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NORMAL FEMALE GERM CELL DIFFERENTIATION REQUIRES THE FEMALE *X* CHROMOSOME TO AUTOSOME RATIO AND EXPRESSION OF SEX-LETHAL IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

In somatic cells of Drosophila, the ratio of *X* chromosomes to autosomes (*X:A* ratio) determines sex and dosage compensation. The present paper addresses the question of whether germ cells also use the *X:A* ratio for sex determination and dosage compensation. Triploid female embryos were generated which, through the loss of an unstable ring-*X* chromosome, contained some germ cells of *2X;3A* constitution in their ovaries. Such germ cells were shown to differentiate along one of two alternative pathways: a minority developed into normal female oocytes and eggs; the majority developed into abnormal multicellular cysts. An *X:A* ratio of 1 is, therefore, required in female germ cell development, at least in the mature ovary after stem cell division.—Abnormal development of female germ cells was also observed when *2X;2A* germ cells which were homozygous or *trans*-heterozygous for mutant alleles at the Sex-lethal locus were transplanted into normal female host embryos at the blastoderm stage. Germ cells homozygous for amorphic alleles failed to give rise to normal eggs. Instead, they formed multicellular cysts, very similar to those formed by *2X;3A* cells. Zygotic Sxl^+ activity is, therefore, also necessary for the development of normal female germ cells. No abnormalities were detected in transplanted germ cells from female embryos whose mothers had been homozygous for the mutation daughterless. When normal *XY* germ cells were transplanted into female embryos, no traces of such cells could be found in the adult ovary. *XY* germ cells seem, therefore, not to develop as far as *2X;3A* or *Sxl* homozygous cells in a female gonad. This indicates that neither *2X;3A* nor *Sxl* homozygous germ cells are equivalent to normal *XY* germ cells.

IN somatic tissues of *Drosophila melanogaster*, both sex determination and dosage compensation occur in a cell autonomous manner (for review see BAKER and BELOTE 1983). For both processes, the ratio of *X* chromosomes to autosomes (*X:A* ratio) acts as the primary signal in each cell (BRIDGES 1921, 1925). In a yet unknown way, an *X:A* ratio of 1.0 causes activation of the Sex-lethal (*Sxl*) locus. This shifts the cells into the female pathway (CLINE 1978, 1980, 1983a). The product of the Sxl^+ gene is thought to control the activity level of a number of other genes, such as transformer (*tra; tra-2*) and double-sex (*dsx*), which are directly responsible for final sex determination (CLINE 1979, 1980, 1983a; BAKER and RIDGE 1980; LUCCHESI and SKRIPSKY 1981;

SANCHEZ and NÖTHIGER 1982). In addition to promoting sex determination, *Sxl* also acts to establish dosage compensation in somatic cells of females (CLINE 1979, 1980, 1983a; LUCCHESI and SKRIPSKY 1981; BAKER and BELOTE 1983; SANCHEZ and NÖTHIGER 1983). It is thought that activation of Sxl^+ prevents the two *X* chromosomes of a female from becoming hyperactivated. An *X:A* ratio of 0.5 would result in inactivation of *Sxl* and thereby cause hyperactivation of the single *X* chromosome. This hyperactivation requires the activity of several male-specific lethal genes (BELOTE and LUCCHESI 1980).

The situation with respect to the cells of the germline is less clear. Autonomous sex determination takes place in some form in the germline, since *XX;2A* cells, when transplanted into a male gonad, fail to produce functional sperm, and *X(Y);2A* cells do not give rise to functional eggs when transplanted to a female gonad (VAN DEUSEN 1976). On the other hand, absence of four of the genes required for normal sex determination in female somatic cells has no detectable effect on sex determination of female germ cells (MARSH and WIESCHAUS 1978; SCHÜPBACH 1982).

Given the important role of *Sxl* for sex determination of somatic cells, it is relevant to determine whether *Sxl* is required in germ cells. Furthermore, it is as yet an unresolved question whether dosage compensation takes place in the germline of Drosophila and whether the ratio of *X* chromosomes to autosomes is indeed the primary signal that is used in germ cells to determine male or female development. To answer these questions, I constructed mosaic animals in which germ cells of particular genotypes and chromosomal constitution found themselves in normal female gonads. Such mosaics should provide germ cells with the maximum opportunity to follow a particular pathway and, therefore, allow a direct assessment of the sexual determination of the tested germ cells themselves.

MATERIALS AND METHODS

Construction of triploid mosaics: Triploid mosaics were obtained by crossing triploid females of the genotype *C(1)RM, y v f mal/Basc* to diploid males carrying the unstable ring-*X* chromosome $R(1)2,w^{vC}$ and a y^+Y chromosome (for a description of marker mutations and balancer chromosomes see LINDSLEY and GRELL 1968, unless otherwise referred to in the text). The line of triploid females was derived from a triploid line ($C(1)RM, w^{co}$ *ec cv/Basc*) that had been given to me by G. LAUGÉ (LAUGÉ and KING 1979). The experimental cross, $\hat{XX}$ *y v f mal/Basc* × $R(1)2,w^{vC}/y^+Y$, yielded two types of mosaic animals which were in part phenotypically wild type ($\hat{XX}/R(1)2$) and in part phenotypically *y v f mal* ($\hat{XX}/O$): those with a triploid autosomal complement (*3X;3A-2X;3A* mosaics), and those with a diploid complement (*3X;2A-2X;2A* mosaics). The former are female-intersex mosaics, the latter are female-female mosaics (SCHÜPBACH, WIESCHAUS and NÖTHIGER 1978). To distinguish the unwanted diploid mosaics from triploid mosaics, the only mosaic flies that were considered were those in which the $\hat{XX}/O$ tissue extended into a sexually dimorphic region and showed some male differentiation there (sex combs, male genital and anal structures or unambiguous male tergite pigmentation in tergites 5 and 6). Such male differentiation had never been observed in more than 300 *3X;2A-2X;2A* mosaics (SCHÜPBACH, WIESCHAUS and NÖTHIGER 1978) and, therefore, clearly identified the intersexual mosaics.

Construction of Sxl *and* da *mosaics*: Germline mosaics for various alleles of Sex-lethal = *Sxl* (formerly called *Fl*, ZIMMERING and MULLER 1961; renamed in CLINE 1978) and of daughterless = *da* (BELL 1954) were constructed by transplanting pole cells, the precursors of the gonadal germ cells, at the blastoderm stage from one donor embryo into one or two host embryos. The method

for these transplantations is described by VAN DEUSEN (1976). In such transplantation experiments, the survival rate of injected host embryos, as well as the frequency of germline mosaicism, varies somewhat from one experiment to the next. Also, usually some of the surviving adult hosts are sterile, probably due to loss of all germ cells during or after the injection. The tables in RESULTS indicate for each experimental series the total number of injected embryos and the resulting number of "normal" hosts. Host animals were considered normal if they survived for at least 1 wk as adults and proved to contain germ cells in their gonads. Their germ cell population was assessed by progeny testing each host singly with appropriate test mates. In addition to the progeny test, egg production was recorded for female hosts by inspecting and counting the eggs of each female every 2 days. After collecting eggs for about 10 days, all female hosts were finally dissected and their ovaries processed and mounted.

Germline-dependent marker mutations: Mosaicism in the germ cell population of the tested flies could often be detected not only by progeny testing but also by distinguishing one germ cell population from another using the markers *fs(1)K10* and *mal*. *fs(1)K10* is a germline-dependent egg marker that alters the shape of the egg and the pattern of the chorion in a very characteristic way (WIESCHAUS, MARSH and GEHRING 1978). In host females that were homozygous for *fs(1)K10*, it was, therefore, possible to detect germline mosaicism, in cases in which the implanted germ cells gave rise to normal eggs, by scoring the morphology of the eggs laid by those females. The mutation *mal* allows detection of mosaicism in many internal tissues, since mutant cells of many tissues lack aldehydeoxidase activity which can be visualized by an easy histochemical staining procedure (JANNING 1972). Lack of aldehydeoxidase activity is cell autonomous in the germ-line such that female germ cells homozygous for *mal* will form oocyte-nurse cell cysts which lack aldehydeoxidase activity and consequently remain unstained, even when surrounded by wild-type tissue, whereas mal^+-carrying germ cells will form staining cysts even when surrounded by mutant tissue (MARSH and WIESCHAUS 1977). Wild-type cysts will stain reliably from stage 1 until stage 9 of oogenesis, whereas staining is usually weak or absent in germarial stages. The mal^+ gene also has a germ line-dependent maternal effect on eye color. Consequently, adult progeny arising from mal^+ germ cells will have mal^+ eye color even when they are homo- or hemizygous for *mal* (MARSH and WIESCHAUS 1977).

In the study of triploid mosaics as well as in all series of pole cell transplants the females were progeny tested for approximately 10 days, and eggs were scored for *fs(1)K10* morphology in those experiments in which *fs(1)K10* was used. Afterward, all of the females, regardless of whether they showed signs of mosaicism, were dissected and their ovaries were fixed and stained for aldehydeoxidase activity. This allowed detection of germline mosaicism even in those cases in which the implanted cells did not form mature eggs but did give rise to cells visible by light microscopy in the ovarian whole mounts.

Genetic markers used for pole cell transplantation experiments: The host and donor genotypes as well as the genotypes of the test partners were specifically chosen to allow unambiguous determination of the genotype of fertile germ cells present in the host flies. The genotypes of the crosses yielding donor and recipient eggs are given in Table 1. A few of these experiments require further explanation:

Series 2: In the donor cross, the female embryos that were homozygous for $Sxl^{f\#1}$ were also homozygous for *mal*, whereas all of the other zygotes from this cross received at least one mal^+ allele. The mal^+ gene translocated to the *Y* chromosome is normally expressed in female germ cells and can be used as histochemical marker (SCHÜPBACH, WIESCHAUS and NÖTHIGER 1978). Upon emergence, host females were crossed to *w/Y* males and host males were crossed to *y w ct f/Basc* females. This allowed identification of all non-*w* progeny as donor derived; in the case of the host females the phenotypes of donor-derived sons as well as presence or absence of progeny carrying *FM6* revealed which of the donor chromosomes was present in the female host.

Series 3: In this donor cross, all male embryos die, because both N^{Nic} and $Df(1)mal^6$ are embryonic lethals (SCHALET and FINNERTY 1968; WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984). This series was originally designed to determine how far *2X;2A* cells homozygous for $Sxl^{f\#1}$ would develop in a potentially empty male gonad given that one-eighth of the hosts were *X/X;tra/tra* and, therefore, transformed into "pseudomales," which usually have empty male gonads (BROWN and KING 1961; SEIDEL 1963). Since all *XY* animals produced by the donor cross die as embryos,

TABLE 1

Genetic markers used in pole cell transplantation experiments

Series	Donor cross	Host cross	Host progeny crossed to
1	*cm* $Sxl^{f\#1}$ *ct/FM6* × *y z* $Sxl^{f\#1}$*sn f/Y*	*fs(1)K10 w* f^{36a} *mal/Basc* × *fs(1)K10 w* f^{36a} *mal/Y*	*y w cm ct f* males or females
2	*cm* $Sxl^{f\#1}$ *ct v f mal/FM6* × *y cm* $Sxl^{f\#1}$*ct f mal/*y^{+}*Ymal*$^{+}$	*fs(1)K10 w/w* × *fs(1)K10 w/Y*	*w/Y* or *y w ct f/Basc*
3	*y* w^{a} N^{Nic} *cm* $Sxl^{f\#1}$ *ct v f mal/In(1)dl-49 + In(1)*B^{M1}*, Df(1)mal*^{6}*v* × *cm* $Sxl^{f\#1}$ *ct f mal/Y*	*y w/y w; Dp(1;3)sc*^{J4}*,* y^{+} *tra/TM3, Sb Ser* × *y w/YB*^{s}*; mwh tra/TM3, Sb Ser*	*w/Y* or *y w ct oc f/FM7c*
4	*y cm* $Sxl^{f\#1}$ *ct f mal/FM6* × *y w* $Sxl^{fm\#1,M\#1}$ *sn/Y*	*fs(1)K10 w* f^{36a} *mal/mal* × *fs(1)K10 w* f^{36a} *mal/Y*	*w/Y* or *y w ct oc f/FM7c*
5	*da pr cn/da pr cn* × *y z* $Sxl^{f\#1}$ *sn f/Y*	*fs(1)K10 w* f^{36a} *mal/Basc* × *fs(1)K10 w* f^{36a} *mal/Y*	*y w* f^{36a} males or females
6	*y cm* $Sxl^{f\#7,M\#1}$*/y cm* $Sxl^{fm\#7,M\#1}$*/*B^{S}*Y ; Dp(1;3)sn*^{13a1}*, cm*$^{+}$ Sxl^{+}*ct*$^{+}$*/+* × *w* $Sxl^{M\#1,fm\#3}$ *sn/Y*	*fs(1)K10 w* f^{36a} *mal/y w* f^{36a} *mal* × *fs(1)K10 w* f^{36a}*mal/*y^{+}*Y mal*$^{+}$	*y cm ct v sn* males or females
7	*y v f mal/y v f mal* × *y v f mal/*y^{+}*Y mal*$^{+}$	*fs(1)K10 w* f^{36a} *mal/y w* f^{36a} *mal* × *fs(1)K10 w* f^{36a} *mal/Y*	*w/Y* or *y w ct f/Basc*

it was hoped that their *XY* germ cells would not be viable after transplantation into the normal host embryos so that only the germ cells homozygous for $Sxl^{f\#1}$ would have a chance of developing in the *tra* homozygous hosts. Among eight surviving *X/X;tra/tra* hosts, three contained spermatocytes and sperm in their gonads. Unfortunately, two of those gave rise to progeny that clearly indicated that these *X/X;tra/tra* hosts contained germ cells of the genotype *y* w^{a} N^{Nic} *cm* $Sxl^{f\#1}$ *ct v f mal/Y*. Therefore, N^{Nic} does not act as a cell lethal in the male germline, in spite of being cell lethal in the epidermis (Ripoll and Garcia-Bellido 1979; E. Wieschaus, personal communication). The third host containing sperm was not fertile and contained immotile sperm. However, *X/X;tra/tra* animals are often infertile even after receiving normal male germ cells (Marsh and Wieschaus 1978). Thus, this series did not allow assessment of the fate of $Sxl^{f\#1}$ homozygous germ cells in male gonads. However, it did yield further evidence concerning the fate of the $Sxl^{f\#1}$ homozygous cells in normal female gonads.

Series 5: The mutation daughterless is somewhat temperature sensitive (Cline 1976). At 18°, some female embryos survive the maternal effect, however, in the donor cross of this series all female embryos also receive a mutant allele of *Sxl*, which, in somatic cells, increases the probability of lethality so that, even at 18°, no female embryos survive (Cline 1976, 1978). Moreover, in this series special care was taken to keep the donor and host flies and embryos at 25°. Since transplantation involved using a room of approximately 20°, dechorionating and mounting of embryos was done in a temperature controlled room at 25°. After the transplantation period of 3 to 15 min, the embryos were put on prewarmed plates to minimize the time they spent at 20°.

Series 6: In this donor cross, a free *Y* chromosome was present in the female parent. Half of the embryos received, therefore, an extra *Y* chromosome. The duplication on the third chromosome allowed the parental females to survive. The *trans*-heterozygous combination $Sxl^{fm\#7,M\#1}$/$Sxl^{M\#1,fm\#3}$ is peculiar in that at 25° approximately 48% of the embryos of this genotype survive,

but all of those survivors are transformed to pseudomales (CLINE 1984; observed and counted here on 105 of 387 *XX* progeny from the donor cross). For host females, the markers *y cm* or *sn* identified donor-derived progeny. In host females homozygous for *fs(1)K10*, all adult progeny were donor derived and could, therefore, easily be classified. In the case of the heterozygous host females, the donor-derived *w sn* males were not distinguishable from the host-derived $w\,f^{36a}$ males, but all other classes of donor-derived progeny were identifiable and allowed me to infer the genotype of the implanted donor germ cells. In particular, in this cross it was crucial to ascertain whether or not the duplication $Dp(1;3)sn^{13a1}$, cm^{+} Sxl^{+} ct^{+} was present in the donor germ cells. Absence of the duplication was inferred only in cases in which two criteria were met. First, at least six *y cm* flies and no *y* cm^{+} flies had to be obtained (in cases of heterozygous host females only *y cm* sons *vs.* *y* cm^{+} sons could be taken into account). In addition, it was required that six *sn* F_1 females did not transmit the duplication when singly taken through a further generation by crossing them to *y cm ct v sn* males. In total, then, at least 12 progeny without the duplication *vs.* none with the duplication was the criterion by which absence of the duplication from the implanted donor germ cells was judged. This seems a fair criterion given that those host females that did receive germ cells carrying the duplication produced duplication-bearing progeny to nonduplication-bearing progeny in a 1:1 ratio (data not shown).

RESULTS

The X *chromosome to autosome ratio*: In *Drosophila melanogaster*, somatic cells containing two *X* chromosomes and three sets of autosomes (*2X;3A*) can differentiate with either a male or a female morphology (HANNAH-ALAVA and STERN 1957; for a review see BAKER and BELOTE 1983). For germ cells, it is not known whether this intermediate *X:A* ratio will likewise lead to a choice between a discrete male or female pathway of differentiation for each cell. It is possible that *2X;3A* germ cells differentiate only one sex or that they develop in an intermediate pattern. Although the morphology of gonads in *2X;3A* intersexes has been described to some extent (DOBZHANSKY and BRIDGES 1928; LAUGÉ 1969; LAUGÉ and KING 1979), it is not clear whether the abnormalities observed in those germ cells reflect an autonomous effect of the chromosomal balance on the germline itself or whether they are caused by the somatic components of the intersexual gonad which may not be capable of supporting the normal development of germ cells.

To determine how *2X;3A* germ cells develop in a female gonad, $\widehat{XX}$/X;3A//$\widehat{XX}$;3A mosaics were recovered as progeny of $\widehat{XX}$, *y v f mal/Basc;3A* females × $R(1)2,w^{vC}/y^{+}Y;2A$ males. *3X;3A* y^{+} v^{+} f^{+} mal^{+} cells had the normal female *X:A* ratio of 1, whereas *2X;3A* cells marked with *y v f mal* had an intermediate *X:A* ratio. Since the mosaic border in such animals is known to separate the somatic parts of the gonads relatively often from the germline (GEHRING, WIESCHAUS and HOLLIGER 1976), a number of triploid mosaics were expected in which somatic gonadal cells of the constitution *3X;3A* would envelope germ cells of the constitution *2X;3A*. The same cross produced nonmosaic *2X;3A* intersexes which served as internal control for the sexual development of *2X;3A* somatic cells under the same genetic background and culture conditions. Differences in genetic background are known to influence the morphology of *2X;3A* intersexes (DOBZHANSKY 1930; LAUGÉ 1969; CLINE 1983a). The intersexes from this cross were of "male-like" appearance: in addition to sex comb teeth on their forelegs and male pigmentation of the abdominal tergites, all of

these flies displayed at least some male elements in their genital and anal apparatus. This indicates that this particular *2X;3A* constitution was perceived by the majority of the epidermal cells as being closer to a 1:2 ratio than to a 1:1 ratio (DOBZHANSKY 1930; LAUGÉ 1969; CLINE 1983a).

In total, 63 triploid mosaics that showed external mosaicism were studied. Of these, 18 had very reduced and abnormal gonads. Another 13 had gonads that more or less resembled male testes, five of which contained a number of spermatogonial cysts including a few later stages (up to the 16-cell cyst stage). None of the gonads had sperm that had reached the advanced stages reported in extreme male-like nonmosaic *2X;3A* flies by DOBZHANSKY and BRIDGES (1928). It is not possible to distinguish between presence or absence of a mal^{+} gene in male germ cells by a histochemical stain. Consequently, the genotype of the apparent male germ cells could not be ascertained. This present study can, therefore, not provide evidence as to the fate of *2X;3A* or *3X;3A* germ cells in a male gonad. It will concentrate solely on the fate of such cells in female gonads.

There were 32 mosaics with at least one normal-looking female gonad which in all cases contained germ cells: 19 contained only mal^{+} (therefore *3X;3A*) egg chambers in their ovaries, ten contained at least one ovary with a mixed germ cell population of mal^{+} and mal-staining cysts, and three mosaics had ovaries in which all of the cysts were mal and, therefore, *2X;3A*. The constitution of the somatic gonadal cells cannot be determined by the histochemical staining method. However, all of the triploid *3X;3A* germ cells observed in mosaic ovaries were of perfectly normal female morphology. The somatic components of those mosaic ovaries were, therefore, sufficiently female to fully support development and differentiation of female germ cells. Within the mosaic ovaries, there were in total 603 *3X;3A* vitellarial cysts and 512 *2X;3A* vitellarial cysts. Germ cells of the constitution *2X;3A*, therefore, survive and divide in a female gonad, form stem cells and produce vitellarial cysts. In those ten flies with a mosaic germ cell population, the number of *2X;3A* germ cell precursors at the blastoderm stage should on average have been the same as the number of *3X;3A* germ cell precursors, given that, in mosaics produced by loss of $R(1)2,w^{vC}$, the mosaic boundary divides a mosaic primordium into randomly sized regions of *3X;3A* and *2X;3A* genotype. The observation that at the end of development the number of *2X;3A* cysts is still very similar to that of *3X;3A* cysts suggests that the *2X;3A* germ cells do not have an overwhelming growth disadvantage.

With respect to their final differentiation, two types of morphology were observed among the 512 *2X;3A* cysts. Thirty-five cysts were apparently of normal female morphology; they had 15 enlarged (presumably polyploid) nurse cells and one oocyte and were in various stages of egg chamber development. There were, however, 477 *2X;3A* cysts which consisted of a large number of small, undifferentiated cells (Figure 1a, b and c). In ten cysts in which the cells could be approximately counted the average number per cyst was 90, the smallest of the ten cysts containing around 45 and the largest about 135. The cells had a diameter of approximately 10–12 μm and were usually surrounded

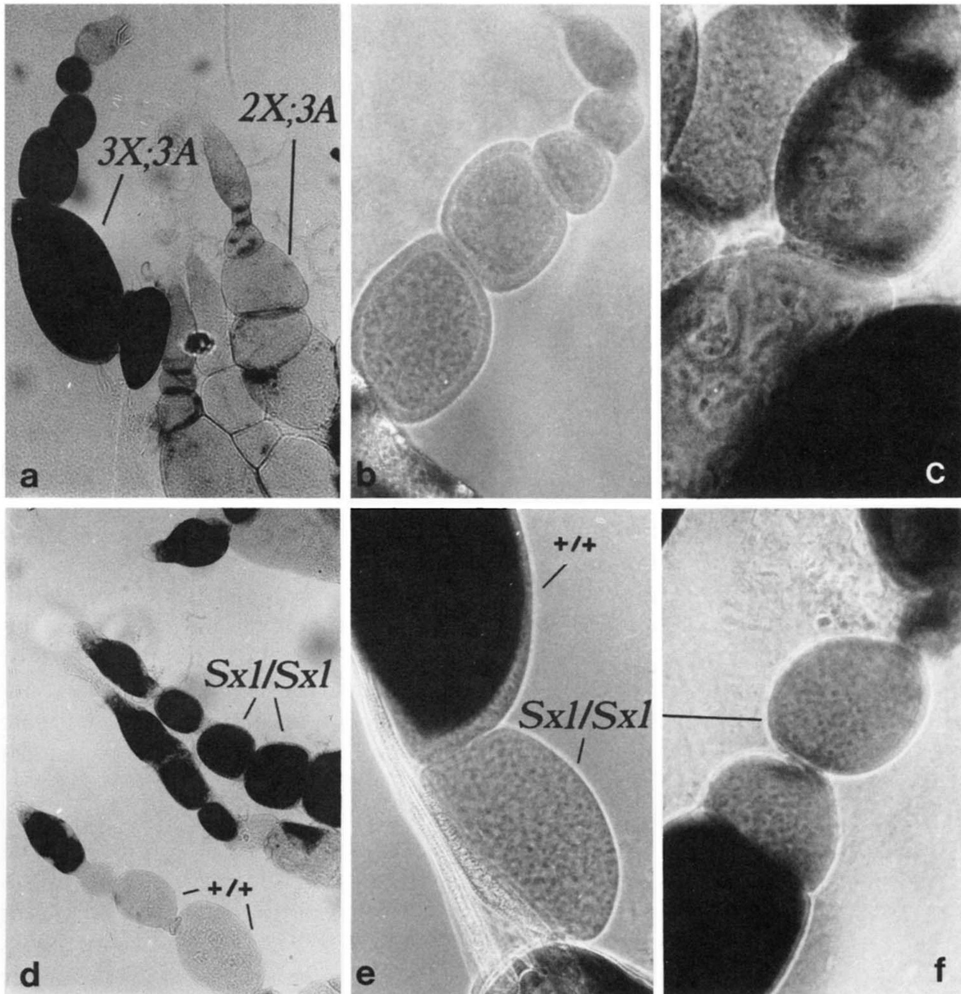

FIGURE 1.—Mosaic ovaries stained for aldehyde oxidase activity. a–c, *3X;3A-2X;3A* mosaics in which *3X;3A* cysts are darkly stained and *2X;3A* cysts are unstained. In a and b, the *2X;3A* cysts are all of multicellular phenotype. In c, *2X;3A* cysts show both the multicellular and the normal 15 + 1 phenotype. d–f, Sxl mosaics. *d*, Ovary from experimental series 1, in which cysts homozygous for $Sxl^{f\#1}$ (Sxl/Sxl) were darkly stained and the normal cysts ($^{+}/+$) of the host remained unstained. e, Ovary from series 2, in which cysts homozygous for $Sxl^{f\#1}$ remained unstained and the cysts of the hosts were darkly stained. f, Ovary from series 3, in which cysts homozygous for $Sxl^{f\#1}$ remained unstained and the cysts of the host were stained.

by a monolayer of follicle cells of normal morphology. In later stages, neighboring cysts often fused and all cysts finally degenerated. The abnormal multicellular cysts were often observed in ovarioles which contained mal (*2X;3A*) cysts preceded or followed by mal^{+} (*3X;3A*) cysts of perfectly normal female morphology. These ovarioles, therefore, had a mixed stem cell population. There were also cases in which *2X;3A* cysts of normal morphology were observed within the same ovariole as *2X;3A* cysts of the abnormal multicellular

phenotype. Multicellular as well as normal cysts were also observed in those three mosaic flies that contained only mal cysts in their ovaries. These three females had in total ten normal female (15 nurse cells and one oocyte) cysts and 188 abnormal multicellular cysts within their ovaries. Six of the females with mosaic ovaries laid eggs, but most of the eggs did not give rise to larvae. This is expected from triploid germ cells which produce many aneuploid zygotes. All of the 53 surviving offspring arose from *3X;3A* germ cells as judged by their maternally determined mal^+ eye color. However, given the small number of offspring these data do not preclude the possibility that the *2X;3A* cysts of normal female morphology may sometimes be able to give rise to functional eggs.

In summary, *2X;3A* germ cells survive well when they find themselves in female gonads. They can develop to functional stem cells in the adult ovary. About the time of the stem cell division, however, the germ cells seem to choose between two pathways: a minority differentiate in a normal female fashion in which four incomplete mitotic divisions give rise to the normal 15 plus one egg chamber. Alternatively, in many cases the cystoblast divisions continue beyond the four divisions, none of the daughter cells become polytene and none of them differentiates into an oocyte which takes up yolk. It is tempting to regard the abnormal cysts as some indication of male differentiation, given that somatic cells of the constitution *2X;3A* make a choice between a male and a female pathway of differentiation. Alternative interpretations of the multicellular cyst phenotype are possible. The results demonstrate, however, that a *2X;3A* constitution results in two distinct classes of differentiation for germ cells. They also show that it is the ratio of *X* chromosomes to autosomes and not simply the number of *X* chromosomes which is crucial for the proper female differentiation of germ cells.

The Sxl *locus*: In somatic cells, an *X:A* ratio of 1.0 results in activation of the *Sxl* locus which is required for both female sexual development and dosage compensation to occur in these cells (CLINE 1979, 1980, 1983a; LUCCHESI and SKRIPSKY 1981). Pole cells that carried various mutations at the *Sxl* locus were transplanted into normal female hosts to determine whether *Sxl* activity is necessary for germ cell perception of the *X:A* ratio.

The first series of experiments were designed to test the effects of $Sxl^{f\#1}$, a strong, presumably amorphic allele (CLINE 1978, 1984). Genetically marked pole cells from embryos heterozygous or homozygous for $Sxl^{f\#1}$ were transplanted into normal host embryos (Table 2). All donor-derived pole cells carried the histochemical marker mal^+. Half of the host females were homozygous for the germline-dependent markers *fs(1)K10* and *mal*; in these females mosaicism could be identified by histochemical staining of their ovaries, by inspection of the morphology of the eggs they produced and by scoring the phenotype of their adult progeny. Three of the homozygous and four of the heterozygous females gave rise to both *FM6*- and $Sxl^{f\#1}$-bearing adult progeny indicating that they arose from implanted $Sxl^{f\#1}/FM6$ germ cells. Among the homozygous host females an additional group of six mosaics were discovered only after dissection and histochemical staining of the ovaries since the im-

TABLE 2

Transplantation of germ cells mutant for Sex-lethal

Series	*Sxl* and *mal* parental donor genotypes[a]	*fs(1)K10* and *mal* parental host genotypes[a]	No. of injected embryos	Normal adult host	No. of hosts with adult progeny from implanted germ cells: *Sxl/Sxl* or *Sxl/Y*	No. of hosts with adult progeny from implanted germ cells: Sxl^+/Sxl or Sxl^+/Y	No. of female hosts with multicellular ovarian cysts and their phenotype
1	$Sxl^{f\#1}$ +/++	*fs(1)K10 mal*/++	628	34 ♀[b]	(1?)	4	6, mal^+
	× $Sxl^{f\#1}$ +/Y	× *fs(1)K10 mal/Y*		78 ♂	11	12	
2	$Sxl^{f\#1}$ *mal*/++	*fs(1)K10* +/++	487	51 ♀	0	3	8, mal
	× $Sxl^{f\#1}$ *mal/* $Ymal^+$	× *fs(1)K10* +/Y		54 ♂	2	7	
3	$Sxl^{f\#1}$ *mal*/+ $Df(1)mal^6$	++/++ × ++/Y	344	28 ♀	0	5	4, mal
	× $Sxl^{f\#1}$ *mal/Y*			27 ♂	3	0	
4	$Sxl^{f\#1}$ *mal*/++	*fs(1)K10 mal*/+ *mal*	224	33 ♀	0	4	5, mal^+
	× $Sxl^{f\#1,M\#1}$ +/Y	× *fs(1)K10 mal/Y*		32 ♂	5	4	

[a]For exact description of genotypes see MATERIALS AND METHODS.

[b]The indicated ♀♀ were *fs(1)K10 mal* homozygotes. An additional 51 host females were heterozygous, three of these gave rise to progeny from *Sxl*/+ implanted germ cells, but none were dissected and stained since staining would not distinguish implanted germ cells from germ cells of those hosts.

planted germ cells did not produce eggs on adults. The ovaries of these six mosaics had mal^+-staining cysts of abnormal morphology in addition to their own mal-negative cysts of normal morphology (Figure 1d). The abnormal mal^+ cysts consisted of a number of small cells surrounded by a monolayer of follicle cells of normal appearance. These cysts increased somewhat in size along the ovariole, although the diameter of the cells within the cysts remained constant at 10–12 μm. In particular, there was no enlargement of cells and nuclei inside these cysts which would have indicated the nurse cell-specific endoreplication. All cells seemed similarly shaped and spaced and of equally indistinct morphology in the light microscope. In ten cysts cell counts were made. The average number of small cells inside the cysts was 65; the smallest cyst contained 27 cells and the largest 110. At later stages, some of the cysts fused and all seemed to degenerate at more posterior locations in the ovariole. At the light microscopic level these cysts were very similar to the abnormal cysts produced by the *2X;3A* cells described in the preceding section. The results of this series of transplantations suggested, therefore, that germ cells homozygous for $Sxl^{f\#1}$ do not give rise to normal eggs and offspring but rather lead to an abnormal multicellular cyst phenotype in the adult ovary.

There was one additional mosaic female among the *fs(1)K10 mal* homozygous hosts in this series that behaved unusually. This female gave rise to ten progeny which might have been produced by germ cells homozygous for $Sxl^{f\#1}$.

Nine of these progeny carried the *cm* $Sxl^{f\#}$ *ct* chromosome of the female donor parent; the other carried the *y z* $Sxl^{f\#1}$ *sn f* chromosome of the male donor parent. There were no crossovers between *y* and *f* as verified by taking all of the ten progeny through another generation of testcrossing. Another unusual feature about this female was that she had in fact laid 107 eggs of normal morphology, in addition to 167 eggs of *fs(1)K10* morphology, but only a few of the normal eggs hatched into larvae. This female might have represented a case in which the $Sxl^{f\#1}$ mutation had a less severe effect on germ cell development. Another likely interpretation for this female is that the implanted germ cells in this case were of triplo-*X* genotype (*3X;2A* = metafemale) due to nondisjunction in a donor female. The rate of nondisjunction in the *FM6* heterozygous donors was indeed rather high (30 *XO* males and 1002 regular males were obtained in a control count). *FM6* in the germ cells would reduce crossovers, and triplo-*X* germ cells are known to cause frequent embryonic lethality (SCHÜPBACH, WIESCHAUS and NÖTHIGER 1978).

To test whether the abnormal multicellular cysts were indeed produced by germ cells homozygous for $Sxl^{f\#1}$, a second series of transplantations was carried out (Table 2, series 2). The donor cross was set up in such a way that only germ cells homozygous for $Sxl^{f\#1}$ would be homozygous for *mal*. All heterozygous germ cells, male germ cells and nondisjunction-derived triplo-*X* germ cells would be mal^{+}, as were all host females. Among the 51 surviving host females, there were eight that carried several cysts that were homozygous for *mal* and consequently for $Sxl^{f\#1}$. These cysts were all of the abnormal morphology (Figure 1e). This shows that the abnormal multicellular cysts represent the usual phenotype of differentiating germ cells homozygous for $Sxl^{f\#1}$ when transplanted into a normal female host.

This result was confirmed in a third series of transplantations in which again $Sxl^{f\#1}$ homozygous germ cells were marked with *mal* (Table 2). In this series, there were four mosaic females found that carried multicellular cysts derived from implanted cells in their ovaries (Fig. 1f). In total in series 1–3, 18 females with multicellular cysts were obtained as compared to 12 host females which had successfully received germ cells heterozygous for $Sxl^{f\#1}$. This indicates that germ cells homozygous for $Sxl^{f\#1}$ when implanted into normal females survive and form dividing stem cells with a frequency similar to transplanted heterozygous germ cells. It suggests that *Sxl* homozygous germ cells become abnormal only after the formation of stem cells.

The first three series of transplantations had exclusively involved the spontaneous mutation $Sxl^{f\#1}$. To ensure that these results indeed reflected the absence of a functional *Sxl* gene and were not the consequence of some unknown aberrations in the vicinity of $Sxl^{f\#1}$, a fourth series of transplantations was carried out which involved the newly induced mutation $Sxl^{fm\#1,M\#1}$, which was generously provided by T. CLINE. $Sxl^{fm\#1,M\#1}$ is also a strong mutant allele with a phenotype similar to $Sxl^{f\#1}$. It was induced on a female viable and fertile *X* chromosome (CLINE 1984). In series 4, the donor cross produced germ cells trans-heterozygous for $Sxl^{f\#1}$ and $Sxl^{fm\#1,M\#1}$ (Table 2, Series 4). Abnormal multicellular cysts were detected in four host females; there was an equal

TABLE 3

Transplantation of germ cells derived from embryos of homozygous daughterless mothers

Series	Parental donor genotypes	*fs(1)K10* and *mal* parental host genotypes	No. of injected embryos	Normal adult hosts	No. of hosts with progeny from implanted germ cells
5	++; *da pr cn/da pr cn*	*fs(1)K10 mal/+,+*	231	36 ♀	12 ♀
	×	×			
	y z $Sxl^{f\#1}$ *sn f/Y; +/+*	*fs(1)K10 mal/Y*		28 ♂	12 ♂

number of host females that had received $Sxl^{fm\#1,M\#1}/FM6$ germ cells. The abnormal cysts of this series had the same morphology as the abnormal cysts produced by $Sxl^{f\#1}$ homozygous germ cells in series 1–3. Absence of the Sxl^{+} gene, therefore, 'eads to a particular abnormality of differentiation of *2X;2A* germ cells.

Interaction of daughterless and Sxl: Proper expression of the *Sxl* gene in female embryos requires maternal activity of a gene on the second chromosome called daughterless (*da*) (CLINE 1978, 1980). The maternal effect of *da* is most severe in *2X;2A* daughters heterozygous for $Sxl^{f\#1}$. All such progeny die regardless of temperature conditions, and even patches of mutant cells behave abnormally in a cell autonomous fashion (CLINE 1978, 1984). Since, in the foregoing experiments, absence of Sxl^{+} caused a detectable effect on female germ cells only at a relatively late stage, the question arises whether in female germ cells activity of Sxl^{+} still requires maternal da^{+} product. In series 5, females homozygous for *da* were mated to males that carried the $Sxl^{f\#1}$ allele on their *X* chromosome and germ cells from their progeny were injected into normal hosts (Table 3). After transplantation 12 among 36 host females were found to be mosaic. They produced normal eggs and gave rise to normal adult progeny carrying the genetic markers of the donor parents. The *mal*-staining test did not reveal any flies among the remaining hosts that contained multicellular cysts, with the possible exception of one case in which a single mal^{+}-staining cyst of very unclear morphology was observed. Therefore, homozygosity for the mutation *da* in the mother does not lead to the same developmental abnormality as homozygosity of germ cells for $Sxl^{f\#1}$. A similar observation has also been made independently by CLINE (1983b).

Defining the role of Sxl: In *2X;2A* somatic cells, presence of Sxl^{+} is required for correct dosage compensation (suppression of *X* hyperactivation) as well as for female sex determination. Since it has been shown that several genes involved in somatic sex determination are not necessary for sex determination of the germline (MARSH and WIESCHAUS 1978; SCHÜPBACH 1982) it would be interesting to know whether Sxl^{+} is required in germ cells both for sex determination and for dosage compensation or only for one of the processes. A sixth series of transplantations was undertaken involving a special combination of *Sxl* alleles (Table 4, series 6). $Sxl^{fm\#7,M\#1}/Sxl^{M\#1,fm\#3}$ flies will survive at 25°

TABLE 4

Transplantation of germ cells mutant or nonmutant for various alleles of Sex-lethal

Series	*Sxl* and *mal* parental donor genotypes[a]	*fs(1)K10* and *mal* parental host genotypes[a]	No. of injected embryos	Normal adult hosts	No. of hosts with adult progeny from implanted germ cells: Females	No. of hosts with adult progeny from implanted germ cells: Males	No. of female hosts with multicellular ovarian cysts
6	$Sxl^{fm\#7,M\#1}$ +/ $Sxl^{fm\#7,M\#1}$ +/Y; *Dp* Sxl^{+}/+ × $Sxl^{M\#1,fm\#3}$ +/Y; +/+	*fs(1)K10 mal*/+ *mal* × *fs(1)K10 mal/Y*	363	63 ♀	4 *Sxl/Sxl* 2 *Sxl/Sxl/Y* 4 *Sxl/Sxl*; *Dp* Sxl^{+}/+ 1 *Sxl/Sxl/Y*; *Dp* Sxl^{+}/+	3 *Sxl/Y* 2 *Sxl/Y/Y* 1 *Sxl/Y/Y*; *Dp* Sxl^{+}/+ 1 *Sxl/Y*[b]	0
				36 ♂			
7	+ *mal*/+ *mal* × + *mal/Y* mal^{+}	*fs(1)K10 mal*/+ *mal* × *fs(1)K10 mal/Y*	344	45 ♀	12 +/+		0
				17 ♂		7 +/Y	

[a]For exact description of genotypes see MATERIALS AND METHODS.
[b]The transplanted germ cells carried the paternal *X* chromosome and the maternal *Y* chromosome.

with a frequency as high as 48% as compared to their siblings, and the survivors appear to be completely transformed into males in their somatic tissues in spite of their two *X* chromosomes (CLINE 1984). This combination of *Sxl* alleles is interpreted as allowing a level of dosage compensation sufficient for partial survival of *2X;2A* cells while still being incapable of directing them into the appropriate female sexual differentiation pathway. For somatic cells, this arrangement of *Sxl* alleles, therefore, separates the sex-determining function and the dosage compensation function of *Sxl*. If the same were true for germ cells, this might allow one to distinguish whether the abnormal multicellular cyst phenotype was primarily caused by incorrect sex determination or by abnormal dosage compensation due to the absence of Sxl^{+}. If the multicellular cysts were caused by an incorrect sex determination, then transplantation of the trans-heterozygous germ cells should lead to multicellular cysts. If, on the other hand, dosage compensation was responsible for the abnormal cysts, then the *trans*-heterozygous combination which partially restores dosage compensation might allow the formation of some normal egg chambers and eggs. In the donor cross of this series, a free *Y* chromosome was present in the female donor parent (Table 4). This supplied half of the *trans*-heterozygous germ cells with a *Y* chromosome, which potentially would have enabled such germ cells to form functional sperm, if they were indeed male determined and properly dosage compensated. However, germ cells of the constitution $Sxl^{fm\#7,M\#1}/Sxl^{M\#1,fm\#3}$ with or without a *Y* chromosome formed normal oocytes and eggs when implanted into female hosts, and no multicellular cysts were observed in any host female (Table 4). Those germ cells seemed, therefore, viable and fully female determined, in contrast to somatic epidermal cells of identical genetic constitution. This result can be explained by assuming that the sex-determining function of *Sxl* which operates in somatic cells is unnecessary for germ cells. Alternatively, it is possible that this particular combination of *Sxl* alleles supplies a level of sex-determining function that is subthreshold for somatic cells but is high enough for proper sex determination of germ cells.

Are 2X;2A *germ cells mutant for* Sxl *equivalent to* XY;2A *germ cells?* If *Sxl* were involved only in sex determination of the germline, but not in dosage compensation, then the $Sxl^{f\#1}$ homozygous germ cells might be equivalent to normal male germ cells. One could then expect that normal male germ cells implanted into a female gonad would also form abnormal multicellular cysts. In series 7, the behavior of normal *XY;2A* germ cells implanted into a female gonad was studied. In this series of transplantations all of the host and donor females were phenotypically mal. Only the donor males carried a mal^{+} gene on their *Y* chromosome. Therefore, only the *XY;2A* (male) germ cells were marked with mal⁺. The mal^{+} gene translocated on to the *Y* chromosome can be expressed in female germ cells (SCHÜPBACH, WIESCHAUS and NÖTHIGER 1978). Among 45 tested host females, none carried any traces of mal⁺-staining cells or cysts in their ovaries, even though 12 such females might have been expected by comparison with the number of female hosts that had successfully received *2X;2A* germ cells in the same experiment (Table 4). Neither were any unstained multicellular cysts observed. One has, therefore, to conclude that

normal male germ cells that are transplanted into a normal female host at the blastoderm stage do not give rise to any cells in the adult ovary recognizable by the histochemical staining method. Such normal male germ cells must abort earlier than the *2X;2A* germ cells homozygous for $Sxl^{f\#1}$. Homozygosity for $Sxl^{f\#1}$, therefore, does not make *2X;2A* germ cells equivalent to an *XY;2A* constitution in the germline.

DISCUSSION

Germ cells will differentiate into eggs in a female animal or into sperm in a male animal. At some time during development germ cells choose either a female or a male pathway of differentiation. The single germ cell could take this decision depending solely on its own genotype in an autonomous fashion. Alternatively, germ cells could be directed into their pathway of differentiation by the somatic tissues that surround them. In *D. melanogaster*, *2X;2A* cells will not form functional sperm in a male soma, nor will *XY;2A* cells produce fertile eggs in a female animal (VAN DEUSEN 1976). The successful sexual differentiation of germ cells in Drosophila depends, therefore, in some way on the chromosomal constitution of the germ cells themselves. Consequently, at least one step that occurs during sexual differentiation of the germline is cell autonomous even if this step would merely represent a response of the germline to somatic signals. Previous experiments indicate that several of the autosomal control genes for somatic sex determination have no function in sex determination of the germline and, thus, at least some aspects of the genetic network used to interpret the *X:A* ratio in somatic cells are not required in the germline (MARSH and WIESCHAUS 1978; SCHÜPBACH 1982; BELOTE and BAKER 1983). The present study was aimed at assessing whether two of the early steps of somatic sexual differentiation and dosage compensation also occur in germ cells: the role of the *X:A* ratio and the function of the *Sxl* locus.

With respect to the *X:A* ratio, it was found that a *2X;3A* constitution of germ cells in a functionally female ovary sometimes allows the production of normal oocytes but in most cases leads to an abnormal development of these cells. Instead of forming a cluster of 15 polytene nurse cells and one oocyte, the stem cell derivatives divide too many times and give rise to cysts containing many small undifferentiated cells. This indicates that having two *X* chromosomes is not sufficient to ensure normal female development in germ cells. If these two *X* chromosomes find themselves in combination with three sets of autosomes, germ cell development will often be abnormal even in a female environment that perfectly supports the development of *3X;3A* sister cells. The precursors of the *2X;3A* cysts observed in the mosaic gonads had survived and divided during larval development and had been integrated as stem cells into the female ovary. The number of *2X;3A* cysts formed was only slightly lower than the number of *3X;3A* sister cells. Once incorporated into the ovarian stem line, they continued to function as active stem cells. The major developmental problem of *2X;3A* germ cells occurred after stem cell division in the cytoblast cell. The observation that after stem cell division *2X;3A* germ cells have two pathway choices is in agreement with observations made by LAUGÉ (1969) and

LAUGÉ and KING (1979), who found cell masses of tumorous appearance in the gonads of *2X;3A* intersexes but mention occasional normal-looking egg chambers. In the gonads of the intersexes described by Laugé, it had not been possible to distinguish whether the tumors were due to the abnormal behavior of germline or of somatic components of the gonad. The ten females with mosaic germ cell population described in the present study must have contained functionally normal female somatic ovarian cells since these ovaries were capable of fully supporting female development of all *3X;3A* vitellarial cysts. This may be the reason why the ovaries contained no huge tumorous cell masses but rather had multicellular cysts with a large (>16) but limited (<150) number of cells per cyst that were, moreover, always enveloped by a layer of follicle cells.

It is tempting to speculate that the two alternative pathways of differentiation observed in *2X;3A* germ cells (either to enter a normal female pathway of differentiation or to develop into multicellular cysts) are analogous to the choice between female and male pathways taken by somatic cells of the same genetic constitution. In the particular cross described in this study, most of the somatic *2X;3A* cells in the nonmosaic intersexes choose the male pathway and most of the *2X;3A* germ cells in the mosaic ovaries differentiated into multicellular cysts. The correspondence between the two pathway choices can at present not be tested, but the striking observation remains that in an ovary *2X;3A* germ cells have two possible alternatives of differentiation and that no intermediates between the two alternatives were observed. The experiments, however, do not address the issue of whether *2X;3A* germ cells would also show two alternative pathways when transplanted into normal male gonads.

Absence of a functional *Sxl* gene leads to an abnormal development of *2X;2A* germ cells even when they are transplanted into a normal female host at an early stage. Autonomous Sxl^+ activity is, therefore, required for normal female germ cell development. Sterility of flies homozygous or *trans*-heterozygous for certain viable alleles of *Sxl* was also reported by ZIMMERING and MULLER (1961), by MARSHALL and WHITTLE (1978) and by CLINE (1984). Since the viable combinations proved to have strong effects on somatic components of the gonads (CLINE 1984), it was not possible to measure a direct effect of *Sxl* on germ cells in such animals. Using germline-dependent marker mutations, the present study has shown that germ cells homozygous for strong "loss-of-function" alleles at the *Sxl* locus survive during embryonic and larval development. They can form stem cells and continue to undergo stem cell division throughout adult life. After stem cell division, the cells enter an abnormal pathway of differentiation and form multicellular cysts. If the *Sxl* gene is active in female germ cells during early development, this early activity can be eliminated without preventing the cells from populating the ovary. It is only after the stem cell division that abnormal germ cell development is detected and, indeed, is very prominent.

The multicellular cysts formed by the *Sxl* homozygous germ cells were at the light microscopic level indistinguishable from the multicellular cysts formed by many of the *2X;3A* germ cells. Given the results, as described here, that

Sxl$^+$ is required in *2X;2A* cells to form oocytes, it seems that the gene was functioning (turned on) in those *2X;3A* cells that produced normal eggs. Given the similarity in phenotype, it is possible that those *2X;3A* cells that differentiated into multicellular cysts did so because *Sxl*$^+$ activity was not induced. This would be very analogous to what has been suggested to happen in somatic cells of *2X;3A* constitution: some perceive this *X:A* ratio as being closer to 1 and turn on their *Sxl* gene (and becomes female cells); others perceive the ratio as closer to 0.5 and do not activate *Sxl* (CLINE 1983a).

The absence of *Sxl*$^+$ function kills female embryos and causes severe growth retardation during larval stages in homozygous mutant clones of somatic cells (SANCHEZ and NÖTHIGER 1982; CLINE 1984). In germ cells, the absence of *Sxl*$^+$ caused detectable abnormalities only after the time of stem cell proliferation, *i.e.*, in the pupal or adult stage. If this late requirement of *Sxl* reflects in fact a late activation of *Sxl* in the germline, this might explain why no effect of the mutation *da* on female germ cell development was observed either in the present study or in an experiment reported by CLINE (1983b). The *da*$^+$ product is deposited in the egg by the mother and is required for activation of *Sxl*$^+$ in female embryos. Possibly *da* only facilitates or speeds up the process in the embryo but is not necessary when there is enough time to activate *Sxl*. At present, only one mutation at the daughterless locus has been identified and it does not represent a complete loss-of-function mutation (MANGE and SANDLER 1973). The failure to observe defects using this particular mutation is, therefore, not fully conclusive.

A similar open question is at the moment why the presumably constitutive mutation *Sxl*$^{M\#1}$ appeared not to have an effect when present in male germ cells (CLINE 1983b). Cline obtained three male chimeras after pole cell transplantation of germ cells bearing *Sxl*$^{M\#1}$. From this result, one could argue that, in contrast to somatic cells, constitutive levels of *Sxl* in germ cells have no effect on male development. Alternatively, *Sxl*$^{M\#1}$ may not be constitutive for germline function.

The results of this study show that *Sxl* is required in germ cells but do not distinguish whether this requirement reflects its role in dosage compensation, sex determination or, strictly speaking, in another yet undiscovered process. A combination of *Sxl* alleles that, in somatic cells, partially supplies dosage compensation function but does not provide (enough) sex determination function still allowed the formation of normal eggs. One interpretation of this result would be that *Sxl*$^+$ is required in germ cells only for dosage compensation, since the combination that supplies just dosage compensation completely rescues the female germ cells. On the other hand, it is also possible that this intermediate combination of *Sxl* alleles was sufficient to supply sex determination function to germ cells in contrast to its behavior in somatic cells.

Germ cells homozygous for Sxl$^{f\#1}$ *transplanted into female hosts are not equivalent to* XY *cells.* Transplanted *XY* germ cells were not able to produce any detectable cell type in the adult ovary and, therefore, do not develop as far as the *Sxl* homozygous mutant cells. In somatic cells, the absence of *Sxl*$^+$ function makes *2X;2A* cells initially equivalent to *X(Y);2A* cells: they embark on a male

pathway and hyperactivate their *X* chromosomes (LUCCHESI and SKRIPSKY 1981). The developmental abnormalities of *Sxl* homozygous *2X;2A* cells presumably result from this hyperactivation. If absence of *Sxal*$^+$ function in the germline had made *2X;2A* cells similarly equivalent to *XY* cells, one would have expected transplanted *XY* germ cells to develop at least as far as, if not farther than, *Sxl* homozygous germ cells. The failure of *XY* germ cells to populate the ovary can not be attributed to the inactivation of *Sxl* normally thought to occur in *XY* cells since the elimination of the gene in *XX* cells does not have that same effect. The observed difference between *XY* germ cells and *Sxl* homozygous *XX;2A* cells could be explained if the mutations used in this study left some residual *Sxl*$^+$ function in *XX;2A* cells which would allow these cells to survive. On the other hand, all available genetic data indicate that these mutations are amorphic (CLINE 1978, 1984).

If the results described in this paper reflect the consequence of total elimination of *Sxl*$^+$ function, then the disappearance of *XY* germ cells could be explained in two ways: One way would be to assume that other, earlier active, genes exist which perform functions analogous to *Sxl* in germ cells during embryonic or larval stages. Such hypothetical genes would read the *X:A* ratio in germ cells and control sex determination and dosage compensation in such a way that, even in the absence of *Sxl*, *XY* cells would behave differently from *XX* cells when transplanted into a female gonad. Such genes might well exist, but, under this hypothesis, the ratio of *X* chromosomes to autosomes would be read twice in the germline—once by those hypothetical genes in early development and once by *Sxl* in the mature gonadal stage. A second, and I believe, simpler hypothesis requires no additional processes and genes but follows directly from the late effect of *Sxl* mutations on female germ cells: if *Sxl* is indeed the key gene for registering the *X:A* ratio in the germline as well as in the soma, and if in germ cells it functions only late in development, then during embryonic and larval stages germ cells would not yet be dosage compensated or sex determined in terms of switches in control genes. During these stages *XY;2A* cells would not synthesize the same amount of *X*-linked products as *2X;2A* cells and the early sex-specific differences observed in germ cells might result directly from this difference: cells with a "double amount" of certain *X*-linked products would develop in a female manner (slow cell divisions, differentiation into stem cells only in pupal stages), whereas cells with a single dose would always develop in a male fashion (more rapid cell divisions, differentiation into stem cells already at larval stages). In a variant of this model, sexual differences in germ cells prior to the action of *Sxl* might depend on signals given by their somatic surrounding. In this case, *XY;2A* germ cells in an ovary would be directed into a female pathway, but since they have only one *X* chromosome and do not undergo dosage compensation, they would not synthesize enough of the necessary *X*-linked products and would die or be outcompeted by their *2X;2A* sisters. A similar germline-autonomous cell competition has been demonstrated in metafemale-female mosaics (SCHÜPBACH, WIESCHAUS and NÖTHIGER 1978).

As discussed in an earlier study (SCHÜPBACH 1982) there is no absolute

necessity to establish dosage compensation in germ cells at all, since male and female germlines behave as different "tissues" at least from larval stages onward and tissue-specific gene regulation alone could regulate expression of *X*-linked as well as of autosomal genes (assuming that all genes have to be regulated with respect to tissue specificity and time). In female germ cells of the mouse, dosage compensation (*i.e.*, *X* chromosome inactivation) does occur but only at a relatively late stage in development, after it has already occurred in other, earlier differentiating tissues (JOHNSTON 1981; KRATZER and CHAPMAN 1981; MONK 1981; MONK and MCLAREN 1981; MARTIN 1982). This situation may be comparable to the late requirement of Sxl^+ in female germ cells of Drosophila; yet in neither case do we understand the function of this late dosage compensation.

In summary, all of the available data are consistent with the following hypothesis: during early germ cell development the germ cells do not dosage compensate. Their sex-specific development at this stage is either a direct consequence of their own chromosomal constitution or is dictated by their somatic surrounding. In a female gonad, the *X:A* ratio is assessed in the daughter cells of the stem cell division such that activation of Sxl^+ results if the ratio is 1, or close to 1. This activation in turn leads to the formation of oocyte-nurse cell cysts of normal 15 + 1 morphology. If at this point, however, Sxl^+ activity is absent, the germ cells will divide abnormally and form multicellular cysts, either because they perceive themselves as being male and/or because they now hyperactivate their *X* chromosomes.

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LITERATURE CITED

BAKER, B. S. and J. M. BELOTE, 1983 Sex determination and dosage compensation in *Drosophila melanogaster*. Annu. Rev. Genet. **17:** 345–393.

BAKER, B. S. and K. A. RIDGE, 1980 Sex and the single cell. I. On the action of major loci affecting sex determination in *Drosophila*. Genetics **94:** 383–423.

BELL, A. E., 1954 A gene in *Drosophila melanogaster* that produces all male progeny. Genetics **39:** 958–959.

BELOTE, J. M. and B. S. BAKER, 1983 The dual functions of a sex determination gene in *Drosophila melanogaster*. Dev. Biol. **95:** 512–517.

BELOTE, J. M. and J. C. LUCCHESI, 1980 Control of X-chromosome transcription by the maleness gene in *Drosophila*. Nature **285:** 573–575.

BRIDGES, C. B., 1921 Triploid intersexes in *Drosophila melanogaster*. Science **54:** 252–254.

BRIDGES, C. B., 1925 Sex in relation to chromosomes and genes. Am. Nat. **59:** 127–137.

BROWN, E. H. and R. C. KING, 1961 Studies on the expression of the transformer gene of *Drosophila melanogaster*. Genetics **46:** 143–156.

CLINE, T. W., 1976 A sex-specific temperature-sensitive maternal effect of the daughterless mutation of *Drosophila melanogaster*. Genetics **84:** 723–742.

CLINE, T. W., 1978 Two closely linked mutations in *Drosophila melanogaster* that are lethal to opposite sexes and interact with daughterless. Genetics **90:** 683–698.

CLINE, T. W., 1979 A male-specific lethal mutation in *Drosophila* that transforms sex. Dev. Biol. **72:** 266–275.

CLINE, T. W., 1980 Maternal and zygotic sex-specific gene interactions in *Drosophila melanogaster*. Genetics **97:** 903–926.

CLINE, T. W., 1983a The interaction between daughterless and Sex-lethal in triploids: a lethal sex-transforming maternal effect linking sex-determination and dosage compensation in *Drosophila melanogaster*. Dev. Biol. **95:** 260–274.

CLINE, T. W., 1983b Functioning of the genes daughterless and Sex-lethal in Drosophila germ cells. Genetics **104:** s16–s17.

CLINE, T. W., 1984 Autoregulatory functioning of a Drosophila gene product that establishes and maintains the sexually determined state. Genetics **107:** 231–277.

DOBZHANSKY, T., 1930 Genetical and environmental factors influencing the type of intersexes in *Drosophila melanogaster*. Am. Nat. **64:** 261–271.

DOBZHANSKY, T. and C. B. BRIDGES, 1928 The reproductive system of triploid intersexes in *Drosophila melanogaster*. Am. Nat. **62:** 425–434.

GEHRING, W. J., E. WIESCHAUS and M. HOLLIGER, 1976 The use of normal and transformed gynandromorphs in mapping the primordial germ cells and gonadal mesoderm in *Drosophila*. J. Embryol. Exp. Morphol. **35:** 607–616.

HANNAH-ALAVA, A. and C. STERN, 1957 The sexcombs in males and intersexes of *Drosophila melanogaster*. J. Exp. Zool. **134:** 533–556.

JANNING, W., 1972 Aldehydeoxidase as a cell marker for internal organs in *Drosophila melanogaster*. Naturwissenschaften **59:** 516–617.

JOHNSTON, P. G., 1981 X chromosome activity in female germ cells of mice heterozygous for Searle's translocation *T(X;16)16H*. Genet. Res. **37:** 317–322.

KRATZER, P. G. and V. M. CHAPMAN, 1981 X chromosome reactivation in oocytes of *Mus caroli*. Proc. Natl. Acad. Sci. USA **78:** 3093–3097.

LAUGÉ, G., 1969 Etude des gonades des intersexues triploides de *Drosophila melanogaster*: description morphologique: ontogenese des structures histologiques. Ann. Soc. Entomol. Fr. (N.S.) **5:** 253–314.

LAUGÉ, G. and R. C. KING, 1979 The ultrastructure of the ovaries of 2X 3A intersexes of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). Int. J. Insect Morphol. Embryol. **8:** 277–288.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **627.**

LUCCHESI, J. C. and T. SKRIPSKY, 1981 The link between dosage compensation and sex differentiation in *Drosophila melanogaster*. Chromosoma **82:** 217–227.

MANGE, A. P. and L. SANDLER, 1973 A note on the maternal effect mutants *daughterless* and *abnormal oocyte* in *Drosophila melanogaster*. Genetics **73:** 73–86.

MARSH, J. L. and E. WIESCHAUS, 1977 Germ-line dependence of the maroon-like maternal effect in *Drosophila*. Dev. Biol. **60:** 396–403.

MARSH, J. L. and E. WIESCHAUS, 1978 Is sex determination in germ-line and soma controlled by separate genetic mechanisms? Nature **272:** 249–251.

MARSHALL, T. and J. R. S. WHITTLE, 1978 Genetic analysis of the mutation female-lethal in Drosophila melanogaster. Genet. Res. **32:** 103–111.

MARTIN, G. R., 1982 X-chromosome inactivation in mammals. Cell **29:** 721–724.

MERRIAM, J. and C. DUFFY, 1972 First multiple seven now contains sn^{X2} for better balancing. Drosophila Inform. Serv. **48:** 43.

MONK, M., 1981 A stem-line model for cellular and chromosomal differentiation in early mouse development. Differentiation **19:** 71–76.

MONK, M., and A. MCLAREN, 1981 X-chromosome activity in foetal germ cells of the mouse. J. Embryol. Exp. Morphol. **63:** 75–84.

RIPOLL, P. and A. GARCIA-BELLIDO, 1979 Viability of homozygous deficiencies in somatic cells of *Drosophila melanogaster*. Genetics **91:** 443–453.

SANCHEZ, L. and R. NÖTHIGER, 1982 Clonal analysis of *Sex-lethal*, a gene needed for female sexual development in *Drosophila melanogaster*. Wilhelm Roux Arch. **191:** 211–214.

SANCHEZ, L. and R. NÖTHIGER, 1983 Sex determination and dosage compensation in *Drosophila melanogaster*: production of male clones in XX females. EMBO J. **2:** 485–491.

SCHALET, A. and V. FINNERTY, 1968 New mutants of *Drosophila melanogaster*. Drosophila Inform. Serv. **43:** 65.

SCHÜPBACH, T., 1982 Autosomal mutations that interfere with sex determination in somatic cells of *Drosophila* have no direct effect on the germline. Dev. Biol. **89:** 117–127.

SCHÜPBACH, T., E. WIESCHAUS and R. NÖTHIGER, 1978 A study of the female germ line in mosaics of *Drosophila*. Wilhelm Roux Arch. **184:** 41–56.

SEIDEL, S., 1963 Experimentelle Untersuchungen uber die Grundlagen der Sterilitat von *transformer* (*tra*) Mannchen bei *Drosophila melanogaster*. Z. Vererbungsl. **94:** 215–241.

VAN DEUSEN, E. B., 1976 Sex determination in germ line chimaeras of *Drosophila melanogaster*. J. Embryol. Exp. Morphol. **37:** 173–185.

WIESCHAUS, E., J. L. MARSH and W. J. GEHRING, 1978 *fs(1)K10*: a germline-dependent female sterile mutation causing abnormal chorion morphology in *Drosophila melanogaster*. Wilhelm Roux Arch. **184:** 75–82.

WIESCHAUS, E., C. NÜSSLEIN-VOLHARD and G. JÜRGENS, 1984 Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. III. Zygotic loci on the X-chromosome and fourth chromosome. Wilhelm Roux Arch. **193:** 296–307.

ZIMMERING, S. and H. J. MULLER, 1961 Studies on the action of the dominant female-lethal *Fl* and of a seemingly less extreme allele, Fl^s. Drosophila Inform. Serv. **35:** 103–104.

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