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Somatic sex-determining signals act on XX germ cells in *Drosophila* embryos

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SUMMARY

In *Drosophila*, the enhancer-trap line *mgm1* is already specifically expressed in male germ cells. Staining is first detected in 10-hour-old embryos and it is found in later stem cells. This line, which reveals the earliest sex-specific gene expression in the germline known so far, is a useful molecular marker to assess the sexual pathway that germ cells have entered before any overt sexual dimorphism is apparent. XY germ cells that develop in feminized animals express *mgm1*, which shows that this marker is autonomously expressed in XY germ cells. However, XX germ cells that develop in masculinized animals also express *mgm1*. Therefore, somatic sex-determining signals have already acted on XX germ cells in 10-hour-old embryos.

Key words: germline, *mgm1*, sex determination, sex-specific marker, *Drosophila*

INTRODUCTION

In *Drosophila*, the sex of germ cells is determined by a combination of somatic and autonomous signals (reviewed in: Steinmann-Zwicky, 1992a,b, 1994a). XX germ cells that had been transplanted into a male host became spermatogenic, which shows that the sex of XX germ cells is determined by somatic signals. However, XY germ cells that developed in a female XX host also became spermatogenic. Since these germ cells do not respond to somatic sex-determining signals, they must possess autonomous information for maleness (Steinmann-Zwicky et al., 1989). In these experiments, the sex of the germ cells was assessed using morphological criteria. Molecular markers to identify the sex of germ cells were not available. Furthermore, it was not determined when the two different types of sex-determining signals act on germ cells; the transplanted germ cells were all analysed in the gonads of adult hosts.

The genes that determine the sex of somatic cells (reviewed in: Baker, 1989; Cline, 1993) control the expression of the somatic sex-determining signals that act on germ cells. We conclude this from the observation that XX animals that are masculinized by a mutation in one of the genes of the sex determination cascade often possess spermatogenic germ cells (Seidel, 1963; Nöthiger et al., 1989; Steinmann-Zwicky, 1994b) and because XX germ cells transplanted into an XY host that is feminized by ectopic expression of one of these genes become oogenic and produce eggs (Steinmann-Zwicky, 1994b).

During the development of *Drosophila* germ cells, two phases can be distinguished. First, during embryogenesis, male and female germ cells look alike. Yet, a difference between male and female germ cells is already detected in embryos around the time when the gonad is formed. At the blastoderm stage, there are similar numbers of germ cells in male and female animals. At 8-10 hours after egg laying, however, males have more germ cells than females (Poirié et al., 1995). In a second phase, germ cells differentiate such that individual cells can be identified as oogenic or spermatogenic. For male germ cells, this is detected during larval stages; for female germ cells it becomes apparent in the adult (reviewed in: Fuller, 1993; Spradling, 1993). Sex-determining signals must act on germ cells before the first sexual dimorphism is seen. To determine whether the early aspects of germline sex determination are controlled by germ-cell autonomous or somatic sex-determining signals, we have analysed the expression of an early sex-specific germline marker in wild-type and sex-transformed animals.

Here we show that the *male germ-line-marker1* (*mgm1*) is already specifically expressed in male germ cells of stage 13 embryos, 10 hours after egg laying. This is the earliest sex- and germline-specifc gene expression known so far. With this molecular marker, we could show that somatic and germ-line autonomous sex-determining signals already act on germ cells in embryos.

MATERIALS AND METHODS

In this paper, we have used the words germ cells for germline cells of all stages: pole cells, germ cell precursor cells and differentiated gametes. Similarly, the word gonad is used for embryonic gonadal anlagen as well as for the differentiated gonads of adults.

Fly strains and culture

All flies were kept on standard fly food at 22°C, unless stated otherwise. Mutations are described in Lindsley and Zimm (1992). Embryonic stages are those of Campos-Ortega and Hartenstein (1985). The enhancer-trap line A507.2M2, which we call *mgm1*, is described in Bellen et al. (1989). β-Gal expression is caused by a *lacZ* gene, which was inserted onto a CyO balancer chromosome. Therefore, *mgm1* cannot be kept in a homozygous condition. However, since, as is the case with many en-trap lines, *mgm1* also

stains parts of the brain and posterior spiracles (Bellen et al., 1989), the presence of *mgm1* can easily be detected in embryos. Adult flies carrying *mgm1* are *Cy*. Germline-less animals were produced by crossing females homozygous for the maternal effect mutation osk^{301} to males carrying *mgm1*. The loss-of-function alleles of *tra* and *dsx* were tra^1 and dsx^1.

Staining procedures

X-Gal staining was performed according to standard protocols. Since the expression of *mgm1* is rather weak in young embryos, X-Gal staining of embryos was done overnight over a period of 16-20 hours. X-Gal and antibody double-stainings were done as described in Poirié et al. (1995). To visualize all germ cells, we used anti-vasa antibody (Lasko and Ashburner, 1990).

Sexing embryos and larvae and identifying homozygotes

Whenever embryos were sexed, we crossed females to males carrying a *Dfd::lacZ* construct inserted on their X chromosome. This construct, which was generously provided by Chaoyang Zeng and Bill McGinnis, is expressed specifically in female embryos, when deriving from the father. A *TM3* balancer chromosome carrying a *ftz::lacZ* construct was used to identify embryos that were homozygous for a mutation on the third chromosome. Larvae were sexed with *y* and y^+ alleles and homozygous larvae mutant for *tra* or *dsx* were identified with p^p.

RESULTS

A male-specific germline marker

The enhancer-trap line A507.2M2 has been reported to be expressed in 'pole cells' of stage 17 embryos (Bellen et al., 1989). To test whether in this en-trap line *lacZ* expression is in fact found in germ cells rather than in somatic cells of the gonads, we constructed agametic animals carrying A507.2M2. Such embryos showed no staining in their empty gonads. Embryos with germ cells, however, that expressed A507.2M2, had blue germ cells.

Sexing the embryos with a paternally introduced *Dfd::lacZ* construct, which is located on the X chromosome, we found that only XY embryos express A507.2M2 in their germ cells. XX embryos, which show the specific *Dfd* pattern of expression of β-galactosidase in their heads, have unstained gonads. Since A507.2M2 is specifically expressed in male germ cells, we named it *male germline-marker 1 (mgm1)*.

mgm1 is not expressed in proliferating female germ cells

In early larval stages, male germ cells divide more than female germ cells (Aboim, 1945; Seidel, 1963; Steinmann-Zwicky, 1994b). The enhancer-trap *mgm1* could therefore reveal the activity of a gene that is required in mitotic germ cells irrespectively of their sex. To test, whether *mgm1* is expressed in proliferating germ cells rather than in male germ cells, we analysed the gonads of female embryos containing excessively proliferating germ cells.

In previously performed experiments, we have observed that a few transplanted germ cells can completely populate ovaries and testes of agametic animals, such that no abnormality is observed in adult gonads (Steinmann-Zwicky et al., 1989; Steinmann-Zwicky 1993, 1994b). To achieve this, the transplanted germ cells must undergo extra rounds of mitoses. Such an upregulation is seen even if only 3-5 germ cells are transplanted into a host embryo (embryos normally have about 40 germ cells). We have searched for a mutation that causes embryos to have few germ cells, in the hope of mimicking the experimental conditions obtained in pole cell transplantation experiments.

Females homozygous for the maternal effect mutation *wkl* yield progeny whose embryonic gonads are largely depleted of germ cell. Yet in some cases, adult daughters have normal ovaries (Daniel St. Johnston, personal communication). Blastoderm embryos revealed that only few embryos deriving from *wkl* mothers possess a small number of pole cells which were stained with anti-*vasa* antibody. When mothers were kept at 18°C, 78.5% of the 14- to 16-hour-old embryos were agametic (*n*=228). At 25°C, 47.5% of the embryos of the same age possessed no germ cells (*n*=160). Those embryos that possessed germ cells had 2.2 (±1.2) (*n*=76) germ cells per gonad, when mothers had been kept at 25°. None of the embryos contained a wild-type number of germ cells, which was found to be 12.2 (±2.3) in female progeny from heterozygous *wkl/+* control mothers. In late first instar larvae, non-agametic gonads of females derived from homozygous *wkl* mothers possessed 8.1 (±4.3) (*n*=10) germ cells, while gonads of control females had 12.9 (±2.4) (*n*=18) germ cells. In second and third instar larvae, most gonads had a wild-type number and fewer than 20% (*n*=20) had a reduced number of germ cells. In adults, gonads were either empty or apparently normal. In females containing germ cells, all ovarioles were filled with germ cells. These data show that germ cells of embryos deriving from *wkl* mothers divide in late embryonic and early first instar stages, while germ cells of control females hardly proliferate at these stages.

Female embryos derived from *wkl* mothers that were kept at 25°C did not express *mgm1* in their gonads (*n*=178), but their brothers did. Of 186 gonads of male embryos tested, 113 possessed 2.5 ±1.3 staining germ cells, a number which is comparable to the total number of germ cells per gonad mentionned above. The other gonads were agametic. This confirms that *mgm1* is expressed specifically in male germ cells and not in proliferating germ cells.

mgm1 is expressed in male germ-line stem cells of embryos, larvae and adults

The earliest germ cell expression of *mgm1* can be seen in male embryos of stage 13, 10 hours after egg laying, just after gonad formation. In 12- to 14-hour-old embryos, all *mgm1*-carrying males possess staining germ cells in their gonads (Fig. 1A). Although the staining is rather weak compared to other enhancer-trap lines, we found on average 11.1±2.6 (*n*=40) blue germ cells per gonad with appropriate staining conditions. This number is slightly lower than the total number of germ cells revealed in such males by anti-vasa antibody in a separate experiment (13.5±1.8, *n*=40), maybe because in a small number of germ cells *mgm1* expression is initiated later than in the majority of the cells.

In larvae that were dissected at 46, 72 and 120 hours after egg laying staining becomes more and more restricted to an anteriorly located subpopulation of cells. In testes of early second and late third instar larvae, about 20-40 germ cells express *mgm1* (Fig. 1B,C). A count of blue cells in gonads of third instar larvae revealed 26.5 (±4.9, *n*=12) blue germ cells, half of which are dark blue, the others lighter. Spermatocytes

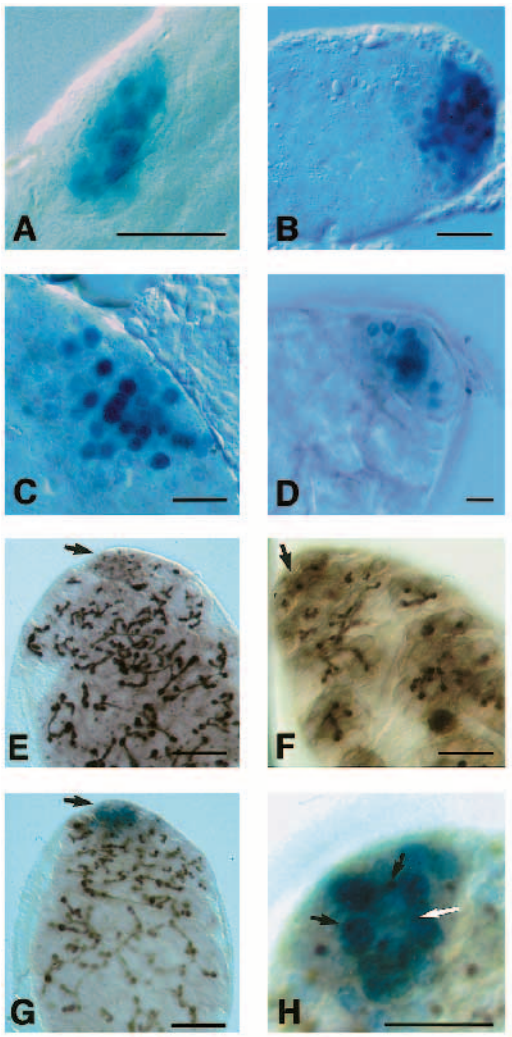

Fig. 1. Expression pattern of *mgm1* in XY embryos, 3rd instar larvae and adults. (A) Gonad of an XY embryo showing *mgm1* expression in male germ cells. (B,C) Testes of larvae containing *mgm1*-expressing germ cells; (B) early second instar larva, (C) late third instar larva. (D) Tip of the testis of an adult male with *mgm1*-expressing germ cells. (E-H) Tips of testes stained with 2C1 antibody revealing fusome structures. Spherical spectrosomes are found in stem cells and their daughters at the very distal tip of the testes (black arrows). Elongated and branched fusomes that hold the cells of cysts together (illustrated in F) are found in more proximal regions of the testes. (G) *mgm1* expression is seen in germ cells with a spherical spectrosome. (H) Dissected tip of a testis photographed from above reveals the radial arrangement of *mgm1*-expressing germ cells around the unstained somatic hub cells (white arrow). Scale bars, 20 μm.

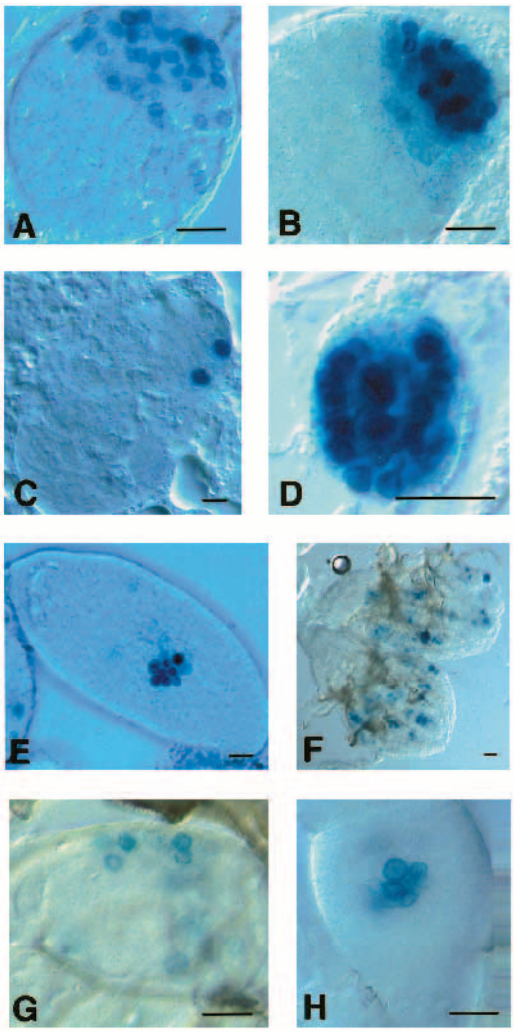

Fig. 2. Expression pattern of *mgm1* in the gonads of sex-transformed XX animals and in females with ovarian tumors. (A) Gonad of a 46-hour-old larva of genotype *X/X; tra/tra*. (B) Gonad of a 46-hour-old larva of genotype *X/X; dsx*^{D}/+. (C) Typical gonad of a 120-hour-old larva of genotype *X/X;dsx/dsx*, with only two *mgm1*-expressing germ cells. (D) Gonad of an early first instar larva of genotype *Sxl*^{M1}*/Y*. (E-H) Gonads of adults; (E) *X/Y; hs::tra/+*, (F) *Sxl*^{f4}*/Sxl*^{f4}, (G) *snf/snf*, (H) *otu*^{1}*/otu*^{1}. Scale bars, 20 μm.

and later spermatogenic stages do not stain. In adult males, staining is seen in 17.5 (±4.1, *n*=14) cells at the tips of the testes (Fig. 1D). Again about half of these cells are dark blue, half of them lighter. In male larvae and adults with agametic testes, no staining was detected.

The fact that embryonic male germ cells express *mgm1* together with the observation that, in adults, *mgm1*-expressing germ cells are located at the tip of the testis indicate that the stained cells are most probably stem cells. Morphological studies have indicated that there are 16-18 germline stem cells per testis at the third larval instar, but that only 5-9 stem cells are found in adults 3 days after eclosion. These stem cells are radially arranged around the hub, the somatic cells to which stem cells are attached (Hardy et al., 1979; Gönczy and DiNardo, 1996). Similarly, *mgm1*-expressing cells were found to be radially arranged around the non-staining central hub region. The number of blue cells, however, was larger than the reported number of stem cells, and more than one row of blue cells were found radially arranged around the hub.

Stem cells divide to give two unequal daughters, one which remains a stem cell that is attached to the hub and one, displaced laterally away from the hub, which becomes a primary spermatogonial cell. Each of the primary spermatogonial cells is the mitotic founder of a cyst of secondary spermatogonia. Four mitotic divisions generate 16 cells which, after early premeiotic DNA replication, enter the primary

spermatocyte stage (reviewed in: Fuller, 1993). Clusters of 16 cells are also generated in the female germline, where a stem cell divides unequally to form a regenerating stem cell and a cystoblast, which divides four times to form 2-, 4-, 8- and 16-cystocyte clusters (reviewed in: Spradling, 1993). Cytokinesis is incomplete during the gonial divisions in both male and females: the cells derived from a single spermatogonial cell or from a cystoblast remain connected by cytoplasmic bridges. Within these bridges, fusomes, large cytoplasmic structures connecting all cells of a cluster, can be visualized by specific antibodies directed e.g. against the product of *hu-li tai shao* (*hts*, Lin et al., 1994). Within stem cells, this antibody detects a spherical structure called spectrosome, which represents an early stage of fusome development. Such a spherical structure is also present in the daughters of the stem cells that have not yet engaged in one of the four mitotic divisions (Lin and Spradling, 1995). Although fusomes have mainly been described in oogenic cells, they can also be found in testes (Lin et al., 1994; McKearin and Ohlstein, 1995). To test whether *mgm1*-expressing germ cells are stem cells and primary spermatogonia with spherical spectrosomes or members of clusters whose 2, 4, 8 or 16 cells are interconnected by elongated branched fusomes, we have used 2C1, an antibody that visualizes these structures (Zaccai and Lipshitz, 1996; and Fig. 1E,F). Blue *mgm1*-expressing cells, which were radially arranged around the unstained somatic hub cells, possessed small sherical dots (Fig. 1G,H). Elongated and branched fusome structures connecting two or more cells were found in cell clusters adjacent to the blue cells, but they were never seen to be associated with *mgm1*-expressing cells. Thus, *mgm1*-expressing cells are stem cells and primary spermatogonia.

An early target gene controlled by somatic signals

To determine whether *mgm1* is controlled by an autonomous germline signal present in XY germ cells, or by signals deriving from somatic tissue, we tested the expression of *mgm1* in XX animals that were masculinized because they were mutant for one of the genes of the sex determination pathway. All tested XX animals that were totally masculinized by the absence of *transformer (tra*) function showed *mgm1* expression in embryonic (*n*=40), larval (*n*=26, Fig. 2A) and adult (*n*=22) stages. XX animals that were partially masculinized, because they lacked *double sex (dsx)* function or because they carried the masculinizing dominant allele dsx^D, also possessed staining germ cells. 40 embryos, 26 larvae and 1 adult of genotype *XX; dsxD/+* possessed blue germ cells; in these animals, the pattern of *mgm1* expression was similar to that observed in males in all embryonic and in most of the larval gonads (Fig. 2B), but some 20% of the larval gonads had fewer blue cells. Eleven adults of the same genotype had no blue germ cells, probably because they had small abnormally differentiated gonads that contained mainly degenerating material (inspection of unstained gonads with phase-contrast microscopy revealed mainly degenerating material and only few cells in 51 testes and 5 undifferentiated bag-like gonads). The inspection of intersexual animals of genotype *X/X; dsx/dsx* revealed that 24 tested embryos, 40 larvae and 10 adults possessed blue germ cells. Although in the embryos all germ cells seemed to be blue, all larvae and adults possessed fewer staining germ cells than male control animals: most gonads had 1-5 blue germ cells (Fig. 2C). 20 embryos, 40 larvae and 22 adults of the same genotype had no blue germ cells. This was expected since *X/X; dsx/dsx* flies contain gonads with either oogenic or spermatogenic cells or with germ cells whose sex cannot be identified or even with no germ cells (Orssaud and Laugé, 1982).

Since XX germ cells that develop in a masculinized animal express *mgm1*, we conclude that *mgm1* is controlled by somatic sex-determining signals. Our results also show that these signals are controlled by the genes of the sex determination pathway and that these signals already act on germ cells in 10-hour-old embryos.

An autonomous component in germline sex determination

We also tested whether genetically male germ cells express *mgm1* when developing in a female environment. XY animals that carried partially or totally feminizing mutations of genes of the sex determination pathway had germ cells that expressed *mgm1*. 40 tested embryos, 64 larvae and 22 adults of genotype *X/Y; dsx/dsx* had blue germ cells (8 adults had no blue germ cells). In the case of genotype Sxl^{M1}/Y, which is feminized by a mutation of the gene *Sex-lethal* that escapes sex-specific control, 60 embryos and 8 first instar larvae that were scored possessed blue germ cells (Fig. 2D). Later larval stages and adults of the same genotype could not be analysed, because the animals died as a consequence of hypoactive X chromosomes, which is the result of the feminizing effect of Sxl^{M1} on the dosage compensation pathway (reviewed in: Cline, 1993). Embryos of genotype Sxl^{M4}/Y, which carry a mutation that is more feminizing than the other tested mutations (Bernstein et al., 1995), also possessed blue germ cells (*n*=40). Due to the lethal effect of Sxl^{M4} on XY animals, no other stages of this genotype could be analysed. Feminized adults, however, of genotype *X/Y; hs::tra/+*, that possess ovaries whose ovarioles contain cysts consisting of spermatogenic cells (McKeown et al., 1988; Steinmann-Zwicky et al., 1989) had blue cells in a middle portion of the cysts when also carrying *mgm1* (Fig. 2E). These results show that XY germ cells express *mgm1* even when developing in a female environment.

mgm1 reveals male-specific gene expression in females with ovarian tumors

Several female-specific mutations lead to uncontrolled proliferation of germ cells, a phenotype called ovarian tumors. Such over-proliferating germ cells can have a spermatogenic appearance (reviewed in Pauli and Mahowald, 1990; Steinmann-Zwicky, 1992a, 1994a) and, in some cases, they have been shown to express male-specific genes (Pauli et al., 1993; Wei et al., 1994). We have analysed the ovaries of females carrying mutations causing ovarian tumors and the marker *mgm1*, to test whether this male-specific marker is expressed in the mutant germ cells. Three female-specific mutations that lead to uncontrolled proliferation of germ cells, because they alter the expression of *Sxl* in the germline (Steinmann-Zwicky, 1988; Bopp et al., 1993), were tested. In all ovaries of genotype Sxl^{f4}/Sxl^{f4} (*n*=20), Sxl^{f5}/Sxl^{f5} (*n*=20) and *snf/snf* (*sans-fille*, *n*=20) blue cells were present. As in genotype *X/Y; hs::tra/+*, staining was found in a subset of germ cells most often located in the middle of cysts (Fig. 2F,G). Similarly, females of genotype otu^1 or otu^3, two hypomorphic mutations of the gene *ovarian tumor*, that also have ovaries containing proliferating

undifferentiated germ cells (reviewed in: King and Storto, 1988) were tested. In all the tested females (n=20+20), *mgm1* expression was seen in a subset of cells found in the middle of cysts (Fig. 2H). These results confirm that germ cells of ovarian tumors can express male-specific genes and that *mgm1* is a useful marker to assess the sex of germ cells.

DISCUSSION

The earliest sex-specific gene expression in the germline

Male 8- to 10-hour-old embryos possess more germ cells than female embryos of the same age (Poirié et al., 1995). Since a sex-specific difference is detected, germ cells might express at least some genes in a sex-specific fashion at this stage. The marker *mgm1* now shows that male germ cells already differ from female germ cells with respect to their gene expression in stage 13 embryos, 10 hours after egg laying.

Zygotic genes are probably not expressed in germ cells before the gonads are formed. We conclude this from the analysis of germline-specific enhancer-trap lines and from studying the appearance of specific gene products in germ cells (S. Staab, A. Heller and M. Steinmann-Zwicky, unpublished). Zygotic *vasa* transcripts were first detected in late stage 12, early stage 13 embryos (Hay et al., 1988). Thus, *mgm1* may be one of the earliest zygotic genes expressed in the germline. In any case, *mgm1* reveals the earliest sex-specific gene expression in the germline known so far. It is also the only sex-specific marker expressed specifically in stem cells. Until now, only *Sxl*, which in addition to its function in somatic cells is also required in the germline, was reported to be expressed in the germ cells of one sex early in development. SXL product was detected in the cytoplasm of germ cells of some females among animals that were 16- to 20-hour-old (Horabin et al., 1995). It was not tested, however, whether this early female-specific germline expression of *Sxl*, which seems to be dispensable for female germline development until metamorphosis (Steinmann-Zwicky, 1994a), is dependent on somatic or germ cell-autonomous control signals.

Somatic sex-determining signals are already acting on XX germ cells in 10-hour-old embryos

Whether *mgm1* is expressed in XX germ cells or not depends on the sex of the somatic tissues surrounding the germline. When *mgm1* is first expressed in male germ cells, XX animals possess *Sxl* activity in all somatic tissues but not in the germ line (reviewed in: Cline, 1993). Therefore, the expression pattern of *mgm1* is complementary to that of *Sxl*, in the sense that it is expressed in male germ cells while *Sxl* is active in female somatic cells. In somatic tissue, SXL protein has a feminizing function both on the sex determination and the dosage compensation pathway. In its presence, *tra* and consequently *dsx* transcripts are spliced in the female mode (Baker, 1989). Furthermore, *msl-2* production is repressed, which renders both X chromosomes hypoactive (Zhou et al., 1995; Kelley et al., 1995; Bashaw and Baker, 1995).

XX embryos whose somatic cells are masculinized by mutations in genes of the sex-determining pathway, possess germ cells that express *mgm1*. Previous work in which adults were analysed had already shown that somatic signals control germline sex-determination. XX germ cells developing in XY male hosts were reported to become spermatogenic (Steinmann-Zwicky et al., 1989). That the somatic signals are controlled by the genes that regulate sex determination in somatic cells was shown in different experiments. (1) Masculinized XX animals often contain spermatogenic cells which can already be recognized in late first instar larvae (Seidel, 1963; Nöthiger et al., 1989; Steinmann-Zwicky, 1994b). (2) XX germ cells that are transplanted into XY hosts are spermatogenic. However, if the hosts are feminized by *hs::tra*, transplanted XX germ cells will make eggs (Steinmann-Zwicky, 1994b). (3) Germ cells of XX flies that are masculinized by a viable combination of hypomorphic *Sxl* alleles are spermatogenic. In flies carrying the same *Sxl* alleles, but also a *hs::tra* construct, germ cells form normal eggs (Steinmann-Zwicky, 1994b). Since *tra* is not required in the germ line (Marsh and Wieschaus, 1978), its function in somatic cells must be crucial.

We now show that somatic sex-determining signals already act on XX germ cells in embryos. In previous experiments, the sex of germ cells was assessed relatively late and using morphological criteria, because no sex-specific molecular markers were available. The marker *mgm1*, which is expressed in germ cells of embryos and in stem cells of later stages now makes it possible to identify sex-specific gene expression in germ cells much earlier. The experiments reported here show a correlation between germ cells that were identified as spermatogenic due to their male appearance and expression of *mgm1*. Therefore, this male-specific marker seems to be an ideal molecular tool to assess the sexual pathway that germ cells have entered.

XX germ cells differ from XY germ cells

XY germ cells became spermatogenic, according to morphological criteria, in the female environment of a feminized XY animal or when transplanted into a female XX host (Steinmann-Zwicky et al., 1989). Using our molecular marker, we now confirm that autonomous signals make XY germ cells male; they express *mgm1* irrespectively of the sex of their environment. Since, in 10-hour-old embryos, XX germ cells respond to somatic sex-determining signals while XY germ cells do not, germline-autonomous signals result in XX germ cells being already different from XY germ cells in the embryo. A counting system that assesses the number of X chromosomes in germ cells must already exist at this stage. Since 2X3A germ cells that develop in a 3X3A female become either oogenic or spermatogenic, the counting system must not only count the number of X chromosomes, but it must relate this number to something which is equally present in XX and XY germ cells (Schüpbach, 1985). Due to the apparent similarity between the X chromosome counting system in the germline and in the soma, the term X:A ratio which was used for the primary sex-determining signal acting in somatic cells was also used for the germline. Later work, however, revealed that an essential somatic numerator gene is dispensable in germ cells of both sexes (Steinmann-Zwicky, 1993), and that the number of copies of such genes is not assessed for sex determination in germ cells (Granadino et al., 1993). Thus, germ-cell-specific elements must be used to assess the X:A ratio of germ cells, i.e. to make XX germ cells different from XY germ cells.

Sex detemination by somatic and germ cell-autonomous signals

Although *mgm1* is a marker for male germ cells, it cannot be considered to be an absolute marker that allows us to extrapolate all sex-specific aspects of a germ cell. Since both autonomous and somatic signals control the sex of germ cells, we expect at least two different classes of genes displaying sex-specific expression in the germline: (1) genes whose expression is controlled by somatic signals, and (2) genes that are controlled by germline-autonomous factors.

The marker *mgm1* shows that a gene, whose expression is controlled by somatic signals, is already active in embryos. Different experiments in which, however, adult flies were analysed revealed that the expression of *Sxl* in the germ line is also dependent on the expression of sex-determining genes in somatic cells. (1) Constitutive expression of *Sxl* rendered XX germ cells oogenic when they developed in the testes of either host XY males (Steinmann-Zwicky et al., 1989) or masculinized XX flies (Nöthiger et al., 1989). (2) Male-specific *Sxl* transcripts were found in germ cells of XX flies masculinized by *tra*, *tra2* or *dsx* mutations (Oliver et al, 1993). In a different study, however, it was reported that XX adult flies that are masculinized because they possess no *tra* or *dsx* function express female-specific transcripts of *Sxl* and *orb* in their germline, while XX animals that were masculinized due to lack of *tra2* function possessed male-specific *orb* transcripts and only little SXL protein in their germ cells (Horabin et al., 1995). The results concerning sex-specific germline expression of *Sxl* are thus conflicting. In one case, however, it seems that germline-autonomous signals control the expression of a gene in germ cells: in adult flies, the sex-specific pattern of expression of *ovo*, another gene required in the germline for oogenesis, was shown to be dependent on the number of X chromosomes present in germ cells, and not on the sex of the surrounding soma (Oliver et al., 1994). As with *Sxl*, however, zygotic *ovo* seems not to be required for germline sex determination until metamorphosis (Staab and Steinmann-Zwicky, 1996).

Outlook

Genes that are sex-specifically expressed in germ cells before any overt sex-specific differentiation takes place, might play an important role in the process of deciding whether germ cells enter the spermatogenic or the oogenic pathway. In any case they can reveal the control mechanisms used in germline sex determination. Here, we describe the expression of such a gene in different genetic backgrounds, which reveals that autonomous and somatic sex-determining signals already act on *Drosophila* germ cells in embryos. Molecular analysis of such genes and a specific analysis of their promoter regions, which contain specific elements that confer sex-specific and germline-specific control, is bound to yield fascinating new insights into the molecular control of germline sex determination in the next few years.

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