Recombination and disjunction in female germ cells of Drosophila depend on the germline activity of the gene Sex-lethal

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SUMMARY

Gametogenesis in males and females differs in many ways. An important difference in Drosophila is that recombination between homologous chromosomes occurs only in female meiosis. Here, we report that this process relies on the correct functioning of Sex-lethal (Sxl) which is primarily known as the master gene in somatic sex determination. Certain alleles of this gene (Sxl\textsuperscript{fs}) disrupt the germline, but not the somatic function of Sxl and cause an arrest of germ cell development during cystocyte proliferation. Using dominant suppressor mutations that relieve this early block in Sxl\textsuperscript{fs} mutant females, we discovered additional requirements of Sxl for normal meiotic differentiation of the oocyte. Females mutant for Sxl\textsuperscript{fs} and carrying a suppressor become fertile, but pairing of homologous chromosomes and formation of chiasmata is severely perturbed, resulting in an almost complete lack of recombinants and a high incidence of non-disjunction events. Similar results were obtained when germine expression of wild-type Sxl was compromised by mutations in virilizer (vir), a positive regulator of Sxl. Ectopic expression of a Sxl transgene in premeiotic stages of male germine development, on the other hand, is not sufficient to allow recombination to take place, which suggests that Sxl does not have a discriminatory role in this female-specific process. We propose that Sxl performs at least two tasks in oogenesis: an ‘early’ function in formation of the egg chamber, and a ‘late’ function in progression of the meiotic cell cycle, suggesting that both events are coordinated by a common mechanism.

Key words: Sex-lethal, Drosophila melanogaster, Oogenesis, Recombination

INTRODUCTION

Germ cells undergo a specialized meiotic cell cycle which coordinates differentiation with nuclear events necessary for production of haploid gametes. Although certain aspects of meiotic cell cycle regulation in higher organisms are understood (Sagata, 1996), little is known about how germ cell progression through meiosis is regulated and coordinated with gametogenesis. The fruitfly Drosophila melanogaster offers a suitable system to experimentally approach this question because many facets of early germ cell regulation and differentiation have already been analyzed in detail. In particular, studies in male gametogenesis led to the discovery of a number of genes that are involved in the coordinate control of the meiotic cycle and spermatid differentiation (reviewed in Maines and Wasserman, 1998). Less is known about the complementary process in females. Like the gonial cells in testes, their female counterparts, the cystoblasts, first undergo four mitotic divisions with incomplete cytokinesis before entering the meiotic prophase. In the resulting 16-cell cysts, cystocytes start assembling structures of the synaptonemal complex (Schmekel et al., 1993) with two cells, the pro-
prophase. For instance, in mutant c(3)G germ cells, the process of synopsis and the formation of the synaptonemal complex are absent (Hall, 1972). As a result, recombination is abolished and non-disjunction highly elevated.

Our report adds an unexpected member to the list of mutations that affect early pachytene functions of meiosis. The corresponding gene, Sxl, is primarily known for its key role in somatic sex determination. In this tissue, it imposes female development when activated whereas male development follows when the gene is inactive. The gene encodes an RNA-binding protein which performs its regulatory function at the post-transcriptional level, either by forcing female-specific splicing pattern or by translational repression of male-specific target genes (Cline and Meyer, 1996; Steinmann-Baker and Baker, 1997; Kelley et al., 1997). Little is understood about its function in the germline. First evidence for an involvement in female germline development came from analysis of loss-of-function alleles in genetic mosaic (Schüpbach, 1985; Steinmann-Zwicky et al., 1989). When mutant germ cells are incorporated in a wild-type ovary, they fail to differentiate and instead display an overgrowth phenotype. Also, a class of sterile alleles exists that are specifically defective for germline, but not for somatic functions (Salz et al., 1987). Germ cells mutant for the recessive alleles Sxl^4, Sxl^5 and Sxl^18 (collectively called Sxl^b) remain small and undifferentiated and continue to proliferate excessively, forming large cysts, a phenotype which is commonly referred to as ovarian tumors or multicellular cysts. The overproliferation phenotype implicated Sxl in playing an essential role in the control of the cystocyte mitotic cycle and subsequent cyst formation.

The cellular mechanism by which Sxl executes this function is not understood. None of the known somatic target genes of Sxl is required in oogenesis (Marsh and Wieschaus, 1978; Schüpbach, 1982). Also, regulation and expression of this gene in the female germline differ from those in the soma. For instance, unlike the soma, germline activity of Sxl depends on non-autonomous cues emanating from the surrounding soma (Steinmann-Zwicky, 1992; Oliver et al., 1993; Horabin et al., 1995). Furthermore, its expression pattern in the germline appears biphasic with a striking change in intracellular distribution (Fig. 1C). Sxl is first expressed in female embryonic germ cells and maintains a high level of cytoplasmic expression until the first cystocyte divisions in the adult ovary (Bopp et al., 1993; Horabin et al., 1995). The level of SXL protein then precipitously declines and reappears in a second phase as nuclear foci in the newly formed 16-cell cyst. From stage 1 onward, it steadily increases in level and localizes to the cytoplasm and nuclei of nurse cells (Fig. 1D). The biphasic nature of this expression pattern is also evident from the differential effects of some positive regulators of Sxl. In germ cells mutant for snf^b21 or for otu alleles of the ONC class, early Sxl expression is prevented (Bopp et al., 1993; Oliver et al., 1993). These cells cannot differentiate and they display the same tumorous phenotype as seen in Sxl mutant germ cells. However, in germ cells mutant for vir, the first phase of cytoplasmic expression is still maintained, but the second expression phase, which commences in early germarial cysts, is abolished (Fig. 1E-F). Absence of this 'late' SXL does not affect formation and differentiation of the egg chamber. The affected cells complete oogenesis and give rise to progeny. It was therefore concluded that early cytoplasmic activity is sufficient to allow formation of a functional egg chamber (Schütt et al., 1998).

In this report, we describe an additional requirement for Sxl activity in the normal progression of the meiotic cell cycle once the egg chamber has been formed. We propose that Sxl plays a role in the coordination of the mitotic and subsequent meiotic cell cycle.

**MATERIALS AND METHODS**

**Culture conditions and mutations**

Flies were reared on standard food (cornmeal, sugar, yeast, agar. Nipagin). All crosses were done at 25°C under controlled population density. For genetic symbols, see Lindsley and Zimm (1992). vir^b2 causes female-specific lethality, and vir^d6 is lethal for females and semilethal for males (Hilfiker et al., 1995). Sxl^4, Sxl^5 and
\textbf{RESULTS}

Dominant suppressors restore fertility in \textit{Sxl} mutant females

The recessive allele \textit{Sxl}\textsuperscript{f5} produces mutant proteins with a single missense mutation downstream of the two RNA-binding RRM domains (Bopp et al., 1993). This change in the protein sequence does not affect somatic functions in females, but renders these sterile. In \textit{Sxl}\textsuperscript{f5} mutant ovaries, germ cells are largely arranged in small clusters of 2 or 4 cells connected by intercellular branches, as seen by antibody staining (Fig. 2).

The detected adducin-like protein of \textit{Hu-li tai shao} (\textit{Hts}) is a component of the fusome, a structure that has been proposed to play a central role in early cyst formation (Yue et al., 1992). It first appears as a spherical organelle in stem cells and cybotelasts and then elongates and branches to connect the dividing cystocytes (Fig. 2D; reviewed in McKearin, 1997). The staining pattern observed in \textit{Sxl} mutant ovaries is typical for cystocytes that have undergone one or two rounds of cystocyte divisions (Fig. 2E). The prevalence of these stages suggests that mutant cells are unable to complete the four mitotic cycles and, therefore, cannot form a functional cyst nor advance into the next stages of oogenic differentiation.

To study the disrupted cellular process, we isolated suppressor mutations that can relieve this block during early oogenesis. From an initial screen using EMS-mutagenized males, we recovered several dominant suppressors that restore fertility of \textit{Sxl}\textsuperscript{f4} and \textit{Sxl}\textsuperscript{f5} mutant females to a significant level (Benjamin I. Arthur and D. B., unpublished results). One of these suppressors, \textit{Su(Sxl}\textsuperscript{fs})46, was mapped to 3-[85]. This extragenic suppressor mutation exhibits the strongest activity in our collection and allows practically normal oogenesis in \textit{Sxl}\textsuperscript{f4} and \textit{Sxl}\textsuperscript{f5} mutant females. Because a significant number of fertile progeny is produced by these females, the mutant stock can be readily propagated. A cytological analysis of the affected ovaries shows mostly normal looking cysts that proceed through all stages of oogenesis without any apparent morphological defects (Fig. 2C). Only rare cases of abnormal morphology were observed, such as for instance mislocalization of the oocyte to the medial region of the egg chamber rather than to the posterior end. The germarial amplification steps leading to the formation of a 16-cell cyst appear normal as judged from the fusome architecture revealed by anti-HTS staining (Fig. 2F).

\textbf{Suppressors of \textit{Sxl}\textsuperscript{f4} uncover a requirement for \textit{Sxl} in meiotic differentiation of the oocyte}

Though no morphological defect was evident in mutant ovaries carrying \textit{Su(Sxl}\textsuperscript{fs})46, we noticed that recombination and proper segregation of homologous chromosomes were severely impaired. Females with a heteroallelic combination of \textit{Sxl}\textsuperscript{f4} and \textit{Sxl}\textsuperscript{f5} carrying \textit{Su(Sxl}\textsuperscript{fs})46 produced only non-recombiant progeny when tested for the \textit{w-f} interval on the \textit{X} chromosomes (Table 1A). Similarly, the recombination values in \textit{Sxl}\textsuperscript{f4}/\textit{Sxl}\textsuperscript{f5} females obtained for markers on the second chromosome were substantially reduced to about 10\% of the expected values (Table 1B). This reduction is caused by lack of \textit{Sxl} wild-type activity and is not due to the presence of the suppressor mutation: replacing one mutant allele by a wild-type copy of \textit{Sxl} restores recombination frequencies to almost normal values (Table 1B). Moreover, the same results were obtained with a different suppressor \textit{Su(Sxl}\textsuperscript{fs})X2 which maps to the \textit{X} chromosome. Testing a heteroallelic combination of \textit{Sxl}\textsuperscript{f4} and \textit{Sxl}\textsuperscript{f18} carrying \textit{Su(Sxl}\textsuperscript{fs})X2 on the \textit{Sxl}\textsuperscript{f18} chromosome, the absence of recombination was even more striking, as none of the experimental females produced any recombinant progeny.
Table 1. Recombination frequencies in Sxl\(^{f4}\); Su(Sxl\(^{fs}\))46 females

<table>
<thead>
<tr>
<th>Recombination frequencies in Su(Sxl(^{p}))46 suppressed females</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. First chromosome</strong></td>
</tr>
<tr>
<td>Maternal genotype</td>
</tr>
<tr>
<td>w Sxl(^{p})+f/+ Sxl(^{p}) c+/+ ; Su(Sxl(^{p}))46/+</td>
</tr>
<tr>
<td>w ++ sn/+ Sxl(^{f4}) Su(Sxl(^{fs}))X2 +</td>
</tr>
<tr>
<td><strong>B. Second chromosome</strong></td>
</tr>
<tr>
<td>Maternal genotype</td>
</tr>
<tr>
<td>Sxl(^{p})/Sxl(^{f4}); al b c/++; Su(Sxl(^{p}))46/+</td>
</tr>
<tr>
<td>Sxl(^{p})/+ ; al b c/++; Su(Sxl(^{p}))46/+</td>
</tr>
<tr>
<td><strong>C. First chromosome</strong></td>
</tr>
<tr>
<td>Maternal genotype</td>
</tr>
<tr>
<td>w Sxl(^{p}}+f/+ Sxl(^{p}) Su(Sxl(^{p}))X2 +</td>
</tr>
<tr>
<td>w ++ sn/+ Sxl(^{f4}) Su(Sxl(^{p}))X2 +</td>
</tr>
<tr>
<td><strong>D. Second chromosome</strong></td>
</tr>
<tr>
<td>Maternal genotype</td>
</tr>
<tr>
<td>Sxl(^{p})/Sxl(^{f4})Su(Sxl(^{p}))X2 ; al b c/++</td>
</tr>
<tr>
<td>Sxl(^{p})+/ ; al b c/++</td>
</tr>
<tr>
<td><strong>E. Third chromosome</strong></td>
</tr>
<tr>
<td>Maternal genotype</td>
</tr>
<tr>
<td>Sxl(^{p})/Sxl(^{f4}) Su(Sxl(^{p}))X2; Kt e/++</td>
</tr>
<tr>
<td>Sxl(^{p})+/ ; Kt e/++</td>
</tr>
</tbody>
</table>

*Recombinants for the two intervals were scored independently. For the first chromosome, the markers white (w; 1-1.5), cut (ct; 1-20.0), singed (sn; 1-21.6) and forked (f; 1-56.7) were used. For the second chromosome, markers aristless (al; 2-0.4), black (b; 2-48.5) and curved (c; 2-75.5) were used and for the third, Kinked (Kt; 3-47.6) and ebony (e; 3-70.7).

\(\text{T} \)The recombination frequency as a number for each interval was calculated as number of recombinants/number of progeny.

\(\|n\) The total number of progeny scored from a cross with w ct f/Y males.

\(\|n\) The total number of progeny scored from a cross with w ct f/Y females.

\(\|n\) The total number of progeny scored from a cross with w ct f/Y males.

\(\|n\) The total number of progeny scored from a cross with w ct f/Y females.

\(\|n\) The total number of progeny scored from a cross with w ct f/Y males.

\(\|n\) The total number of progeny scored from a cross with w ct f/Y females.

We performed the recombination tests independently with different intervals on the X, second or third chromosome (Table 1C-E). Again, presence of one wild-type copy of Sxl was sufficient to restore almost normal recombination frequencies in Su(Sxl\(^{p}\))X2 females (Table 1C).

In addition to a severely impaired recombination, we also observed a high incidence of non-disjunction (Table 2). For instance, among 178 descendants of females of the genotype Sxl\(^{f4}\)/Sxl\(^{f4}\) Su(Sxl\(^{p}\))X2, 39% were products of X-chromosomal non-disjunction events. Besides the occurrence of XXY and XO individuals, we observed a substantial number of nonviable embryos. This lethality is most likely caused by aneuploidy resulting from non-disjunction of autosomes. Adding a single wild-type copy of Sxl in the mother significantly reduced the frequency of non-disjunctive offspring (Table 2B) which indicates an involvement of Sxl in proper segregation. The still elevated frequency of non-disjunction which is observed even in presence of a wild-type copy of Sxl could be due to the occasional presence of an extra Y chromosome (Fig. 4B.E) resulting from a non-disjunctive event in a previous generation. An extra Y chromosome causes secondary non-disjunction of non-exchange X chromosomes. This phenomenon could also explain the rather high frequency of X-chromosomal non-disjunction in Su(Sxl\(^{f}\))46 females carrying one wild-type copy of Sxl (Table 2A). In the presence of two wild-type copies, however, no single case of X chromosomal non-disjunction was recovered among 820 descendants (Table 2A). This result excludes the possibility that Su(Sxl\(^{f}\))46 itself is responsible for the segregation defects. The results from this analysis indicate that Sxl wild-type activity is needed in the female germline for exchange and normal progression of the meiotic cell cycle.

**Oocyte nuclei in Sxl\(^{fs}\); Su ovaries display multiple abnormalities in meiotic stages**

To investigate whether the failure to proceed through normal meiosis is accompanied by a cytologically visible defect, we microscopically examined squashed preparations from different oogenic stages. The first conspicuous difference between germarial cysts of wild type and those of the Sxl\(^{f4}\)/Sxl\(^{f4}\); Su(Sxl\(^{f}\))46 genotype is an apparent lack in the latter of nuclei that could be identified as pro-oocytes. Unlike in wild-type cysts (Fig. 3A), no distinction can be made between the presumptive oocyte and the nurse cell nuclei. Late pachytene nuclei which are typical for stage 3 wild-type egg chambers were not found (Fig. 3B). The first signs of meiotic differentiation appear around stage 7. However, the appearance of oocyte nuclei is accompanied by a variety of defects in karyosomal organisation. Chromosome threads seem to be decondensed and dispersed (Fig. 3D) or defectively packed in the karyosome (Fig. 3E). Occasionally, two or three loose bodies of chromatin are present instead of a single compact karyosome which is typical for wild-type oocytes (Fig. 3F-J). Such a loose organisation could be due to a defect in pairing and/or formation of the chomocentre during meiotic prophase. The same types of aberrations were also detected in vitellogenic cysts of the Sxl\(^{f4}\)/Sxl\(^{f4}\) Su(Sxl\(^{p}\))X2 genotype.

Consistent with the lack of recombination, no chiasmata are present in stage 13-14 oocytes of either Sxl\(^{f4}\)/Sxl\(^{f4}\) Su(Sxl\(^{p}\))X2 or the Sxl\(^{f4}\)/Sxl\(^{f4}\); Su(Sxl\(^{f}\))46 females (Fig. 4A-F). Consequently, chromosomes are usually seen in anaphase-like arrangement in the first division spindle. Another unusual feature in these oocytes is the occurrence of ‘cleft spindles’. In many instances, chromosomes are arranged in two or three spindles instead of one (Fig. 4G). This abnormality probably relates to the loose organisation of chromatin in defective karyosomes and suggests that more than one spindle-organising center may be present. An extra chromosome, presumably a Y, is seen in some meiotic plates and must derive from a previous non-disjunction (Fig. 4E,F).

The described results may all be attributed to pairing defects of early meiotic prophase chromosomes which in turn would explain the absence of chiasmata and defects in segregation during the first meiotic division.
Compromised germline expression of Sxl leads to reduced recombination and increased non-disjunction frequencies

The described meiotic abnormalities usually occur when a specific class of sterile Sxl alleles is suppressed by our dominant suppressor mutations. It is thus possible that these mutant SXL proteins interfere with a process in meiosis that is normally not regulated by Sxl. As an additional test for a role of Sxl in meiotic differentiation, a female genotype was constructed in which germline activity of Sxl is affected not by a mutation in the coding sequence, but at the level of its expression.

It was previously reported that germline expression of Sxl is in part controlled by vir. Germ cells mutant for vir differentiate into functional eggs, although expression of SXL protein is no longer maintained after the apparently normal expression in early germarial stages (Schütt et al., 1998). The vir-independent expression of Sxl in the germarium is sufficient for germ cells to advance into and to complete the oogenic pathway. It was of obvious interest to test if absence of vir-dependent expression of Sxl in these germ cells may have any bearings on normal meiotic differentiation of the oocyte. To this end, vir mutant germ cells were generated using the FLP-DFS system, and the derived progeny was examined for possible defects in recombination and segregation. In germ cells homozygous for the female-specific mutation vir2f,

Table 2. X-chromosome non-disjunction in SxlBf; Su(Sxl fs )46 females

<table>
<thead>
<tr>
<th>Maternal genotype*</th>
<th>N</th>
<th>XY</th>
<th>XX</th>
<th>Non-disjunction</th>
<th>Frequency†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. in Su(Sxl Bf )/46 suppressed females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sxl Bf /Sxl Bf ; Sxl Bf /46/+</td>
<td>896</td>
<td>366</td>
<td>462</td>
<td>42</td>
<td>26</td>
</tr>
<tr>
<td>Sxl Bf /+ ; Su(Sxl fs )46/+</td>
<td>781</td>
<td>262</td>
<td>425</td>
<td>58</td>
<td>36</td>
</tr>
<tr>
<td>+/+ ; Su(Sxl fs )46/+</td>
<td>820</td>
<td>423</td>
<td>397</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. in Su(Sxl Bf )/X2 suppressed females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sxl Bf /Su(Sxl fs )/X2+Sxl Bf</td>
<td>178</td>
<td>64</td>
<td>71</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>Sxl Bf /Su(Sxl fs )/X2/+</td>
<td>354</td>
<td>68</td>
<td>256</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

*Progeny were scored from a cross of +B'/Y males. Exceptional XO males and XXY females can be distinguished from normal disjunction products by the absence resp. presence of B'/Y. In addition, y, w and markers onSxl Bf were used for scoring non-disjunction XO males.

†Frequency of non-disjunction was calculated as 2 x [XO+XXY]/[XY+XX+2XO+2XXY]. The sum of XO males and XXY females was multiplied by 2 to account for YO and XXX products.

Fig. 2. Su(Sxl Bf )/46 restores differentiation of Sxl Bf mutant ovaries. (A-C) Optical sections of nuclear staining of ovaries from wild-type OrR females (A), Sxl Bf /Sxl Bf females (B), and Sxl Bf /Sxl Bf ; Su(Sxl Bf )/46/+ females (C). g: germarium, nc: nurse-cell nuclei, fc: follicle cells, oc: oocyte. (D-F) AntisHTS-staining of germaria of the same genotypes: OrR (D), Sxl Bf /Sxl Bf (E) and Sxl Bf /Sxl Bf ; Su(Sxl Bf )/46/+ females (F). Arrowheads point to spherical fusome organelles in stem cells and cystoblasts (D-F), and small arrows to the branched extensions of fusomes in proliferating cytocysts. 16-cell cysts are surrounded by follicle cells stained with anti-HTS. Note absence of staining in germ cells of the stage 1 cysts (S1).
recombination frequencies for X chromosomal intervals appeared only slightly reduced when compared to those in heterozygous control females (Table 3A). A clear and significant reduction in recombination frequencies, however, was obtained with the stronger allele vir 6. In this case, recombination values for at least one interval collapsed to 25% of that obtained in control females (Table 3B). The reduction in recombination was accompanied by a substantial increase in the frequency of non-disjunction (XO males in Table 3B). The dose-dependent interaction with Sxl suggests that recombination and segregation defects in vir mutant germ cells are primarily based on insufficient expression of Sxl activity. Thus, the normal process of meiosis is not only disturbed by presence of mutant SXL protein as shown before, but also by compromising its wild-type expression. Taken together, these results convincingly prove Sxl to be required in normal meiosis.

**Ectopic expression of Sxl does not allow recombination in male germ cells**

In *Drosophila*, recombination is a female-specific trait. Cross-over events do not occur in spermatogenesis. As shown in this report, recombination in females depends on the activity of Sxl, a gene which is not active in the male germline. This raises the possibility that Sxl acts as a discriminating factor that permits meiotic recombination in germ cells to occur when the gene is active. If so, we expect that forced Sxl expression in the premeiotic stages of male germ line development may provoke exchange between homologous chromosomes. Misexpression studies by Hager and Cline (1997) have shown that, in contrast to the soma, ectopic SXL is tolerated in male germ cells and does not affect the normal course of spermatogenic differentiation.

We constructed a transgene that expresses the female-specific activity of Sxl in spermatocytes. The construct contains the SxlcF1 open reading frame (Bell et al., 1988) under the control
of mst59Da promoter sequences, a potent driver of male- and germline-specific transcription (Fig. 5 and H. H., unpublished results). 18 independent lines were obtained carrying a stable insertion of this construct. All lines show substantial expression of the corresponding 38 kDa SXL protein variant in testes extracts, but not in extracts prepared from the remaining carcasses, confirming the germline specificity of the mst59Da promoter (Fig. 5C). As shown by immunolocalization studies in larval testes, transgenic males produce abundant amounts of SXL protein in early primary spermatocytes (Fig. 5B). The expression is maintained into the late stages of meiotic prophase. As in the cases reported by Hager and Cline (1997), ectopic SXL activity is not sufficient to allow recombination and, thus, argues against a discriminatory role of Sxl in the sex-specific control of this process.

**DISCUSSION**

**Sxl controls two distinct processes of oogenesis**

A requirement for Sxl for normal female germline development has been demonstrated by Schüpbach (1985) who produced Sxl mutant germline clones in a wild-type ovary by pole cell transplantation. The failure of these cells to form an egg...
compromised (Table 3). Hence, the two types of mutation permit produced when the expression of normal SXL protein is germline (Schütt et al., 1998), we show that similar results are homologous chromosomes.

undergo normal synapsis, recombination and segregation of germ cells to form functional eggs, the oocyte nucleus fails to mutations. Although these suppressors now allow cystocyte divisions was relieved by dominant suppressor
differentiation takes place.

normal course of cystocyte amplification prevents the cells into one multicellular cyst. This precocious exit from the 4 cells are produced which become enveloped by follicle cells for stages in cystocyte proliferation, are consistent with
expression (Bopp et al., 1993). The temporal parallels are intriguing, and we may speculate that the early cytoplasmic expression which persists into the first rounds of cysotocyte divisions is necessary and sufficient for the formation of a 16-cell cyst and subsequent differentiation of the oocyte. If this early expression fails, as for example, in germ cells mutant for snf621 or for the ONC class of otu alleles (Bopp et al., 1993; Oliver et al., 1993), differentiation is prevented and multicellular cysts are formed. The reappearance of protein in nuclear foci of the early 16-cell cyst in wild-type ovaries and subsequent expression in differentiating cysts might then be essential for proper meiotic development. In line with this idea is the observation (Schütt et al., 1998) that early cytoplasmic expression of Sxl in the germarium is not discernibly affected in vir mutant cells, thus permitting normal cyst formation and differentiation, whereas the second phase of expression is strongly reduced or even abolished, affecting meiotic differentiation. In a preliminary report Cook et al. (1992) noticed weak but significant reduction of recombination frequencies in females with only one wild-type copy of Sxl expression in differentiating cysts might then be essential for this 'late' function.

What is the function of Sxl in the meiotic cell cycle?

Genetic evidence shows that meiotic cell division in the male germine is governed by well known regulators of the mitotic cell cycle. For instance, Dmcdc2 and the cdc25 homolog twine, components of the p34/cdc2 kinase family and the cdc25 phosphatase family, are essential for regulating the onset of the meiotic cell divisions, most likely by triggering the G2/M transition (Jiménez et al., 1990; Lehner and O’Farrell, 1990; Alphey et al., 1992; Courtot et al., 1992; Stern et al., 1993). These genes in turn are believed to be controlled by other components of the Twine class, pelota (Eberhart and Wasserman, 1995), and boule (Eberhart et al., 1996). Mutations in pelota or boule prevent execution of meiosis, but still allow a remarkable degree of (postmeiotic) spermatid differentiation. Some weaker alleles of pelota, which allow the production of sperm, cause defects in segregation and spindle formation.

### Table 4. Recombination in male germ cells expressing SXL

<table>
<thead>
<tr>
<th>Paternal genotype</th>
<th>n</th>
<th>Recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Second chromosome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p[mst-Sxl]11; cn bw/+ +</td>
<td>210*</td>
<td>0</td>
</tr>
<tr>
<td>p[mst-Sxl]113; cn bw/+ +</td>
<td>202*</td>
<td>0</td>
</tr>
<tr>
<td>p[mst-Sxl]114; cn bw/+ +</td>
<td>40*</td>
<td>0</td>
</tr>
<tr>
<td>p[mst-Sxl]122; cn bw/+ +</td>
<td>205*</td>
<td>0</td>
</tr>
<tr>
<td>B. Third chromosome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p[mst-Sxl]11; cu red sbd st el+++++</td>
<td>419§</td>
<td>0</td>
</tr>
<tr>
<td>p[mst-Sxl]113; cu red sbd st el+++++</td>
<td>283§</td>
<td>0</td>
</tr>
<tr>
<td>p[mst-Sxl]114; cu red sbd st el+++++</td>
<td>631§</td>
<td>0</td>
</tr>
<tr>
<td>p[mst-Sxl]122; cu red sbd st e+++++</td>
<td>634§</td>
<td>0</td>
</tr>
</tbody>
</table>

*Number of progeny scored from a cross to cn bw females.
§Independent lines carrying one copy of transgene on:
\[ p[mst-Sxl]11 chromosome 2 \]
\[ p[mst-Sxl]113 chromosome 3 \]
\[ p[mst-Sxl]114 chromosome 3 \]
\[ p[mst-Sxl]122 chromosome 3 \]

§Number of progeny scored from a cross to cu red sbd st e females.
The cell cycle regulators, *twine* and *pelota*, are also required in oogenesis (Alphey et al., 1992; Courtot et al., 1992; Eberhart and Wasserman, 1995). Meiotic spindles, although abnormal in appearance, do form in *twine* mutant females. However, the meiotic divisions are not arrested at metaphase I as in wild type, but continue repeatedly, leading to high frequencies of non-disjunction (White-Cooper et al., 1993). Thus, unlike the situation in males, where meiosis is completely thwarted, *twine* in females affects only certain aspects of normal progression of the meiotic cell cycle.

This behavior resembles that found in females mutant for *Sxl*+/Su(Sxl*) or *vir* where some aspects of meiosis are disturbed, but neither meiosis itself is abolished nor formation of the egg. It is thus conceivable that the ‘late’ function of *Sxl* acts in the same pathway that controls entry into meiosis, a process that we have shown to be genetically separable from cyst formation. The apparent lack of pachytene configurations in early cysts of *Sxl*+/Su(Sxl*) ovaries suggests that the meiotic function of *Sxl* may be to provide correct cues for the oocyte nucleus to enter the extended prophase of meiosis. *Sxl* may elicit these cues by regulating meiosis-promoting factors which are necessary to coordinate the transition from the mitotic to the meiotic cell cycle. A checkpoint mechanism can thus be envisioned which is responsible for this transition after four mitotic divisions. We propose that this mechanism includes *Sxl* as an important regulatory component. A complete loss of *Sxl* activity would prevent entrance into the postmitotic stage as no cues are provided to trigger the transition, a situation that is observed in *Sxl*+/Su(Sxl*) mutant ovaries. In *Sxl*+/Su(Sxl*) and in *vir* ovaries, however, the transition does take place, but may be retarded compared to wild type. Thus, the pairing and condensation defects observed during karyosome formation may be a consequence of impeded or diminished function of *Sxl* which affects the correct timing of this process. Alternatively, the process of cystocyte mitoses and entry into meiosis may not be coupled and may be controlled by different cues. In this case, the suppressor mutations may specifically rescue the *Sxl*+/Su(Sxl*) defect in cyst formation, but cannot supply the cues for correct meiotic differentiation of the oocyte.

**Meiosis in females versus males**

Differentiation of gametes and the process of generating a haploid genome follow different pathways in males and females. Differences in the architecture of the end products, eggs and sperm, and their differential contribution of nutrients and information to the next generation are traits that account for the need of distinct pathways. Less obvious is why the process of meiosis is different in males and females. In *Drosophila*, two major female-specific features stand out. First, only one cell of the 16 cells of a cyst will enter and complete meiosis, while the 15 sister cells undergo endomitosis. Thus, in contrast to the male where all cells execute the meiotic program, different fates have to be assigned to cells of the female cluster. Second, recombination between paired chromosomes only occurs in females, not in males. The differences in meiotic pathways are also in part reflected by the need for different sets of genes. For instance, coordinate control of meiotic cell cycle and spermatid differentiation is achieved by four genes, *spermatocyte arrest*, *cannonball*, *always early* and *meiosis I arrest* (Lin et al., 1996) which are not needed in oogenesis. In females, on the other hand, it has been shown that three members of the *RAD52* DNA repair pathway coordinate meiotic DNA metabolism and patterning of the oocyte (Ghabrial et al., 1998). This report now adds *Sxl* to the list of genes specifically required in the female germline for meiosis, in particular for recombination and segregation.

In view of its master-switch role in sexual development of the soma, it is conceivable that, in the germline, an active *Sxl* gene is also sufficient to impose certain female-specific traits, such as recombination. However, our results show that no recombination occurs in male germ cells expressing SXL at the premeiotic stage. It can be argued that our cDNA construct was not active early enough or that it did not express the specific protein variants needed for this process. The latter argument may be less of a concern, as it has been shown that expression of a *Sxl* transgene in the male germline can transactivate the otherwise silent endogenous gene (Hager and Cline, 1997). We consider it more likely that the lack of recombination in male germ cells is due to absence of other female-specific activities which normally participate in this process. Thus, it is possible that *Sxl* is neither the main switch for the choice of the sexual pathway of germ cells (Steinmann-Zwicky, 1992; Horabin et al., 1995; Hager and Cline, 1997) nor for female-specific traits such as recombination.

It remains to be investigated what the precise regulatory role of *Sxl* is in the germline. Not only would we like to understand how it performs this function, but also which target genes are controlled. Candidate genes are cell cycle regulators that allow transition from the mitotic phase into meiosis, and those involved in normal progression through the meiotic prophase. For decades, only two meiotic mutations were known, c(3)G (Gowen, 1928), and *ca* (Sturtevant, 1929). Since 1968, systematic searches have discovered a still growing number of new meiotic mutations (Sandler et al., 1968; Baker and Carpenter, 1972; Sekelsky et al., 1999). Testing whether germline activity of *Sxl* and *vir* is required for correct expression of these meiotic genes should eventually help to understand the genetic system regulating female meiosis and to define the position of *Sxl* in this pathway.

As this process is fundamentally different from that regulated by *Sxl* in the soma, it is conceivable that these tissue-specific functions evolved independently. It will therefore be interesting to investigate whether the germline function of *Sxl* is conserved in other dipteran insects. In line with a conserved role in this tissue is the observation that the *Sxl* homologue of *Musca domestica* is expressed in germlar germ cells (D. B., unpublished results). Of particular interest is the finding that the *Sxl* homologue in *Megaselia scalaris* is expressed only in the germline, but not in the soma of adult flies (Sievert et al., 1997). This result suggests an exclusive role in the germline of this fly. Unlike the phylogenetically recent acquisition of a sex-determining function in the soma of *Drosophila*, the germline function may thus be more widely conserved indicating a possible ancestral role of *Sxl* in germline development. Comparing expression and, whenever possible, function of this gene in other dipteran species should give insights into the evolution of the pathways regulating gametogenesis and sexual differentiation and into the mechanisms of how genes are recruited for various tasks in different developmental pathways.

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REFERENCES


