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In *Drosophila*, female gonadal cells repress male-specific gene expression in XX germ cells

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Abstract

In *Drosophila*, the sex of XX germ cells is determined by somatic signals. Whether sex-specific genes, however, are activated or repressed by somatic signals is not known. We have used *mgm1*, a germline-marker which is specifically expressed in male germ cells to analyze sex-specific gene expression of embryonic germ cells. We found that XX and XY germ cells that do not contact gonadal tissue can express *mgm1*. In contrast, XX germ cells that were associated with female somatic gonadal cells never expressed *mgm1*. Our results suggest that XX germ cells express male-specific genes, unless these genes are repressed by feminizing short range signals produced by the somatic cells of the prospective ovary. © 1998 Elsevier Science Ireland Ltd. All rights reserved

Keywords: Default pathway; Germline; Gonad; *mgm1*; Sex determination

1. Introduction

In *Drosophila*, the sex of somatic cells is determined in a cell-autonomous manner. A set of genes, that together form a genetic hierarchy, make sure that XX cells become female, while XY cells become male (reviewed by Baker, 1989; Cline, 1993). Germ cells, however, respond to sex-determining signals that are different from those acting in somatic cells (reviewed by Steinmann-Zwicky, 1992a,b, 1994a). XY germ cells are autonomously male, as they become spermatogenic and display male-specific gene activity even when developing in a female soma (Steinmann-Zwicky et al., 1989; Staab et al., 1996). The sex of XX germ cells is determined by somatic signals, as XX germ cells developing in a male environment become spermatogenic. This has been shown by transplanting XX germ cells into XY agametic hosts (Steinmann-Zwicky et al., 1989) and by analyzing germ cell differentiation in masculinized XX animals (Nöthiger et al., 1989; Steinmann-Zwicky, 1994b; Staab et al., 1996). Thus, the sex of germ cells is determined both by cell-autonomous information that makes XX cells different from XY cells, and by somatic signals that act on XX germ cells.

It is not known, whether male or female sex-determining signals can act on XX germ cells. Four models can explain the observations reported so far: (1) XX germ cells are inherently female, but they become masculinized when exposed to male signals, (2) XX germ cells are inherently male, but become feminized by somatic signals when developing in a female, (3) XX germ cells express both male- and female-specific genes, unless these are repressed by sex-specific somatic signals, (4) XX germ cells do not express sex-specific genes, unless such genes are activated by sex-specific somatic signals. With our experiments, we want to test whether XX germ cells express male-specific genes, when they are not exposed to somatic sex-determining signals.

A first sexual dimorphism already appears in embryonic germ cells, as male embryos possess more germ cells than female embryos around the time of gonad formation (Poirié et al., 1995). The earliest sex-specific gene expression, however, was detected slightly later, just after gonads have been formed. At stage 14, male germ cells express the enhancer-trap *mgm1*, which is a *male germline-marker*. This marker is expressed in nearly all male embryonic germ cells and in male germline stem cells of older animals (Staab et al.,

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1996). The marker *mgm1* is not exclusively expressed in XY germ cells, but also in XX germ cells that develop in a male environment. Its expression thus is regulated by somatic sex-determining signals.

Somatic sex-determining signals that act on germ cells may originate from the somatic cells of the gonads or they may be long range signals sent to the germ cells from tissue farther apart. Although the former is generally believed (Steinmann-Zwicky, 1994a), no evidence proving the existence of short range signals has been reported. Here we show that XX and XY germ cells that are not imbedded in gonads can express *mgm1*, while XX germ cells that are associated with female gonadal cells do not express this marker. These results suggest that, in *Drosophila*, germ cells express male-specific genes by default, unless these genes are repressed by female gonadal signals.

2. Results

2.1. Germ cells can express germline-specific zygotic marker genes when not imbedded in a gonad

Since germ cells start expressing zygotic genes much later than somatic cells (reviewed by Williamson and Lehmann, 1996), it was important to determine, whether germ cells reach a stage of competence at which they can express zygotic genes, when developing in embryos lacking gonads. Such embryos were obtained by two methods: either they were homozygous for *infra-abdominal-4*, or they derived from mothers homozygous for *nanos*. Mutations in *iab-4*, one of the *cis*-regulatory regions of *abdominal-A* (*abd-A*), a homeotic gene within the *bithorax complex* which is required for determining the correct identity of the second through the eighth abdominal segments, transform epidermal structures of the fourth abdominal segment into those of the third segment. Flies carrying a strong mutation, such as *iab-4*^{302} or *iab-4*$^{4-5}$, have no gonads (Karch et al., 1985). In these animals, the mesodermal cells fail to encapsulate the pole cells when gonadogenesis is normally initiated (Cumberledge et al., 1992). In the second type of experiments, we analyzed embryos in which not only the gonads are absent, but in which the whole abdomen is not formed properly. Seven maternal genes participate in forming the posterior pattern of *Drosophila* embryos. Two of these, however, *nanos* (*nos*) and *pumilio* (*pum*), are not involved in germ cell formation, as in the absence of either of them, the abdomen is not differentiated, but the pole cells are nevertheless formed (Nüsslein-Volhard et al., 1987). Although it has been reported that gene expression begins prematurely in germ cells of embryos lacking *nos* product (Kobayashi et al., 1996), more recent studies showed that the timing of the onset of transcription within germ cells is similar in wildtype embryos and in embryos deriving from *nos* females (Seydoux and Dunn, 1997; Heller and Steinmann-Zwicky, 1998).

Our results show that in animals that lack gonads, many germ cells are present (Table 1A,D). These cells are recognized by anti-*vasa* antibody, which binds to all germ cells, since these have inherited maternal VASA product (Lasko and Ashburner, 1990). We tested BC69, an enhancer-trap line with a *lacZ* gene that is controlled by the *vasa* promoter (J.-L. Couderc, F. Laski, pers. commun.), and that is expressed specifically in germ cells. When such a construct is inherited from the father, which excludes maternal product, most germ cells of 14–16 h old wildtype embryos

Table 1
Expression of marker genes in XY and XX germ cells of embryos without gonads

Genotype	Sex	Marker	*n*	Embryos with blue cells	No. of blue cells/embryo	Total no. of germ cells/embryo
(A) *iab-4/iab-4*	Unsexed	*vasa*-ab	42	–	–	27.7 ± 1.5
	Unsexed	BC69	69	31 (45%)	3.9 ± 1.8	–
(B) *iab-4/iab-4*	X/Y	*mgm1*	99	40 (40%)	3.5 ± 1.2	–
	X/X	*mgm1*	77	26 (34%)	1.8 ± 0.6	–
(C) *iab-4/iab-4*	X/Y	*vasa*-ab/*mgm1*	51	28 (55%)	3.3 ± 1.1	nd
	X/X	*vasa*-ab/*mgm1*	65	23 (35%)	1.4 ± 0.7	nd
(D) Derived from *nos* mothers	Unsexed	*vasa*-ab	62	–	–	13.1 ± 5.1
	Unsexed	BC69	31	3 (10%)	2.7 ± 1.5	–
(E) Derived from *nos* mothers	X/Y	*mgm1*	34	22 (65%)	4.1 ± 1.8	–
	X/X	*mgm1*	30	19 (63%)	3.8 ± 2.1	–
(F) Derived from *nos* mothers	X/Y	*vasa*-ab/*mgm1*	68	42 (62%)	4.4 ± 1.7	nd
	X/X	*vasa*-ab/*mgm1*	106	61 (58%)	4.5 ± 1.4	nd
(G) Derived from *pum* mothers	X/Y	*vasa*-ab/*mgm1*	48	24 (50%)	2.2 ± 1.4	10.6 ± 3.6
	X/X	*vasa*-ab/*mgm1*	60	27 (45%)	2.3 ± 1.6	8.4 ± 4.2
(H) *srp/srp*	X/Y	*mgm1*	44	41 (93%)	9.5 ± 5.6	34.5 ± 6.3
	X/X	*mgm1*	49	47 (96%)	10.8 ± 5.9	33.0 ± 6.3

The expression of BC69 or *mgm1* was monitored by staining for β-galactosidase, which also reveals the marker we use for sexing. In (A), embryos were 14–16 h old. In all other experiments, embryos were 12–14 h old. As BC69 expression is first detected in 13 h old embryos, this might explain the low number of blue cells found in (D). In (A,C,D,F,G) embryos were stained with anti-*vasa* antibody to visualize all germ cells. This antibody recognized the germ cells of all animals. *n*, number of embryos analysed; no. of blue cells/embryo means per embryo with blue cells; nd, not determined.

express this marker. In embryos without gonads, only few germ cells expressed BC69 (Table 1A,D). Thus, many germ cells may not reach a stage of competence at which they start expressing zygotic germline-specific genes when developing outside of a gonad. The fact, however, that some germ cells did express a germline-specific gene encouraged us to continue our experiments.

2.2. XX germ cells can express a male-specific marker gene when not imbedded in a gonad

The male-specific germline marker *mgm1* is autonomously expressed in XY germ cells of 12–14 h old embryos irrespective of the sex of the surrounding somatic tissue (Staab et al., 1996). We therefore expected it to be expressed in XY germ cells that develop in the absence of gonads. To test, however, whether XX embryos also possess *mgm1* expressing germ cells, we performed crosses in which we could identify the sex of each individual embryo. A large number of male and female mutant embryos lacking gonads (because they were mutant for *iab-4* or because they derived from *nos* mothers), but carrying *mgm1* contained one or several blue cells (Fig. 1A–D; Table 1B,E). The mutant embryos deriving from the same cross but lacking *mgm1* were also analysed: none of them contained blue cells. Thus, in embryos without gonads, both XY and XX germ cells can express *mgm1*. As expected, only few staining germ cells were found, probably because in the absence of a gonad, not all germ cells reach the stage of competence required to express zygotic genes.

The cells that were blue look very much like germ cells: they are round and larger than somatic cells. To test whether the *mgm1*-expressing cells in embryos lacking gonads really are germ cells, we stained embryos homozygous for *iab-4* and embryos deriving from *nos* mothers with both X-Gal which reveals *mgm1* expression and anti-*vasa* antibody, which specifically recognizes all germ cells. A third experiment was performed, in which embryos without gonads but carrying *mgm1* derived from *pum* mothers. In all three experiments, the sex of the embryos was assessed using the paternally introduced *Dfd::lacZ* marker. As expected, only few of the germ cells that were present in mutant embryos expressed *mgm1*. All cells, however, that were blue were also stained with the *vasa* antibody (Fig. 1E; Table 1C,F,G). This shows that the blue cells expressing *mgm1* in mutant embryos lacking gonads are germ cells.

We analysed sexed embryos lacking germ cells, but carrying *mgm1*, to test whether agametic embryos possess blue staining cells. The embryos were obtained by crossing females homozygous for osk^{302} (Lehmann and Nüsslein-Volhard, 1986) to males carrying the X-chromosomal marker *Dfd::lacZ* and one copy of *mgm1*. After staining with X-Gal, we analysed 59 XY embryos and 57 XX embryos carrying *mgm1*. None of these possessed the typical blue cells, which again confirmed that *mgm1* is specifically expressed in germ cells.

2.3. In germ cells, mgm1 is activated by default

When migrating towards the gonads, germ cells of wild-type embryos interact with cells of the midgut, they contact mesodermal cells and finally lie close to those cells that will form the gonads. We found that germ cells can activate *mgm1* in 12–14 h old embryos, and BC69 in 14–16 h old embryos, even if these embryos do not possess gonads or if they lack the abdomen. This suggests that germ cells are not required to migrate properly and to receive somatic signals from adjacent tissue to start transcription. Rather, germ cells seem to possess an internal clock that tells them when to activate zygotic genes. To test whether interactions with adjacent somatic cells are required for germ cells to express *mgm1*, we decided to analyze the expression of this male-specific marker in embryos that specifically lack the midgut, and in which interactions between germ cells and mesoderm are not possible. Our test embryos were homozygous for the mutation *serpent* (*srp*) (Reuter, 1994). In these animals, cells that would normally form the midgut undergo homeotic transformation, such that foregut is differentiated instead of anterior midgut and hindgut is differentiated instead of posterior midgut. The embryos thus lack the entire midgut. In the mutant embryos, germ cells are internalized into the forming gut at the beginning of gastrulation. Then, however, many germ cells remain entrapped in the pocket formed by the abnormal gut cells. About 50% of the germ cells manage to exit the gut. These cells are found in random positions within the embryos (Fig. 1F). If germ cells of *srp/srp* embryos that do not exit the abnormal gut activate *mgm1* after 12–14 h, when germband retraction is completed, we might conclude that they possess an internal timing which controls activation of zygotic genes and that no interaction with any specific somatic tissue, like midgut, mesoderm or gonad, is required for this activation. We analysed XX and XY embryos that were homozygous for *serpent*. In embryos that were 12–14 h old, cells that expressed *mgm1* were found in animals of both sexes in similar numbers (Fig. 1G; Table 1H). On average, a third of all germ cells expressed the male-specific marker gene. There was no difference in the percentage of blue cells when germ cells that remained in the gut where compared with those that had exited the gut. Blue cells were never found in embryos that had not completed germband retraction. Neither were blue cells found in embryos from the same cross that were mutant for *srp* and that lacked the marker *mgm1*. This shows that, in animals of both sexes, many germ cells specifically express the male marker *mgm1* by default after 12–14 h of development.

2.4. Female gonadal tissue represses mgm1

Our results show that XY and XX germ cells can express the male germline-marker *mgm1* when they are not associated with gonadal cells. This suggests that, in wildtype females, the ovary produces signals that repress male-

specific genes in XX germ cells. Table 1 reveals that in embryos of genotype *srp/srp* and in embryos deriving from *nos* or *pum* mothers, a similar number of blue cells are found in XY and XX embryos. In embryos that are

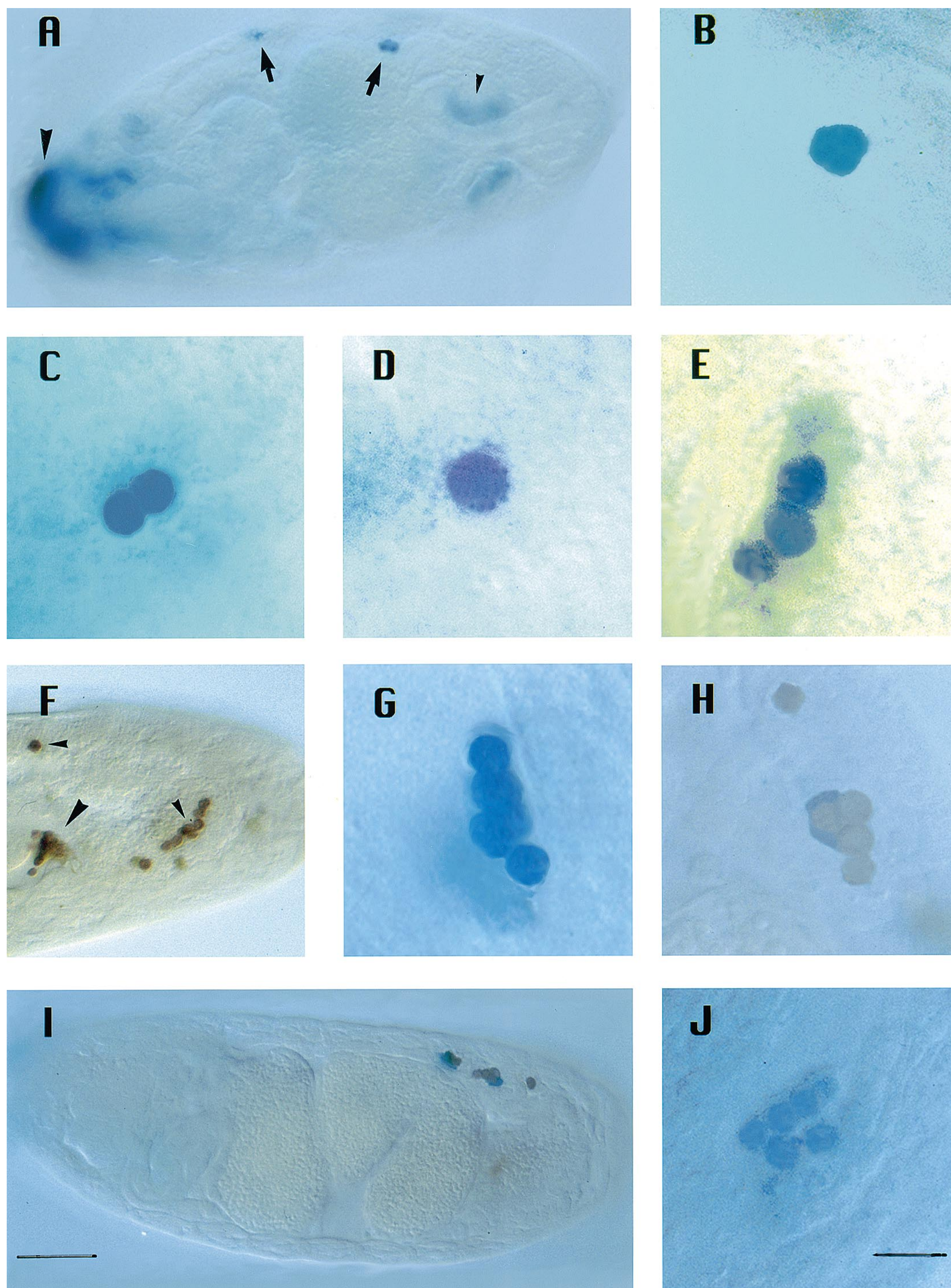

Fig. 1. Embryos with stained germ cells. (A) Female embryo derived from *nos* mother and carrying *mgm1*. The *Dfd* expression in the head region (large arrowhead) reveals that the embryo is a female, the staining in the telson (small arrowhead) reveals that *mgm1* is present. The arrows point to blue cells within the embryo. These are germ cells that express *mgm1*. (B–E) *mgm1* expressing cells in: (B) XX embryo homozygous for *iab-4*, (C) XX embryo derived from *nos* mother, (D) XY embryo derived from *nos* mother, (E) XX embryo derived from *pum* mother, double stained with antibodies recognizing *vasa*. Germ cells therefore have a brown color, (F) female *srp/srp* embryo treated with anti-*vasa* antibody which reveals that some 50% of the germ cells remain entrapped in the abnormal gut (large arrowhead), while 50% find their way to a random place within the body cavity (small arrowheads), (G) X/X;*srp/srp* embryo with germ cells expressing *mgm1*, (H,I) XX embryos homozygous for *iab-4* with somatic gonadal tissue revealed by the expression of a specific enhancer-trap line (blue); such tissue is always associated with a cluster of germ cells (stained brown by *vasa*-ab), that do not express *mgm1*, (J) XY embryo homozygous for *iab-4* showing a cluster of *mgm1* expressing cells in the gonad region. Scale bar: (A,F,I) 90 μm, (B–E,G,H,J) 20 μm.

homozygous for *iab-4*, however, more blue staining germ cells are found in males than in females. This is expected if gonadal cells are not totally absent in the mutant embryos, so that some communication between somatic gonadal tissue and germ cells can still take place. It has been reported that some somatic gonadal cells are in fact present in these animals. Somatic cells of the gonads derive from at least two different subpopulations of precursor cells. One group of cells derives from the mesoderm of parasegments 11–13 and is migrating anteriorly together with the germ cells. The other group of precursor cells originates from the mesoderm of parasegments 10 and 11: they do not migrate, as they arise at the places where the gonads will form. In *iab-4/iab-4* embryos, the expression of *abd-A* is abolished in the group of precursor cells from parasegments 10 and 11. As a result, the second group of precursor cells are not formed, but at least some somatic gonadal precursor cells from parasegments 11–13, which migrate together with germ cells, are intact (Boyle and DiNardo, 1995).

Using an enhancer-trap line that is specifically expressed in somatic cells of female gonads (line 1252B, Sergeev, P., Heller, A., Hollmann, M., Schäfer, U. and Steinmann-Zwicky, M., unpublished data), we found that some gonadal cells are present in 56% ($n = 80$) of our female *iab-4/iab-4* embryos, while no staining gonadal cells were present in embryos deriving from *nos* ($n = 170$) or *pum* ($n = 150$) mothers. In female *iab-4/iab-4* embryos, gonadal cells were always associated with a cluster of germ cells (Fig. 1H,I). Cells from such clusters never expressed *mgm1*. Germ cells that were not close to such a cluster sometimes expressed the marker. Blue *mgm1* expressing cells were found in 34 of the 80 females tested. The cells were either alone or in pairs of two, but more than two blue cells were not found together, and none of the blue cells were associated with somatic gonadal cells that were present in roughly half of the females. In males, 46 out of 87 embryos analysed contained *mgm1*-expressing germ cells. Of these 46 embryos, 16 had clusters with three or more (up to seven) blue cells where the gonads normally form (Fig. 1J). These results suggests that in females, those precursor cells of the gonad which are still present in *iab-4* embryos produce a short range signal that represses the expression of *mgm1* in adjacent germ cells.

3. Discussion

3.1. The initiation of transcription in the germline

Our results show that germline-specific enhancer-trap lines are expressed in embryos irrespective of the presence of gonads, midgut tissue or even abdominal structures. The tested enhancer-trap lines were expressed in embryos of the same stage whether the embryos were wildtype or whether they were lacking one of the mentioned tissue. Previously, it has been reported that in the germ cells of embryos deriving from homozygous *nos* mothers, gene expression begins prematurely (Kobayashi et al., 1996). This conclusion was drawn because, in mutant embryos, germline-specific enhancer-trap lines were apparently expressed at stage 7–8, while in wildtype embryos, these lines were expressed after gonad formation, at stage 13–14. However, newer studies showed that in embryos derived from homozygous *nos* females neither the germline-specific enhancer-trap lines tested nor other lines are expressed prematurely (Heller and Steinmann-Zwicky, 1998). To test whether germ cells of embryos deriving from *nos* females transcribe genes prematurely, another study was performed. Polymerase II is specifically phosphorylated upon transcription. An antibody recognizing the phosphorylated epitope was therefore used to detect gene transcription in *Drosophila*. When the onset of polymerase II phosphorylation was studied in *Drosophila* germ cells, no difference was found between the germ cells of wildtype embryos and germ cells of embryos deriving from homozygous *nos* females (Seydoux and Dunn, 1997). In agreement with these results, the data presented in this paper show that the two enhancer-trap lines *mgm1* and BC69 are not prematurely expressed in embryos lacking *nos*, but that the timing of expression is the same as in wildtype.

Our results suggest that germ cells are not required to migrate properly through the midgut and to receive somatic signals from adjacent tissue (midgut, mesoderm or gonadal tissue) to start transcription. Rather germ cells seem to possess an internal clock that tells them when to activate zygotic genes. In contrast, different experiments have shown that migration is not controlled by a germline autonomous timing. Both germ cells that have been kept in vitro culture for a few hours and germ cells of heterochronic embryos were able to populate the gonad and produce functional germ cells when transplanted into a host gonad (Allis et al., 1979; Jaglarz and Howard, 1994). Somatic cells will send signals, one of them being the repulsive signal from the gene *wunan* (Zhang et al., 1997) or they will interact with the germ cells and help them to find their way to the gonads (Boyle and DiNardo, 1995).

3.2. The male marker mgm1 is activated by default in germ cells

Our aim was to analyze the sex-specific gene expression of germ cells that were not influenced by somatic signals that could derive from the gonads. We analysed the germ cells of embryos that lacked gonads in three different sets of experiments. In each of them, lack of gonads was caused by a different mutation. In embryos homozygous for *iab-4*, only the gonads are absent (Table 1A–C). In embryos deriving from *nos* or *pum* mothers, the abdomen is not formed (Table 1D–G). Furthermore, we analysed germ cells of *srp/srp* embryos which do not interact with somatic gonadal cells because they remain entrapped in the gut (Table 1H). The different sets of experiments yielded comparable

results. In all cases, expression of *mgm1* was seen in a number of germ cells of 12–14 h old embryos (stage 14–16), irrespective of the sexual genotype of the embryos. This is best explained by postulating that *mgm1* is activated by default in XX and XY germ cells.

If *mgm1* is activated by default in the germ cells of XX and XY embryos, why do not all germ cells express this marker in the mutant animals analysed? The highest number of *mgm1* expressing germ cells was observed in XX and XY embryos homozygous for *srp*. In these, roughly a third of all germ cells expressed the male-specific marker. Similar results were obtained with embryos lacking *nos* or *pum*. These embryos possess fewer *mgm1* expressing cells, but, since the total number of germ cells of these embryos is much reduced, it appears that in these embryos too, roughly a third of the germ cells express *mgm1*. What about the other two-thirds of the germ cells? Do they not express the marker? We think that this is unlikely. A large number of germ cells (maybe all) might be competent to transcribe germline-specific genes even in mutant animals. Enhancer-trap lines, however, might not yield enough gene product for detection in some germ cells before the cuticle is formed, which is when the animals have to be analysed. Not all germ cells are stained in wildtype males carrying *mgm1* or in embryos with BC69 when these animals are treated with X-Gal. Furthermore, germ cells might need more time to express the markers in suboptimal conditions. In *srp* embryos and in embryos lacking *nos* or *pum* product, germ cells might have more time than in embryos that are wildtype or homozygous for *iab-4*. We observed that development of these embryos is slightly delayed compared with *iab-4* and wildtype embryos.

3.3. Female gonadal cells feminize XX germ cells by repressing male-specific gene expression

Our results show that XX germ cells can express a male-specific marker gene when developing outside of a gonad. This suggests either that the default pathway of *Drosophila* XX germ cells is male or, alternatively, that XX germ cells express male and female genes in the absence of somatic sex-determining signals. In either case, we can conclude that XX germ cells depend on a feminizing somatic signal for female development.

It has previously been claimed that evidence for the existence of a somatic positive feminizing signal for germline development was found (Granadino et al., 1993). The experiments presented, however, did not address this question and the conclusions drawn did not follow from the results presented. The authors found that germ cells of genotype Sxl^{f7M1}/Sxl^{fc} differentiated functional eggs when transplanted into host females. Since females of genotype Sxl^{f7M1}/Sxl^{fc} had been claimed to possess no gonads, the authors concluded that germ cells degenerate in the mutant animals, because they do not receive a feminizing signal from the soma. Germ cells, however, cannot survive and be identified in adult animals lacking gonads. The observation that no germ cells are found in animals lacking gonads, therefore gives no information about the sex-specific requirements of germ cells.

With our experiments, we have analysed the sex of germ cells directly in embryos. There we have tested for sex-specific gene expression before germ cells die because they do not receive proper somatic signals to support their development. So far there is only one sex-specific marker, *mgm1*, whose expression can be tested for at this early stage. Since XX germ cells express *mgm1* when not imbedded in a gonad, we conclude that XX germ cells require a feminizing signal to become female. Since XX germ cells that are associated with female somatic gonadal cells never expressed *mgm1*, we can conclude that this feminizing sex-determining signal is produced by somatic cells of the prospective ovary and that this signal represses male-specific genes within germ cells.

In mice, XX and XY germ cells become oogenic (by morphological criteria) by default, i.e. when not influenced by somatic sex-determining signals. Masculinizing signals, that force germ cells to become spermatogenic, are secreted by somatic cells of the testis (Zamboni and Upadyay, 1983; reviewed by McLaren, 1993; McLaren and Southee, 1997). Thus, similarities and differences appear when mechanisms used for germline sex determination are compared. With our studies, we confirm the importance of somatic signals in germline sex determination, but we also show that the default sexual pathway of germ cells can be different depending on the species analysed.

4. Experimental procedures

4.1. General experimental conditions

Flies were kept on standard fly food at 22°C. Mutant embryos were homozygous for *iab-4*^{302} or *srp*^{3}, or they derived from mothers that were homozygous for *nos*^{L7}, or *pum*^{680}. In the two former cases, embryos carrying a balancer were identified due to an *ftz::lacZ* construct that was present on the TM3 balancer. The presence of *mgm1* was detected due to a staining of telson structures in embryos with this enhancer-trap (Staab et al., 1996). Embryos were sexed by introducing from the father an X-chromosome carrying a *Dfd::lacZ* construct (F1-70.2, Zeng et al, 1994), which causes all daughters to display a blue *Dfd* pattern in the head.

BC69 is an enhancer-trap line reflecting *vasa* activity (Couderc and Laski, pers. commun.). Staining for β-galactosidase was performed according to standard protocols. X-Gal and antibody double-stainings were performed according to Poirié et al. (1995). BC69 expressing cells are first detected in 13–14 h old embryos (Heller and Steinmann-Zwicky, 1998). *mgm1* staining cells already appear in 10–12 h old embryos (Staab et al., 1996).

4.2. Staining of progeny deriving from nos mothers

Degenerating germ cells located outside of *nos* derived embryos display a pH-dependent blue staining when treated with X-Gal, irrespective of the presence of a *lacZ* gene and irrespective of the stage of the embryos (Heller and Steinmann-Zwicky, 1998). Because of these 'pole cells', embryos often appear younger than they really are. Germ cells that have managed to enter the embryos do not display this artefactual unspecific staining. Therefore we have, in our experiments, only analysed the staining of cells within the mutant embryos. Since we use a *Dfd::lacZ* construct to sex our embryos, and since the pattern of expression of this construct very precisely reveals the stage of the embryos (Heller and Steinmann-Zwicky, 1998), we were able to compare embryos of different genotypes, but of the same stage.

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References

Allis, C.D., Underwood, E.M., Caulton, J.H., Mahowald, A.P., 1979. Pole cells of *Drosophila melanogaster* in culture. Dev. Biol. 69, 451–465.

Baker, B.S., 1989. Sex in flies: the splice of life. Nature 340, 521–524.

Boyle, M., DiNardo, S., 1995. Specification, migration and assembly of the somatic cells of the *Drosophila* gonad. Development 121, 1815–1825.

Cline, T.W., 1993. The *Drosophila* sex determination signal: how do flies count to two?. Trends Genet. 9, 385–390.

Cumberledge, S., Szabad, J., Sakonju, S., 1992. Gonad formation and development requires the *abd*-A domain of the bithorax complex in *Drosophila melanogaster*. Development 115, 395–402.

Granadino, B., Santamaria, P., Sanchez, L., 1993. Sex determination in the germ line of *Drosophila melanogaster*: activation of the gene *Sex-lethal*. Development 118, 813–816.

Heller, A. and Steinmann-Zwicky, M., 1998. No premature gene expression in germ cells of embryos deriving from *nos* females. Mech. Dev. 72, 169–173.

Jaglarz, M.K., Howard, K.R., 1994. Primordial germ cell migration in *Drosophila melanogaster* is controlled by somatic tissue. Development 120, 83–89.

Karch, F., Weiffenbach, B., Peifer, M., Bender, W., Duncan, I., Celniker, S., Crosby, M., Lewis, E.B., 1985. The abdominal region of the bithorax complex. Cell 43, 81–96.

Kobayashi, S., Yamada, M., Ashoka, M., Kitamura, T., 1996. Essential role for the posterior morphogen nanos for germline development in D*rosophila*. Nature 380, 708–711.

Lehmann, R., Nüsslein-Volhard, C., 1986. Abdominal segmentation, pole cell formation, and embryonic polarity requires the localized activity of *oskar*, a maternal gene in *Drosophila*. Cell 47, 141–152.

Lasko, P.F., Ashburner, M., 1990. Posterior localization of vasa protein correlates with, but is not sufficient for, pole cell development. Genes Dev. 4, 905–931.

Nöthiger, R., Jonglez, M., Leuthold, M., Meier-Gerschwiler, P., Weber, T., 1989. Sex determination in the germ line of *Drosophila* depends on genetic signals and inductive somatic factors. Development 107, 505–518.

Nüsslein-Volhard, C., Frohnhöfer, H.G., Lehmann, R., 1987. Determination of anteroposterior polarity in *Drosophila*. Science 238, 1675–1681.

McLaren, A., 1993. Germ cell sex determination. Semin. Dev. Biol. 4, 171–177.

McLaren, A., Southee, D., 1997. Entry of mouse embryonic germ cells into meiosis. Dev. Biol. 187, 107–113.

Poirié, M., Niederer, E., Steinmann-Zwicky, M., 1995. A sex-specific number of germ cells in embryonic gonads of *Drosophila*. Development 121, 1867–1873.

Reuter, R., 1994. The gene *serpent* has homeotic properties and specifies endoderm versus ectoderm within the *Drosophila* gut. Development 120, 1123–1135.

Seydoux, G., Dunn, M., 1997. Transcriptionally repressed germ cells lack a subpopulation of RNA polymerase II in *C. elegans* and *Drosophila* embryos. Development 124, 2191–2201.

Staab, S., Heller, A., Steinmann-Zwicky, M., 1996. Somatic sex-determining signals act on XX germ cells in *Drosophila* embryos. Development 122, 4065–4071.

Steinmann-Zwicky, M., Schmid, H., Nöthiger, R., 1989. Cell-autonomous and inductive signals can determine the sex of the germ line of *Drosophila* by regulating the gene *Sxl*. Cell 57, 157–166.

Steinmann-Zwicky, M. 1992a. How do germ cells choose their sex? *Drosophila* as a paradigm. BioEssays 14, 513–518.

Steinmann-Zwicky, M. 1992b. Sex determination of *Drosophila* germ cells. Semin. Dev. Biol. 3, 341–347.

Steinmann-Zwicky, M. 1994a. *Sxl* in the germ line of *Drosophila*: a target for somatic late induction. Dev. Genet. 15, 265–274.

Steinmann-Zwicky, M. 1994b. Sex determination of the *Drosophila* germ line: *tra* and *dsx* control somatic inductive signals. Development 120, 707–716.

Williamson, A., Lehmann, R., 1996. Germ cell development in *Drosophila*. Annu. Rev. Cell Dev. Biol. 12, 365–391.

Zamboni, L., Upadyay, S., 1983. Germ cell differentiation in mouse adrenal glands. J. Exp. Zool. 228, 173–193.

Zhang, N., Zhang, J.-P., Purcell, K.J., Cheng, Y., Howard, K.R., 1997. The *Drosophila* protein Wunen repels migrating germ cells. Nature 385, 64–66.