sxl*, is sufficient to support developing XX germ cells through oogenesis.

Key words: *double sex*, germ cells, induction, sex determination, *transformer*

INTRODUCTION

In somatic cells of *Drosophila*, sex is determined by a cell-autonomous primary signal called X:A ratio. The number of X-chromosomes is related to the number of sets of autosomes, and this X:A ratio forms a signal that regulates the gene *Sex-lethal* (*Sxl*). *Sxl* becomes active in female cells that have an X:A ratio of 1 and remains inactive in male cells that have an X:A ratio of 0.5. An active product of *Sxl* achieves the female-specific splicing of the *transformer* (*tra*) pre-mRNA whose product in turn, together with the constitutive product of *transformer 2* (*tra2*), leads to female-specific expression of the gene *double sex* (*dsx*) (reviewed in Baker, 1989; Steinmann-Zwicky et al., 1990; Belote, 1992; Cline, 1993).

In *Drosophila* germ cells, sex is determined by both cell-autonomous and inductive signals (reviewed in Pauli and Mahowald, 1990; Steinmann-Zwicky, 1992a,b). XX cells become spermatogenic when developing in a male host which shows that their sexual identity is imposed on them by their somatic environment. In contrast, XY cells are spermatogenic even when developing in the ovary of a female host. They must have autonomous information for maleness (Steinmann-Zwicky et al., 1989). The cell-autonomous signal that makes XX cells different from XY cells depends on the number of X chromosomes and autosomes of the germ line, as XX; AAA germ cells often become spermatogenic even in a female fly (Schüpbach, 1985). The 'germ line X:A ratio', however, is formed by elements that are different from those building the X:A ratio in somatic cells. The gene *sisterless-b* (*sis-b*) which is an important X-chromosomal element of the X:A ratio in somatic cells, is not required in germ cells (Steinmann-Zwicky, 1993). Furthermore, germ cells that are simultaneously heterozygous for *sis-b*, *sis-a*, *runt* and *Sxl* can become oogenic in a female host although somatic cells of the same genotype are male (Granadino et al., 1993). The number of these genes or gene products is therefore not counted to assess the X:A ratio in germ cells.

To become oogenic, XX germ cells require an active *Sxl* gene, whereas the products of *tra*, *tra2* and *dsx* are dispensable within the germ cells (Marsh and Wieschaus, 1978; Schüpbach, 1982, 1985; Steinmann-Zwicky et al., 1989). The inductive signal that determines the sex of XX germ cells appears to be controlled by genes of the sex-determining hierarchy, as the testes of XX pseudomales lacking the female-specific function of *tra*, *tra2* or *dsx* can contain spermatogenic germ cells. Since oogenic cells, however, are also found in such animals, the function of these genes is not clear (Seidel, 1963; Nöthiger et al., 1989). The inductive signal seems to regulate the expression of the gene *Sxl*. This follows from the observation that XX germ cells carrying a constitutive mutation of *Sxl* become oogenic even when developing in the testes of male hosts (Steinmann-Zwicky et al., 1989). Further-

more, XX flies carrying masculinizing *tra* or *dsx* mutations contain male-specific splice products of *Sxl* within their gonads (Oliver et al., 1993).

First sexual dimorphisms of germ cells are already detectable in first instar larvae (Kerkis, 1931; Aboïm, 1945). At this stage, the gonads of males are much larger than the gonads of females. This difference arises as a consequence of the sex-specific development of germ cells. Agametic gonads of both sexes contain all the somatic cells required to form a gonad. They, however, are similar in size throughout larval development (Aboïm, 1945). XY germ cells undergo more mitotic divisions than XX germ cells (Sonnenblick, 1941) and they differentiate spermatocytes, which can already be recognized in the posterior region of the testes in late first instar larvae (Fig. 1B). At this time, XX germ cells have not divided much and they all have a similar appearance (Aboïm, 1945; Fig. 1A). The sex-specific development of germ cells of first instar larvae can therefore be recognized when gonads are scored for size, number of germ cells and presence or absence of spermatocytes.

To test when germ cells become affected by inductive sex-determining signals, I analysed the gonads of larvae carrying mutations for *tra* or *dsx*. I found that *tra*- and *dsx*-dependent inductive signals determine the sex-specific characteristics of XX germ cells already in first instar larvae. Unexpectedly, even XY germ cells respond to *dsx*-dependent signals.

To test whether somatic *tra* expression is sufficient to direct XX germ cells into oogenesis, I analysed the gonads of pseudomales that were feminized by a female-specific cDNA of *tra* (McKeown et al., 1988). I also transplanted XX germ cells into XY hosts feminized by the same construct. In XX and XY animals expressing the female-specific function of *tra*, XX germ cells were able to enter oogenesis and to differentiate eggs.

MATERIALS AND METHODS

Sexing germ cells in larvae

No molecular marker is available to test the sex-specific development of germ cells in first instar larvae. Even if such a marker were available, it would only reflect one sex-specific characteristic of the germ cells. Since germ cells respond to autonomous and inductive signals, it is possible that male- and female-specific genes are expressed in young larvae carrying mutations such as *tra*. As there is a striking sex-specific dimorphism between male and female germ cells already during the first larval instar, I chose to assess the sex of germ cells using morphological criteria.

Flies and larvae were kept at 25°C. Egg collections were made for 2 hours on standard *Drosophila* food. Larvae of late first (46 hours), second (70 hours) and third (120 hours) instar were collected and dissected. Their fat bodies including the gonads were put on a slide in a drop of Ringer's solution and covered with a cover slip. Excess ringer was sucked off. The morphology of the germ cells and the size of the gonad was scored. To obtain a value for gonad size, which reflects the sex-specific development of germ cells, the largest and the shortest diameter of the gonads were measured on a scale with arbitrarily chosen units. In some cases, 46 hours old larvae were cut in two, turned inside out and stained with Sudan III which gives a red color to the fat body. Larvae were fixed in 4% paraformaldehyde, washed in PBS, left for 3 minutes in 50% ethanol and stained for 30-45 minutes in Sudan III solution. With this procedure, the gonads could easily be identified.

Crossing *y*/*y*; *tra* p^p/TM6 females to *tra* p^p/TM6 males yielded pseudomales marked with y^+ p^p. Crossing *y*/*y*; *dsx* p^p/TM6 females to *dsx* p^p/TM6 males yielded XX and XY animals lacking *dsx* function marked with y^+ p^p and *y* p^p, respectively. Crossing *y*/*y*; hs-*tra*-female *tra* p^p *Ki*/*TM6*, *e* females to p^p *e* males yielded *X*/*Y*; hs-*tra*-female larvae that could be identified due to the markers *y* p^p. The *hs-tra*-female construct constitutively expresses the female-specific function of *tra* even at 25°C so that XY animals are transformed into pseudofemales at normal temperatures (McKeown et al., 1988). Crossing *y*/*y*; hs-*tra*-female *tra* p^p *Ki*/TM6 females to *tra* p^p/TM6 males yielded y^+ p^p larvae with two X chromosomes lacking the endogenous *tra* function, but carrying the hs-*tra*-female construct.

Pole cell transplantations

Donor XX pole cells were obtained by crossing *cn bw* females to males of genotype $^+$/*Dp(2;Y)CB25-4*, $y^+Y^S.Y^L$ Rsp^s B^S;*SD-ARM 28*/*In(2L)Cy*, *Cy E(SD)*Rsp^i *bw*. These males carry mutations causing a segregation distortion and only transmit sperm carrying the X chro-

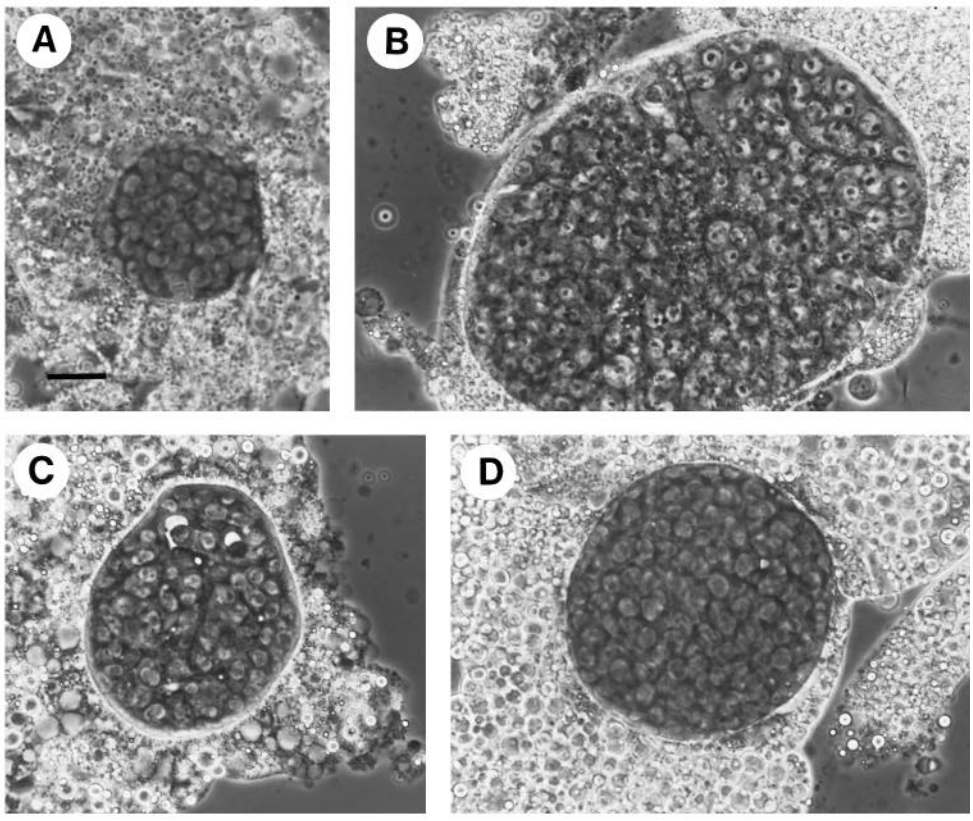

Fig. 1. Gonads of 46 hours old late first instar larvae. (A) Wild-type female; (B) wild-type male; (C) *X*/*X; dsx*/*dsx*; (D) *X*/*Y; dsx*/dsx, Bar, 20 μm.

mosome (Lyttle, 1989). Agametic host embryos were obtained by crossing p^p osk^{301} hs-*tra*-female/p^p osk^{301} e^s females to *FM6*/*Y* males. At 18°C, the females produce embryos devoid of germ cells due to a maternal effect of the mutation osk^{301}. XY progeny from this cross were wild type; XX progeny had *B* eyes. Crossing p^p osk^{301} hs-*tra*-female/*TM6* females to *X*/*YB*^{S};*bw;e* males yielded host embryos with own germ cells. Adult XY flies from this cross were B^S; XX flies were wild type.

Pole cells were transplanted as described by van Deusen (1976) and Steinmann-Zwicky et al. (1989). Best results are obtained when all steps of the experiments are done at 18°C. Because the temperature of my transplantation room could not be reliably kept at 18°C in the summer in which I performed the first experiment, I obtained a poorer survival rate than in other similar experiments. In the second experiment, there were fewer pseudofemale adult hosts and more males than expected. Many XY animals apparently lost their hs-*tra*-female construct. Loss of the hs-*tra*-female construct was also observed several times in the stock from which the host females were derived.

Mutations and balancer chromosomes are described in Lindsley and Zimm (1992). Criteria used to identify the sex of germ cells in adults are listed in Steinmann-Zwicky et al. (1989).

RESULTS

Somatic *tra* and *dsx* functions control the sex-specific development of XX germ cells in larvae

To learn when XX germ cells become affected by inductive signals, the sexual development of germ cells in larvae of various genotypes was investigated. Pseudomales of genotype *X/X; tra/tra* have a male soma but their germ cells produce functional eggs when transplanted into a female host (Marsh and Wieschaus, 1978). Any effect of *tra* mutations on the sex-specific development of germ cells must therefore arise from lack of *tra* function in somatic cells. Seidel (1963) described the development of *X/X; tra/tra* gonads through all larval stages. In first instar larvae, the mutant gonads have a male-specific size. They contain a male-specific number of germ cells that, by morphological criteria, look like spermatogonia. Germ cells therefore display male characteristics. During later larval stages, the mutant gonads grow less than testes but more than ovaries. Some spermatogenic germ cells grow while others degenerate. In some cases, oogenic cells are identified

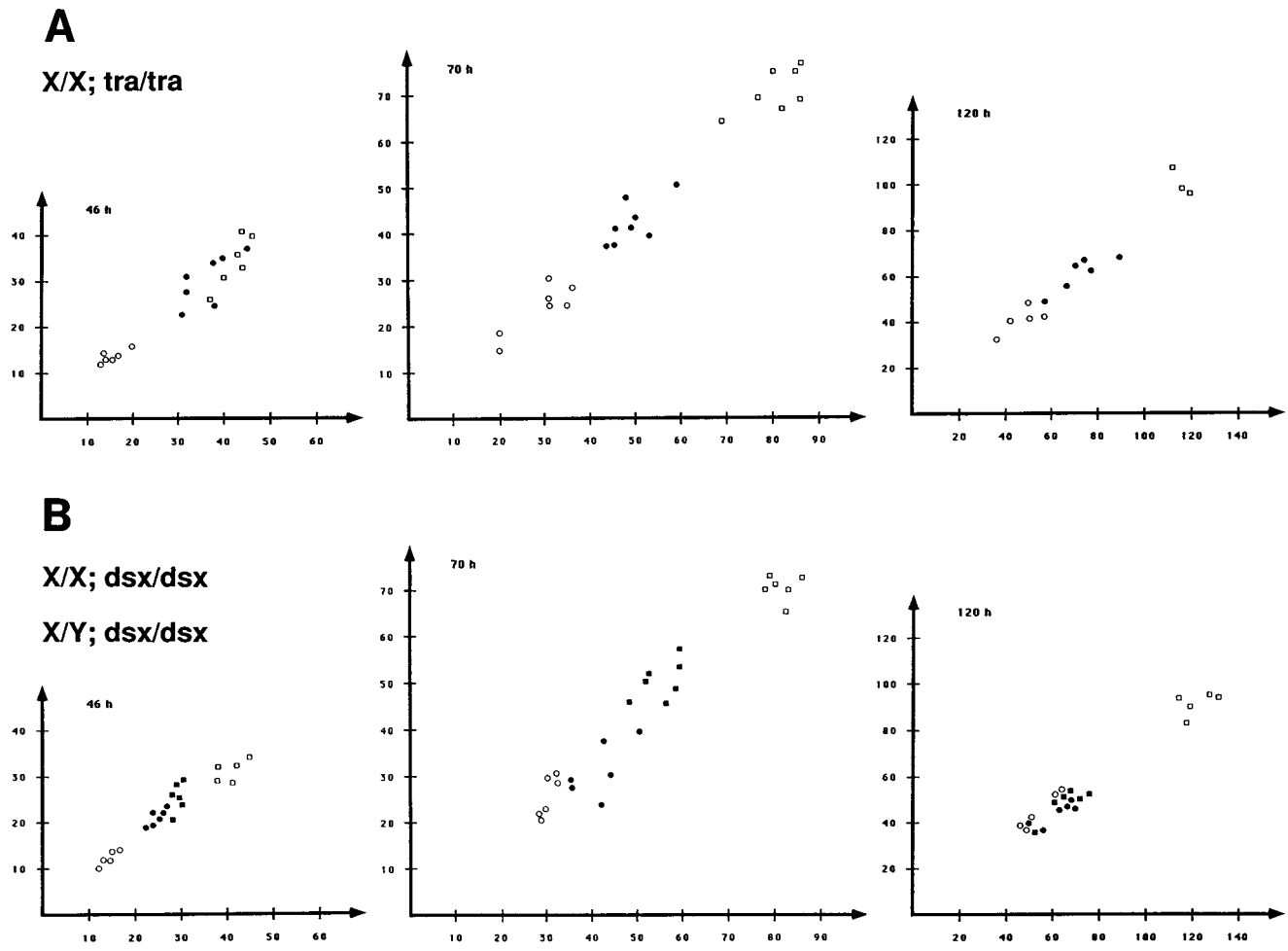

Fig. 2. Size of gonads of larvae mutant for *tra* or *dsx*. At the end of each larval instar (46 hours, 70 hours, 120 hours), animals of various genotypes were dissected. To obtain a value for gonad size that reflects the sex-specific development of germ cells, the largest (abscissa) and shortest (ordinate) diameter of each gonad was measured. Units are chosen arbitrarily. Since agametic male and female gonads show similar sizes throughout development, the somatic component can be neglected. In each cross yielding mutant XX test animals (●) or mutant XY animals (■), gonads from female (○) and male (□) sibs were also measured to obtain internal control values.

after metamorphosis. I have reanalysed the size and morphology of larval *X/X; tra/tra* gonads. In general, I could confirm Seidel's observations. In first instar larvae, the gonads were nearly male-like in size and morphology. In later stages, they grew less than control testes (Fig. 2A). Some germ cells degenerated while others showed the typical morphology of spermatogenic cells (Fig. 3C). These observations show that the early sex-specific characteristics of XX germ cells are dependent on somatic *tra* expression. I conclude that *tra* controls an early inductive signal that determines the sex of young germ cells carrying two X chromosomes.

The TRA protein is normally made only in females. It regulates the somatic sex-determining gene *dsx* which can express two opposing functions that are regulated by alternative splicing of a pre-mRNA (Burtis and Baker, 1989). A female-specific protein, DSX^F, is made in the presence of TRA and with the help of the constitutive TRA2 product; a male-specific protein, DSX^M, is made in the absence of either TRA or TRA2. XX and XY animals lacking *dsx* function develop as intersexes. Germ cells require no autonomous *dsx* function; mutant XX cells form functional eggs when transplanted into a wild-type female host, and mutant XY cells complete spermatogenesis in a wild-type male soma (Schüpbach, 1982).

To test whether the early inductive signal that dictates sex-specific characteristics to larval XX germ cells is controlled by *dsx*, I analysed the gonads of *X/X; dsx/dsx* larvae. If somatic DSX^F product is required for XX germ cells to enter the female pathway, XX larvae lacking this product should possess gonads displaying male characteristics. First instar larvae of genotype *X/X; dsx/dsx* carrying a mutation that abolishes both the female-specific and the male-specific *dsx* function have gonads that are distinctly larger than female gonads. They, however, are smaller than male gonads (Figs 2B, 1C). During the second and the third larval instar, these gonads grow little such that they finally show a nearly typical female size (Fig. 2B). Germ cells remain small and do not show the morphology of spermatocytes even in third instar larvae (Figs 3E, 4C). Since gonads of XX animals mutant for *dsx* are neither completely female nor male, somatic DSX^F is required for XX germ cells to display typical female-specific characteristics in larvae. Somatic DSX^M, however, which is expressed in *X/X*; *tra/tra* pseudomales, masculinizes XX germ cells. Thus, *tra* and *dsx* control one or several inductive signals to which XX germ cells of first instar larvae respond by displaying male or female characteristics.

Larval XY germ cells also respond to somatic signals

Gonads of *X/X; tra/tra* larvae are male because they are masculinized by DSX^M, not because they lack DSX^F. If somatic DSX^M can masculinize XX germ cells, the question arises whether DSX^M also acts on XY germ cells. Interestingly, XY germ cells developing in larvae lacking *dsx* function do not display a typical male phenotype. In first and second instar

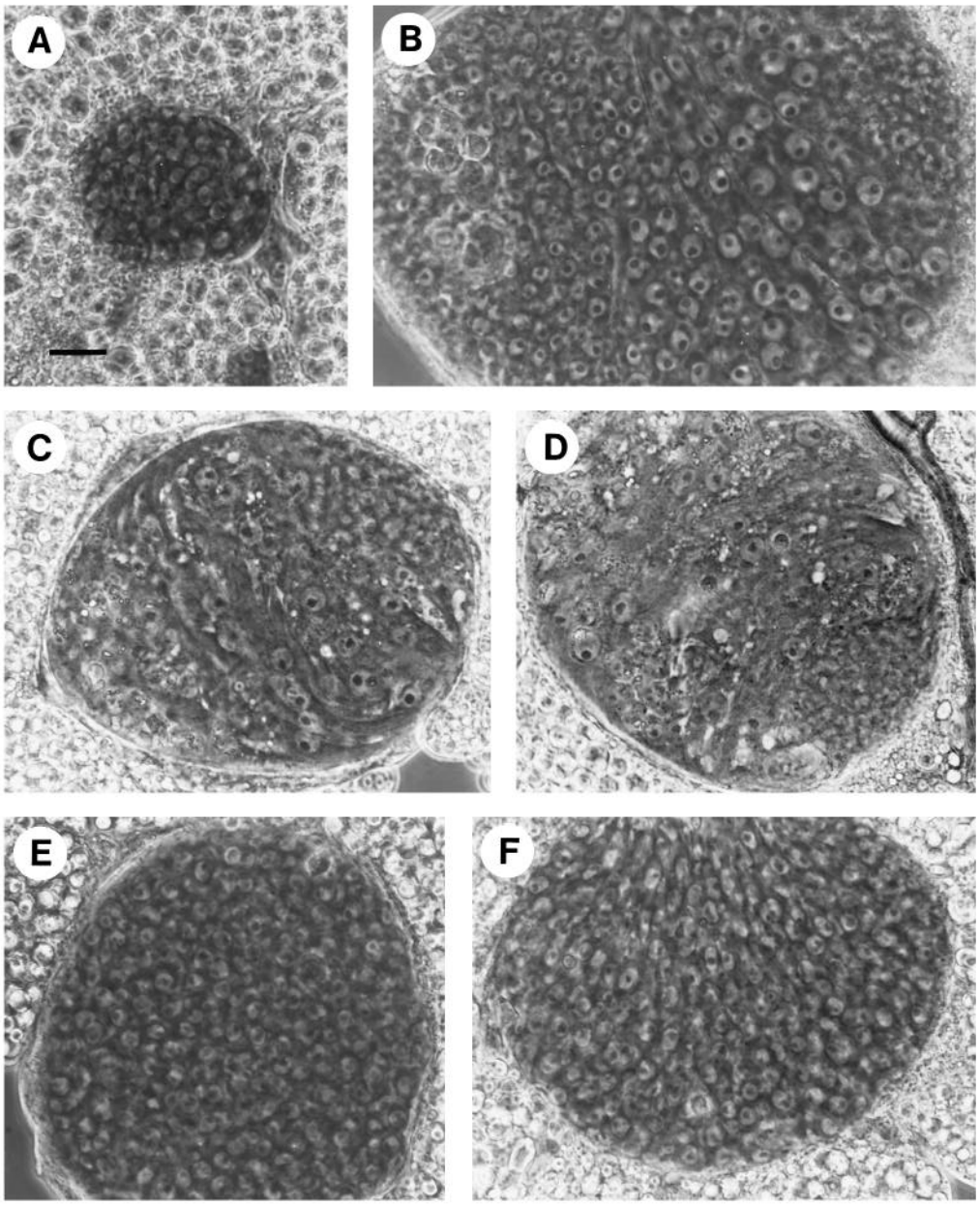

Fig. 3. Gonads of 70 hours old late second instar larvae. (A) Wild-type female; (B) wild-type male; (C) *X/X; tra/tra;* (D) *X/X; tra/tra*, hs-*tra*-female; (E) *X/X; dsx/dsx*; (F) *X/Y; dsx/dsx*. Bar, 20 μm.

larvae, gonads display a size that is intermediate between the size of male and female gonads. In third instar larvae, gonads almost stop growing such that they finally show a nearly female size (Fig. 2B). Germ cells remain small and do not differentiate large spermatocytes (Figs 1D, 3F, 4D). Similar results were obtained when larvae of genotype *X/Y; dsx/dsx*^{11} were analysed (Fig. 4E). The allele *dsx*^{11} is specifically defective for the male DSX^{M} function. In the mutant XY animals, however, DSX^{F} is not expressed as no TRA protein is present. Since the gonads of *X/Y*; *dsx/dsx* and *X/Y*; *dsx/dsx*^{11} are similar, we can exclude a mutation different from *dsx* or an allele-specific defect affecting the development of germ cells in the tested larvae. Therefore, XY germ cells require DSX^{M} to display typically male characteristics in larvae.

To test whether female-specific gene functions feminize XY germ cells in larvae, I analysed the gonads of XY animals carrying the construct hs-*tra*-female. These animals express female-specific functions of the gene *tra* (McKeown et al., 1988). First instar larvae of this genotype contain gonads of male size (Fig. 5A). During the subsequent larval stages, these gonads grow far less than testes of control males, and by the end of the third larval instar, their size is comparable to the size of female gonads. The morphology of the germ cells is male-like throughout development and large spermatocytes can be identified in the small gonads of third instar larvae. The finding that gonads of *X/Y;* hs-*tra*-female larvae are more male than the gonads of *X/Y; dsx/dsx* larvae was unexpected. The former animals develop as females; they express *tra* and the female-specific function of *dsx*, DSX^{F}. The latter develop as intersexes; they express neither DSX^{F} nor DSX^{M}. The observation, however, is understandable if one assumes that the *tra* gene from the hs-*tra*-female construct becomes active later than the endogenous *tra* gene of females. Male-specific DSX^{M} product might be present during early development of these animals before TRA is made. This early DSX^{M} product could masculinize the germ cells of *X/Y;* hs-*tra*-female animals, such that these initially show a male-specific division rate. The early effect of DSX^{M} would furthermore drive some germ cells into differentiating large spermatocytes.

To test this hypothesis, I analysed the gonads of *X/X; tra/tra* larvae carrying the construct hs-*tra*-female. If the construct hs-*tra*-female provides TRA and DSX^{F} activity later than what is required for normal ovary development, young XX larvae carrying this construct, but lacking endogenous *tra* activity, might contain masculinized gonads. Fig. 5B shows that this is indeed the case. *X/X;tra/tra* hs-*tra*-female gonads are variable, but some are similar to *X/X*; *tra/tra* gonads in size and morphology. They start development displaying male-specific features. In a few larvae of the second and the third instar, spermatocytes can be recognized amid degenerating material (Fig. 3D). The hs-*tra*-female construct thus seems to expresses *tra* function later than the endogenous *tra* promoter.

The female-specific *tra* function feminizes XX germ cells

Adult flies of genotype *X/X*; *tra/tra* carrying the construct *hs-tra*-female develop as females. Their ovaries are oogenic and contain eggs that are, however, not laid (McKeown et al., 1988). Inspecting ovaries of such flies revealed that they exclusively contain oogenic cells. These cells apparently develop normally, except for an occasional egg chamber that contains a reduced number of nurse cells. Of 20 animals dissected, none contained spermatogenic cells or otherwise abnormal ovaries. The *hs-tra*-female construct therefore provides all somatic *tra* functions required to direct XX germ cells into the oogenic pathway, at least when adult flies are analysed.

The gonads of pseudomale flies of genotype *X/X; tra/tra*, *X/X*; *tra2/tra2* or *X/X*; *dsx*^{D}*/dsx* contain male, female or degenerating germ cells (Seidel, 1963; Nöthiger et al., 1989). Somatic *tra* function seems therefore not to be absolutely required for XX germ cells to become oogenic. Pseudomales

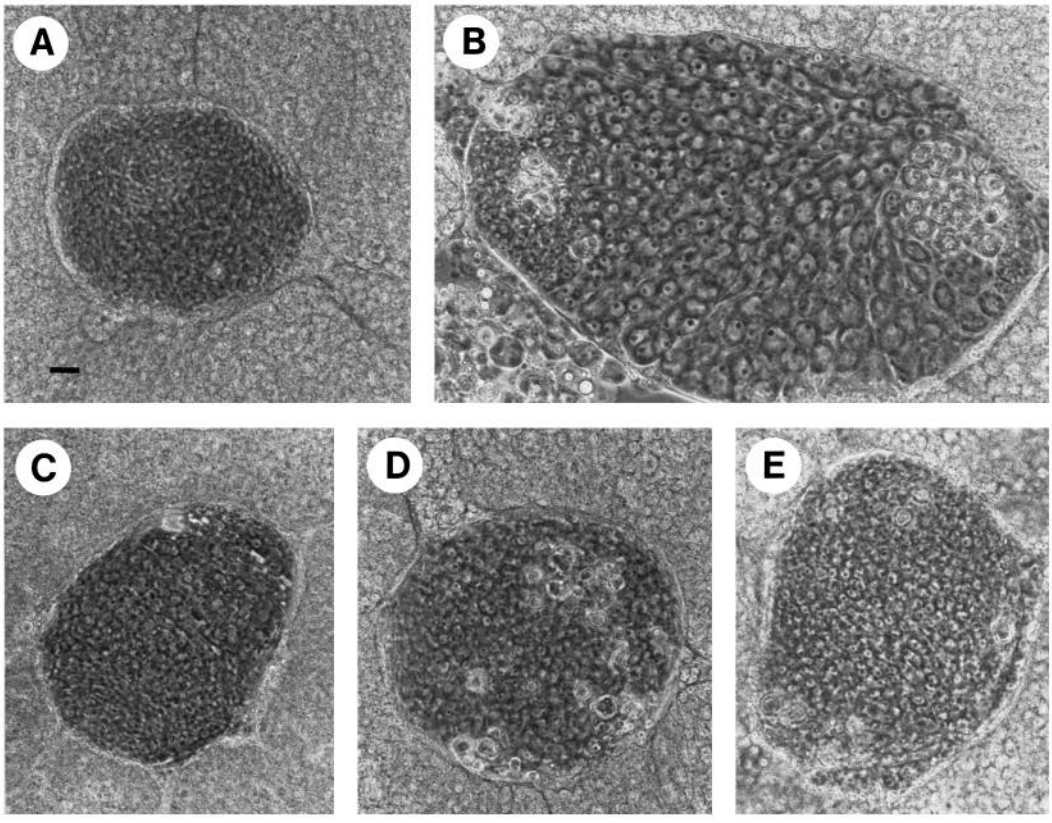

Fig. 4. Gonads of 120 hours old late third instar larvae. (A) Wild-type female; (B) wild-type male; (C) *X/X; dsx/dsx*; (D) *X/Y; dsx/dsx*; (E) *X/Y; dsx/dsx*^{11}. Bar, 20 μm.

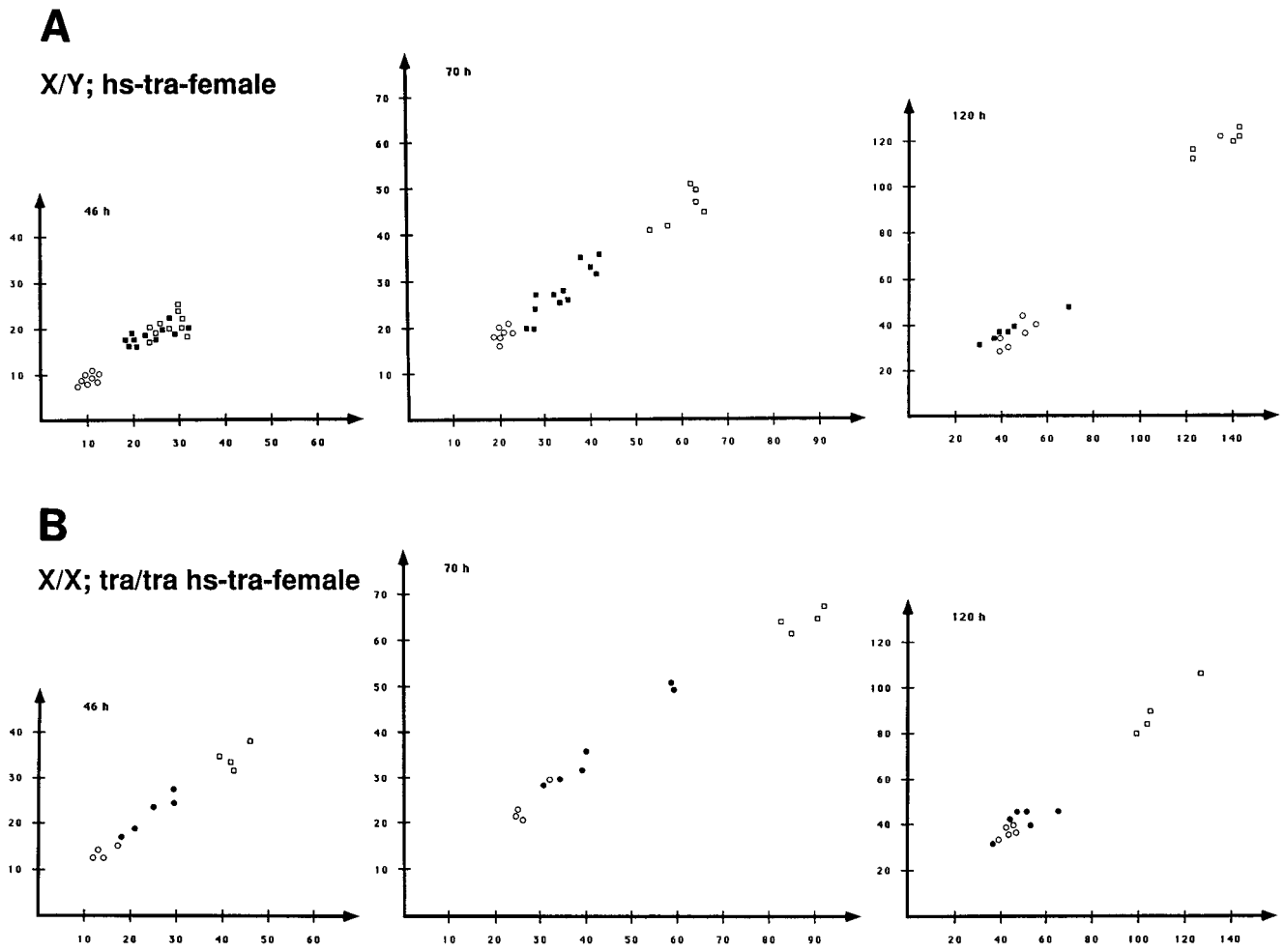

Fig. 5. Size of gonads of larvae carrying hs-*tra*-female. For symbols see legend to Figure 2.

carrying an allelic combination that specifically eliminates the somatic sex-determining function of *Sxl* ($Sxl^{M1,\,fm3}/Sxl^{fm7,\,M1}$), in contrast, do not contain any oogenic germ cells (Nöthiger et al., 1989). This suggests that, in somatic cells, *Sxl* might control the expression of an inductive signal that determines the sex of XX germ cells by a pathway that is independent of *tra*. To test this hypothesis, I analysed the gonads of flies of genotype $Sxl^{M1,\,fm3}/Sxl^{fm7,\,M1}$ carrying the *hs-tra*-female construct. If the inductive signal that determines the sex of XX germ cells is controlled by *tra* we expect these flies to contain normal oogenic ovaries. If the inductive signal is controlled by an alternative pathway that does not include *tra* and *dsx*, we expect them to contain spermatogenic or degenerating germ cells.

Animals of genotype $Sxl^{M1,\,fm3}/Sxl^{fm7,\,M1}$; hs-*tra*-female develop as females. The 20 flies dissected contained apparently normal ovaries with oogenic cells of all stages including eggs that were not laid. None of the ovaries contained spermatogenic cells or degenerating material. This shows that *tra* controls an inductive signal that is sufficient to direct XX germ cells either into oogenesis or into spermatogenesis. The experiments, however, do not exclude the existence of an alternative pathway.

XX germ cells developing in an XY host that expresses *tra* function can become oogenic

Somatic cells of genotype $Sxl^{M1,\,fm3}/Sxl^{fm7,\,M1}$; *hs-tra*-female contain two X chromosomes. The female X:A ratio signal of these cells might feminize the XX germ cells by controlling an alternative pathway independent of *tra*, *dsx* and *Sxl*. To test whether somatic female-specific *tra* function is sufficient to feminize XX germ cells that develop in an XY host, I transplanted XX pole cells into *X/Y*; hs-*tra*-female pseudofemales. These animals express female-specific functions of the gene *tra* and of genes that act downstream of *tra*. In their cells, however, the X:A ratio and the state of activity of *Sxl* are male-specific. In a first experiment, in which I used agametic hosts, one pseudofemale had ovaries that looked wild type. They were full of oogenic cells of all stages (Table 1; Fig. 6). Mature eggs were present but were not laid. In a second experiment, I used pseudofemale hosts that had germ cells of their own. One adult *X/Y*; hs-*tra*-female host had apparently normal ovaries containing oogenic egg chambers. Close inspection showed 3 cysts that seemed to contain undifferentiated germ cells. Such cells are normally observed in pseudofemales (McKeown et al., 1988; Steinmann-Zwicky et al., 1989). They could be the host's own XY germ cells or they could be undifferentiated

Table 1. Development of XX germ cells in XY pseudofemales

		XY hosts								
		Pseudofemales			Males			XX hosts		
	Transplanted embryos	Oogenic ovaries	Spermatogenic ovaries	Empty	Sperm	Spermatocytes	Empty	Oogenic ovaries	Spermatogenic	Empty
Agametic hosts	775	(1)		9		(3)	6	(5)	(1)	11
Hosts with own germ cells	898	(1)	6		44			48		

XX germ cells were injected into agametic hosts or into host embryos that possessed own germ cells (see Materials and Methods). Some of these hosts were pseudofemales, i.e. XY animals that were feminized by a hs-*tra*-female construct. Animals with positively identified donor germ cells are circled. In the first experiment, one XY pseudofemale contained large ovaries filled with oogenic XX germ cells. One XX host contained ovaries with spermatogenic cells. These were probably of genotype XO and arose by non-disjunction in the donor cross. The donor cross, which was used specifically to generate XX donor embryos, yields a majority of XX animals and a few XO animals carrying the paternal X chromosome. In the second experiment, one pseudofemale had oogenic ovaries.

From seventeen surviving host pseudofemales, only two had oogenic ovaries, showing that they had integrated XX germ cells. Two out of seventeen host animals that integrate transplanted germ cells is a much lower number than that obtained when other hosts are used. The two experiments that yielded pseudofemale hosts also gave three out of nine XY male hosts and thirty out of forty-eight XX female hosts that had integrated XX germ cells (The 48 XX female hosts were tested for progeny and in 30 cases progeny from the donor germ cells were obtained). It therefore seems that XX germ cells are best integrated into XX female hosts, second best into XY male hosts and very poorly into XY pseudofemales. In other experiments too, XX germ cells were always better integrated into XX hosts than into XY hosts. XY germ cells, in contrast, integrate well in XX and XY hosts (Steinmann-Zwicky et al., 1989; Steinmann-Zwicky, 1993).

XX germ cells. Eggs were not found in this animal: it had to be dissected shortly after emergence because it was caught in the food. The animal, however, contained oogenic cells of all previtellogenic stages. Pseudofemales without transplanted XX germ cells, in contrast, never contained any oogenic germ cells; nor did XY males containing transplanted XX germ cells.

Since somatic *tra* expression can be sufficient to drive XX germ cells developing in XY hosts into oogenesis, I conclude that the inductive signal from the soma that determines the sex of XX germ cells is controlled by *tra* and consequently *dsx*, or by genes that act downstream of *dsx*.

DISCUSSION

In first instar larvae, the sex of germ cells is dependent on *tra* and *dsx*

Germ cells already display sex-specific characteristics in first instar larvae. These features are dependent on *tra* and *dsx* expression. At the end of the first instar, gonads of *X/X; tra/tra* larvae are nearly male-like in size and morphology. In later stages, many spermatogenic XX germ cells degenerate such that the size of the gonads does not any more reflect the sex of germ cells. Some germ cells, however, differentiate spermatocyte-like cells, which can be seen throughout larval development. The somatic *tra* function thus controls the sex-specific division rate and the differentiation fate of XX germ cells developing in larvae. The TRA product also seems to control a pathway whose gene products are required for the survival of XX germ cells.

Gonads of *X/X; dsx/dsx* larvae are intermediate in size. Their germ cells do not differentiate large spermatocytes and they do not degenerate. This shows that the male-specific phenotype of *X/X; tra/tra* germ cells and the death of such cells is caused by the presence of DSX^M and not by the absence of DSX^F in these animals. Adult flies of genotype *X/X; dsx/dsx* contain either oogenic gonads or gonads with germ cells whose sex cannot be identified. Rarely, abortive testes with small spermatogenic germ cells or agametic gonads are seen (Orssaud and Laugé, 1982). It thus seems that XX germ cells that display some male characteristics in larvae (division rate higher than in female XX germ cells) can nevertheless become oogenic in adults. To enter unequivocally the female pathway, however, XX germ cells require DSX^F, the female-specific function of *dsx*. From this I conclude that *dsx* function is not absolutely required for XX germ cells to become oogenic. DSX^F and DSX^M, however, have a feminizing and a masculinizing role, respectively, on ambivalent XX germ cells that in absence of these products can enter the male or the female pathway.

When developing in adult XX female hosts, transplanted XY germ cells become spermatogenic (Steinmann-Zwicky et al., 1989). It was therefore assumed that XY germ cells do not respond to inductive signals and that they autonomously enter the male pathway. The results presented here, however, show that XY germ cells do respond to somatic signals. Through all larval stages, XY germ cells display sex-specific characteristics that are dependent on *dsx*. In the absence of *dsx*, gonads with XY germ cells are intermediate in size. In these gonads, germ cells divide more often than female but less than male germ cells, they form small spermatogenic cells, but they do not differentiate large spermatocytes. Adult *X/Y; dsx/dsx* flies contain gonads with spermatogenic and undifferentiated germ cells. In some cases, agametic gonads are found (Orssaud and Laugé, 1982). The differentiation potential of XY germ cells developing in an *X/Y; dsx/dsx* soma seems to be similar to that of XY germ cells developing in the female soma of an XX host; some spermatocytes, but no spermatids and no sperm are formed. Many germ cells remain undifferentiated (Steinmann-Zwicky et al., 1989). From these observations, I conclude that DSX^M is required to drive XY germ cells fully into spermatogenesis. DSX^F, in contrast, seems to have little or no effect on the sex-specific differentiation of XY germ cells, at least when

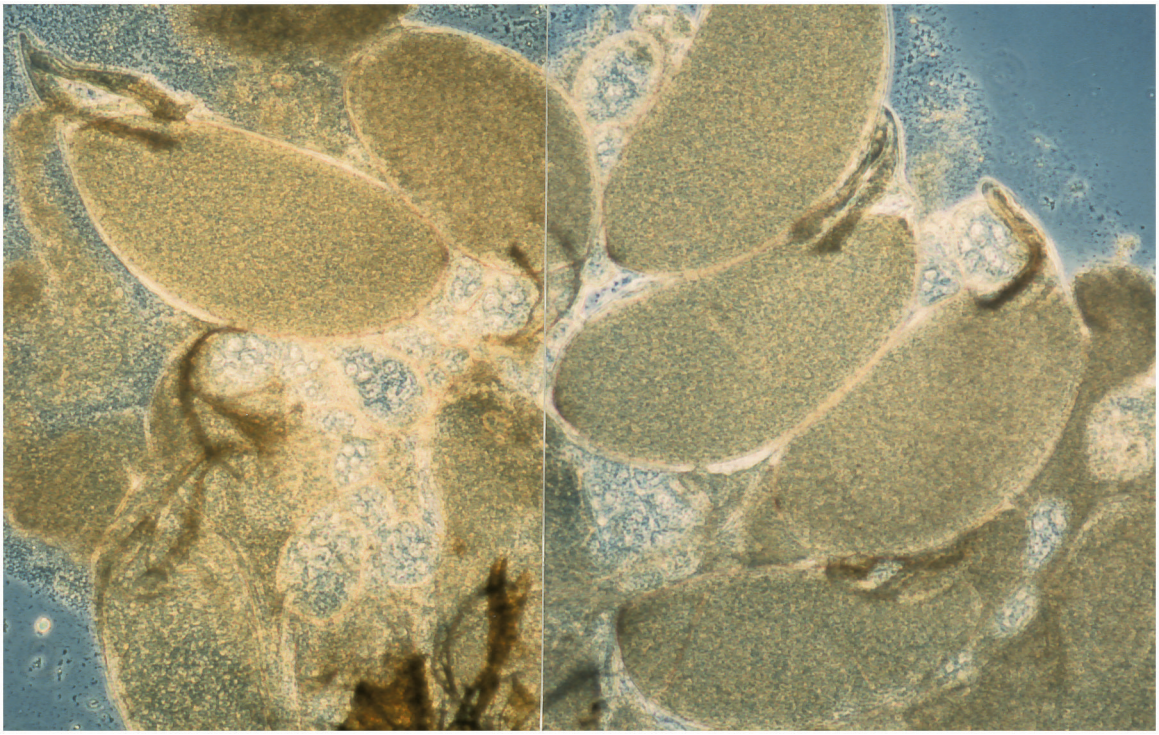

Fig. 6. XX germ cells in an XY pseudofemale. Ovary of an adult XY; hs-*tra*-female pseudofemale that contains apparently normal oogenic XX germ cells after pole cell transplantation.

adults are scored. How DSX^F affects the larval development of XY germ cells is unclear, as XY animals feminized by hs-*tra*-female still seemed to express some early DSX^M function.

tra and *dsx* control sex-determining inductive signals

XX and XY germ cells lacking *dsx* complete gametogenesis and form functional eggs and sperm, respectively when transplanted into appropriate hosts (Schüpbach, 1982). In *XX; dsx/dsx* animals, however, XX germ cells can become oogenic or spermatogenic or they can remain undifferentiated. In *XY; dsx/dsx* animals, XY cells can enter spermatogenesis but they do not form sperm (Orssaud and Laugé, 1982). Thus, *dsx* functions that masculinize or feminize germ cells are expressed in somatic cells and not in the germ line. To control the sexual development of germ cells, the DSX products of somatic cells must regulate the production of inductive signals.

It has been shown that, in somatic cells, *dsx* regulates the expression of sex-specific differentiation genes by repression and activation, respectively. In absence of *dsx*, male- and female-specific differentiation genes seem to be weakly expressed constitutively. Yolk proteins e.g. are made in XX and XY flies lacking *dsx* function. Interestingly, both DSX^M and DSX^F bind to an enhancer that directs sex- and tissue-specific transcription of yolk protein genes. DSX^M represses and DSX^F activates transcription of these female-specific genes (Coschigano and Wensink, 1993). Thus, male and female DSX proteins seem to act directly on target genes to control their expression. Evidence that DSX^M activates some aspects of male differentiation and represses female differentiation was obtained when flies ectopically expressing the male-specific product of *dsx* were analysed (Jursnich and Burtis, 1993).

In analogy to the situation just described, we can postulate that the sex-specific products of *dsx* repress and enhance the production of inductive signals that determine the sex of germ cells. These inductive signals might be weakly expressed constitutively in the absence of *dsx*. We thus have to be aware that germ cells developing in a dsx^- environment do not necessarily display their default sexual phenotype. They may be exposed to weakly expressed male and female signals and, not receiving clear instructions, differentiate cells of ambiguous sexuality.

So far, we do not know what sexual pathway XX germ cells would enter in the complete absence of inductive signals. Such germ cells could be male or female or they could express male- and female-specific genes simultaneously. Since XY germ cells enter spermatogenesis even in female hosts their default pathway must be male. To complete spermatogenesis, however, XY germ cells require somatic DSX^M. This product might enhance the expression of a masculinizing inductive signal, or it might repress a female signal. Alternatively, to complete spermatogenesis XY germ cells might depend on testicular supporting signals that have no sex-determining properties.

Somatic female-specific *tra* function is sufficient to direct XX germ cells into oogenesis

Earlier experiments have shown that XX germ cells developing in XX animals lacking the somatic sex-determining function of *Sxl* are spermatogenic (Schüpbach, 1985; Nöthiger et al., 1989). The same is true for XX germ cells developing in an XY host, which never become oogenic but differentiate spermatocyte-like cells (Steinmann-Zwicky et al., 1989). Here, I show that female-specific *tra* function expressed in somatic cells of animals lacking the female-determining function of *Sxl* is sufficient to drive XX germ cells into oogenesis. When XX flies carrying a viable but mutant allelic combination of *Sxl* are

feminized by female-specific *tra* functions, they contain apparently normal oogenic ovaries. Furthermore, transplanted XX germ cells became oogenic in two XY pseudofemales expressing *tra*.

Table 1 shows that XX germ cells are integrated poorly when transplanted into XY pseudofemales. The partially male phenotype of gonads of larvae carrying the hs-*tra*-female construct, however, suggests that this transgene does not express *tra* as early as the endogenous *tra* gene. Gonads of *XY;* hs-*tra*-female embryos might therefore masculinize transplanted XX germ cells initially, and it is known that spermatogenic XX germ cells have a reduced viability (Seidel, 1963; Steinmann-Zwicky et al., 1989; Nöthiger et al., 1989; this paper). Nevertheless, since, in two positive cases, XY pseudofemales did contain oogenic XX germ cells, it is clear that XX germ cells can enter the female pathway in XY animals expressing *tra*. Since the presence or the absence of somatic *tra* expression makes the difference between an XY soma that can support oogenesis and an XY soma that does not allow for any oogenic differentiation of XX germ cells, I conclude that *tra* via *dsx*, controls an inductive signal that is sufficient to determine the sex of XX germ cells.

Are there inductive signals that are not controlled by *tra* and *dsx*?

XX animals that lack *tra* function develop as males whose adult testes contain spermatogenic, oogenic or degenerating germ cells (Seidel, 1963; Nöthiger et al., 1989). These observations suggest that XX germ cells do not absolutely require somatic *tra* function to become oogenic. How can *tra* function be sufficient in somatic XY cells but not necessary in somatic XX cells for XX germ cells to enter oogenesis?

In addition to somatic induction that is controlled by *tra* and *dsx*, which is sufficient to specify the sex of XX germ cells, yet another inductive signal seems to control the fate of germ cells. XX germ cells that develop in XY hosts never formed oogenic stages. XX germ cells that develop in the masculinized soma of an *X/X; tra/tra* animal, however, can form female-specific nurse cells after metamorphosis. Thus, the soma of an XY male seems to be different from the soma of an XX pseudomale. Since neither expresses *tra* and both express DSX^M, the difference must lie upstream of *tra*. The XX constitution might therefore support the development of XX germ cells by a pathway that bypasses *tra* and *dsx*.

Evidence for an X:A-dependent signal that determines the fate of the gonad by a pathway that bypasses *dsx* can be seen in flies lacking *dsx* function. If *dsx* were to control the differentiation of all somatic sex-specific organs, XX and XY animals mutant for *dsx* should differentiate similar organs. This is true for most sex-specific structures like genitalia, analia or sex comb region which become intersexual in such animals. For the differentiation of the gonad, however, the situation is different. *X/X;dsx/dsx* flies can contain ovaries, testes-like structures or abnormal gonads. *X/Y;dsx/dsx* flies, in contrast, never differentiate ovaries: they form abnormal testes (Orssaud and Laugé, 1982; Schüpbach, 1982). The X:A signal therefore seems to participate in the decisions that lead to the sex-specific differentiation of gonads by controlling the expression of genes that are not regulated by *dsx*. Even XY cells can form female gonads, as shown by *X/Y*; hs-*tra*-female flies that possess ovaries. The TRA and DSX^F functions, therefore, are sufficient to promote ovary formation. In the absence of DSX^F, XX cells may still receive an ovary-determining signal from a female X:A ratio signal such that they can occasionally form an ovary (in *X/X; dsx/dsx* flies). Thus, an XX constitution seems weakly to feminize directly gonadal cells and indirectly XX germ cells by a pathway that bypasses *dsx*.

I have shown that *tra* function is sufficient to control the expression of inductive signals such that XY animals tolerate or promote the development of oogenic XX germ cells. I have also shown that *tra* and *dsx* control an inductive signal that acts on germ cells already in first instar larvae. Future work will be required to test whether a minor contribution to sex determination of germ cells is provided by the somatic X:A ratio through a pathway that does not include *tra*.

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