The involvement of *ovarian tumour* in the intracellular localization of Sex-lethal protein

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Abstract

The Drosophila *ovarian tumour* gene is required at multiple times in the germline for oogenesis. A second gene, *Sex-lethal*, controls sex determination in the soma and also has a separate germline function affecting similar oogenic stages as *ovarian tumour*. We demonstrate that *ovarian tumour* is not required for early *Sex-lethal* gene expression in the female germline, as had been previously reported. Instead, we provide evidence that *ovarian tumour* has a specific role in the developmentally regulated accumulation of SEX-LETHAL protein within the cytoplasm and nucleus. Furthermore, the examination of nurse cell polytene chromosomes produced by certain ovarian tumour mutations showed that SEX-LETHAL protein can associate with discrete chromosomal sites in the germline and that this pattern appears to change as the egg chamber matures. This is the first indication that SEX-LETHAL is capable of direct physical interactions with chromosomes (albeit in a mutant background) and is consistent with the developmentally regulated nuclear localization of SEX-LETHAL being important for oogenesis.

Keywords: oogenesis, *transformer-2*, *ovarian tumour*, *transformer*, *Sex-lethal*

Introduction

The *ovarian tumour* (*otu*) gene is required during several stages of female germline development (King & Riley, 1982; Storto & King, 1988). During normal oogenesis, germline stem cells undergo asymmetric divisions to form daughter stem cells and cystoblasts. These two cell types can be identified by the presence of a spherical, spectrin-rich spectrosome (Lin & Spradling, 1995). The cystoblast undergoes a set of four mitotic divisions to produce 16 cystocytes connected by ring canals through which passes the fusome, a branched derivative of the spectrosome (Lin & Spradling, 1995; Lin et al., 1994). Shortly after the formation of the 16-cystocyte cluster, the fusome disappears and the cyst becomes enveloped by the somatically derived follicle cells to form the egg chamber. 

*X/X* germ cells mutant for null *otu* alleles abort oogenesis at about the first cystoblast division (Rodesch et al., 1997). This produces either a ‘quiescent’ phenotype where ovarioles are devoid of egg chambers (but still typically contain germ cells in their germerial regions) or small *ovarian tumours* in which egg chambers are filled with hundreds of poorly differentiated germ cells (Geyer et al., 1993; Rodesch et al., 1995; Storto & King, 1988). Severe hypomorphic *otu* mutations produce larger ovarian tumours associated with arrest during later cystocyte divisions, while weaker alleles allow the formation of differentiated egg chambers arrested during late vitellogenic stages (King et al., 1986; King & Riley, 1982; Rodesch et al., 1997).

Certain mutant alleles of *Sex-lethal* (*Sxl*) can also cause ovarian tumours similar to those produced by *otu* mutations, indicating an early role in oogenesis (Perrimon et al., 1986; Salz et al., 1987). In the soma, *Sxl* controls sex determination by acting as a sex-specific regulator of RNA splicing (Bell et al., 1988; Inoue et al., 1990; Sakamoto et al., 1992; Valcârcel et al., 1993) and translation efficiency (Gebauer et al., 1998; Kelley et al., 1997). It is not known which or if either of these functions is involved in the regulation of oogenesis.

The germline-specific SXL protein displays a complex and dynamic pattern of expression and subcellular localization during oogenesis (Bopp et al., 1993). It is initially found in the cytoplasm of *X/X* stem cells and continues to accumulate until the first cystoblast division (which forms two cystocytes). This is followed by a precipitous decrease in detectable SXL levels during the subsequent three cystocyte divisions. Shortly after the formation of the 16-cell cyst, SXL re-accumulates, but this time in the nucleus. Additional intracellular shuffling of SXL protein occurs in postgerminal stages (Bopp et al., 1993). Given the somatic examples
of Sxl functioning in both the nucleus and cytoplasm, it is likely that this germline distribution pattern is important for the regulation of oogenesis.

Regulatory and functional interactions have been observed between Sxl and otu. Mutations in otu were reported to prevent the early expression of SXL protein and disrupt female-specific Sxl RNA processing, leading to suggestions that otu might regulate Sxl gene expression (Bopp et al., 1993; Oliver et al., 1993; Pauli et al., 1993). In addition, dominant, semiconstitutive Sxl alleles can improve the phenotypes of certain otu mutant combinations, leading to suggestions that Sxl may act downstream of otu in a germline genetic pathway controlling oogenesis (Bae et al., 1994; Pauli et al., 1993). However, this Sxl-induced rescue is incomplete and often allele-specific, complicating interpretations of these data. How otu might interact with Sxl has not been determined.

In this paper, we investigate how otu acts with Sxl to control oogenesis. Our studies demonstrate that otu is not necessary for early Sxl expression. Instead, otu mutations can arrest germ cell development at stages with different patterns of SXL localization, including at least one early period when SXL does not normally accumulate. Furthermore, this interaction between otu and Sxl appears specific, as conditions that allow otu mutant germ cells to undergo further oogenic differentiation do not alleviate the disruption of SXL localization. Finally, we show that SXL can bind to specific chromosomal sites in a pattern that appears to vary as the germ cells differentiate. This suggests that the regulated nuclear localization of SXL product is an essential regulator of early oogenesis.

Results

Two otu-dependent Sxl expression patterns are observed during early oogenesis

It was previously reported that the tumour-susceptible germ cells produced by otu mutations in females contained aberrantly high levels of the male-specific (non-functional) Sxl RNA splice form, leading to lower than normal amounts of SXL protein and the conclusion that otu is required for regulation of Sxl RNA splicing (Bopp et al., 1993; Oliver et al., 1993; Pauli et al., 1993). However, a complication of these studies is their dependence on partially functional otu alleles, which we have previously demonstrated to block oogenesis at a later stage than the null condition (Rodesch et al., 1997). Hence the effect of the absence of otu function on Sxl expression could only be inferred. To directly characterize the null phenotype we examined germ cells mutant for the otuP31 allele, a deletion of the entire otu coding region.

Homzygous otuP31 adult ovaries form quiescent ovarioles lacking egg chambers or, less frequently, small tumorous chambers (Geyer et al., 1993). In both phenotypes, clusters of poorly differentiated germ cells are present that can be unambiguously identified by germline-specific markers (Rodesch et al., 1995). In contrast to reports on hypomorphic otu alleles, we found that SXL protein was typically expressed and accumulated in the cytoplasm of the mutant germ cells (Fig. 1A). These results indicate that at least the initial expression of SXL protein in the germline is not dependent on otu function.

To reconcile our results with past studies, we re-examined the SXL distribution pattern in otuP32 mutants, a hypomorphic allele previously reported to greatly reduce SXL protein levels (Bopp et al., 1993). In agreement with these studies, SXL expression was reduced or absent in the germ cells of the tumorous chambers that make up the bulk of the otuP32 mutant ovary (Fig. 1B). However, we consistently found cytoplasmic SXL in the germ cells of the germarium (Figs 1–2). This was particularly evident in young ovaries (1–2 days posteclosion) where germaria were most prominent. As the mutant ovaries aged, there was a reduction in germarium size, making their detection and analysis difficult (data not shown). To confirm that the SXL-expressing cells were of germline origin, we demonstrated that they were connected by fusomes, a germline-specific structure (Fig. 1C1). Therefore, otuP32 does not prevent the initial expression of SXL in the germline as previously reported, but blocks oogenesis at a stage when SXL is mostly absent.

Figure 1. SXL distribution in otu mutant X/X ovaries. All preparations were treated with anti-SXL antibodies (Oregon Green). (A) An otuP32 tumorous chamber showing germ cells and follicle cells expressing substantial levels of cytoplasmic SXL protein (cytoplasmic SXL is observed in follicle cells during normal oogenesis, Bopp et al., 1993). (B) otuP32 tumorous chamber showing reduced levels of cytoplasmic SXL in the germ cells. (C) otuP32 mutant germarium labelled with anti-SXL (Oregon Green) and polyclonal antispectrin (Texas Red). (C1) View with Texas Red channel alone showing fusomes (arrow). (C2) Oregon Green channel alone showing expression of SXL in germ cells. Bars = 10 µm.
Interactions between sex-lethal and ovarian tumour

These results are consistent with our previous descriptions of the otu\(^{P31}\) and otu\(^{P33}\) mutant phenotypes. The otu\(^{P31}\) mutation disrupts oogenesis prior to or during the first cystoblast division (Rodesch et al., 1997) when cytoplasmic SXL levels are normally relatively high (Bopp et al., 1993; Fig. 2A). In comparison, otu\(^{P33}\) mutant germ cells show abortive development during the later cystocyte divisions (Rodesch et al., 1997), when cytoplasmic SXL levels markedly decline (Bopp et al., 1993; Fig. 2A). Therefore, otu is required during two early developmental stages that can be defined by distinctive SXL expression and localization patterns.

The involvement of otu on SXL distribution during later oogenic stages

In wild-type ovaries, SXL protein accumulates in the nurse cell cytoplasm prior to vitellogenesis, but decreases significantly by stage 8 (Bopp et al., 1993; Fig. 2B,C). The maturation of egg chambers to vitellogenic stages can be determined by several phenotypes, including the elongated shape of the egg chamber, the presence of yolk in the oocyte, and the expression pattern of the staufen gene product (STAU), which accumulates in the oocyte beginning at stage 8 and subsequently becomes anchored to the posterior pole by stage 10 (Clark et al., 1994; Giniger et al., 1993; St. Johnson et al., 1991; Fig. 3A). By these criteria, ovaries mutant for the hypomorphic otu\(^{P35}\) allele contain chambers typically reaching stages 8–9, and on rare occasions stage 10 (Fig. 3B,C). Despite this degree of egg maturation, the otu\(^{P35}\) mutant germ cells displayed high SXL levels in the cytoplasm as well as in portions of the nucleus, a pattern more consistent with previtellogenic nurse cells (Fig. 2E,F, compare with Fig. 2B).

These results indicate that not only does the effect of otu mutations on SXL localization persist into later oogenic stages, but it can also be separated from other aspects of egg chamber development. This suggests a specific interaction between the two genes. We tested this possibility by using a hypomorphic otu allele combination that can be partially suppressed by dominant, semiconstitutive alleles of Sxl (Bae et al., 1994; Pauli et al., 1993). If the interaction between otu and Sxl is specific, then the improvement in the ovarian phenotype of chambers still mutant for otu should not affect the aberrant SXL pattern.

The otu\(^{P35}\) allele is a deletion in the promoter region of otu that greatly reduces, but does not eliminate, otu function (Geyer et al., 1993). When otu\(^{P33}\) is made heterozygous with the severe otu\(^*\) allele, tumorous chambers are produced, along with a few (10–20%) more mature eggs (Bae et al., 1994). The tumorous phenotype was associated with high levels of SXL in germ cell cytoplasm, similar to that observed with otu\(^{P31}\) mutants (data not shown). In comparison, females mutant for the same otu alleles, but now carrying one copy of the semiconstitutive Sxl\(^{M2}\) allele (Sxl\(^{M2}\) otu\(^*/Sxl^{*}\) otu\(^{P35}\)), produced primarily (80–90%) egg chambers with differentiated nurse cells, and oocytes based on morphology and the incorporation of stage-specific components of the ring canals (Bae et al., 1994). In this genotype, STAU protein was expressed in the oocyte of the more mature mutant chambers, but was not
anchored to the posterior pole (Fig. 3D), providing physiological evidence for at least partial maturation to stage 8 and arrest shortly thereafter.

Once again, this improvement in oogenic differentiation did not alleviate the aberrant SXL distribution pattern. Cytoplasmic SXL remained high in the germ cells of all stages, including those in vitellogenic chambers (Fig. 2H,I). Importantly, this was also the case for the germarium, which in the suppressed genotype was typically abnormally elongated (Fig. 2G). The germarium contained apical cells with high levels of cytoplasmic SXL, indicative of stem cells and cystoblasts, as well as persistent SXL accumulation in the more distal dividing cystocytes. A similar result was often observed in otu<sup>-M</sup> germaria, although more variably as would be expected with weak hypomorphic alleles (Fig. 2D). In comparison, when Sxl<sup>M</sup> was present in an otu<sup>+</sup> background, SXL cytoplasmic levels were markedly reduced in the dividing cystocytes (Fig. 2A). Therefore, even in a genotype capable of vitellogenic differentiation, the germ cells were abnormal with respect to SXL localization both at early germarial and later vitellogenic developmental stages. Taken together, these results demonstrate that the germline distribution pattern of SXL protein reflects the level of otu activity present, regardless of the state of germ cell differentiation.

**SXL associates with discrete chromosomal sites in the developing germline**

In our studies with otu<sup>RAS</sup> we consistently observed abnormally prominent foci of nuclear SXL localization (Fig. 2G–I). otu<sup>RAS</sup> is one of several hypomorphic otu alleles that cause the formation of polytene chromosomes in the mutant nurse cells (King et al., 1981), suggesting the possibility that the SXL foci may be due to this more organized chromosomal arrangement. We examined this nuclear distribution of SXL in more detail. In the nurse cells of previtellogenic chambers, we found SXL protein concentrated within a narrow band at what appears to be a single chromosomal location (Fig. 4A,B). In more elongated chambers (indicating differentiation to stage 7), SXL is associated with multiple chromosomal sites, as well as in a spherical concentration that might be extrachromosomal (Fig. 4C,D). Therefore, SXL may undergo stage-specific changes in its localization within the nucleus.

**Sensitivity of SXL localization to increased levels of otu**

To further study the correlation between otu activity and SXL protein distribution, we examined X/X pseudomales produced by mutations in the somatic sex regulatory gene transformer-2 (tra-2). Loss of tra-2 function in X/X flies results in somatic male differentiation, producing pseudo-testes that are reduced in size and contain X/X germ cells arrested early in gametogenesis (Nöthiger et al., 1989). The germ cells are of variable phenotypes, but can be readily identified by the expression of the germline-specific VASA product (Fig. 5A). This provides an opportunity to examine the behaviour of female germ cells blocked early in development, but that are genetically wild-type for otu and the other germline-specific genes regulating oogenesis.

It was previously reported that tra-2 expression is required for the accumulation of SXL protein in the female germline (Horabin et al., 1995). However, we found that this requirement is not absolute, as 60% of the tra-2 mutant gonads examined contained at least one cluster of germ cells with detectable levels of SXL protein (Table 1A). These almost always (in 17/18 gonads) displayed cytoplasmic SXL that co-localizes with the germline-specific VASA protein (Fig. 5A). This pattern is consistent with arrest prior to the cystocyte divisions.

The levels of both Sxl and otu were simultaneously increased by the introduction of Sxl<sup>RM</sup> and a heat-shock
inducible \textit{otu} transgene (\textit{hs-otu}) to \textit{tra-2} mutant pseudomales. This resulted in a substantial increase in testis size due to the further differentiation of the germ cells (Hinson & Nagoshi, in press). This phenotypic change correlated with an increase in SXL accumulation such that 95% of gonads now contained SXL-expressing germ cells, with most now showing nuclear localization of the protein (Table 1B3, Fig. 5B). In contrast, Sxl\textsuperscript{M} or \textit{hs-otu} alone did not significantly alter the pseudomale phenotype and resulted in only a modest increase in nuclear localization, to 15% and 20% of gonads, respectively (Table 1B1–B2; Hinson & Nagoshi, in press).

A similar result was observed in the germline of \textit{X/X} pseudomales produced by mutations in another somatic sex regulatory gene, \textit{transformer (tra)}. In the presence of \textit{otu\textsuperscript{P}} only 12% of the pseudomale gonads contained germ cells with predominantly nuclear SXL localization (Table 1C1). The majority displayed no SXL expression, reflecting the high frequency of gonads lacking germ cells produced by this \textit{tra} allele combination, with most of the remainder displaying cytoplasmic accumulation (Fig. 5C). We previously showed that in the case of \textit{tra} mutants, the addition of one copy of \textit{hs-otu} alone was sufficient to cause an increase in oogenic differentiation (Hinson & Nagoshi, 1999; Nagoshi et al., 1995). This change in phenotype correlated with a high frequency of nuclear SXL localization in the germline (Table 1-II), and a close association of SXL protein with the chromatin of these cells (Fig. 5D). Taken together, we conclude that despite the different methods of arresting germ cell development, these results are consistent with those from studies on \textit{otu} mutant ovaries. Changes in \textit{otu} expression influence the pattern of cytoplasmic and nuclear localization of SXL protein within the \textit{X/X} germline.

**Discussion**

Mutations in \textit{otu} can block early oogenetic differentiation at two stages which are distinguishable by their intracellular SXL protein distributions. In the absence of \textit{otu} function,
the mutant germ cells display a cytoplasmic SXL accumulation which is consistent with morphological criteria indicating arrest at or before the first cystocyte division, while a hypomorphic *otu* allele that disrupts later cystocyte divisions results in germ cells with little cytoplasmic SXL. Similar results were obtained from experiments using *X*/*X* pseudomales. In this case, female germline development was arrested not by *otu* mutations, but by a male transformation of the soma that disrupts essential, but largely uncharacterized, soma–germline interactions. Yet even in this markedly different and non-ovarian background, increased *otu* activity was still necessary (and in the case of *tra* pseudomales sufficient) for germ cells to reach a stage where SXL becomes nuclear rather than cytoplasmic.

We considered the possibility that the aberrant SXL localization phenotype is a non-specific and secondary consequence of the arrested development caused by the *otu* mutations. If correct, then the SXL distribution pattern should correspond to the differentiated state of the arrested germ cell, such that manipulations suppressing the developmental arrest will lead to a more normal SXL expression pattern. However, this was not observed in experiments using hypomorphic and suppressed *otu* allele combinations. In each case, despite the mature morphology and physiology of the egg chambers with respect to ring canals, nurse cell differentiation, yolk protein accumulation, and the transport of STAU to the oocyte, the intracellular distribution of SXL remained aberrant and characteristic of less mature stages (Fig. 2). Therefore, the SXL distribution pattern is more dependent on the level of *otu* activity than the differentiated state of the germ cell or egg chamber.

Additional evidence comes from the *tra-2* mutant pseudomale experiments which demonstrated that an increase in *otu* and *Sxl* expression together, but not separately, causes a substantial improvement in germ cell differentiation and in the frequency of nuclear SXL localization (Table 1). These pseudomale results are analogous to those obtained from genetic epistasis studies between certain *otu* and *Sxl* alleles, which were the first indications of functional or regulatory interactions between these two genes (Bae et al., 1994; Pauli et al., 1993). The semiconstitutive *Sxl* alleles could suppress the phenotype of partially functional *otu* allele combinations, but had no effect on the most severe *otu* mutations. We interpret these findings as indicating that in *X*/*X* germ cells arrested either by *otu* mutations or somatic sexual transformation, increased levels of SXL protein can support further oogenic development only if sufficient *otu* activity is present. Therefore, an *otu*-dependent process is required to establish conditions conducive for the optimal localization and function of the germline *Sxl* product.

How the dynamic redistribution of SXL during oogenesis relates to its function in the germline is unknown. In somatic cells, *Sxl* acts in the nucleus to regulate sex-specific RNA processing, including that of its own transcript (reviewed in Cline & Meyer, 1996). A similar autoregulatory mechanism has been suggested to occur in the germline (Bopp et al., 1993; Hager & Cline, 1997), in which case a block in nuclear SXL localization by *otu* mutations should result in an increase in the male-specific splice form and a concomitant decrease in SXL protein levels. Such a phenotype has been reported for certain hypomorphic *otu* alleles (Bopp et al., 1993; Oliver et al., 1993; Pauli et al., 1993). Therefore, the shuttling of SXL between the nucleus and cytoplasm may be a critical means of regulating early oogenic stages.

An unexpected finding was that SXL protein associates with one or more discrete sites on the chromosome in a pattern that appears to change as the egg chamber matures. We did not observe a similar localization on somatic

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**Table 1. SXL protein distribution in *X*/*X* pseudomales**

<table>
<thead>
<tr>
<th>Cross</th>
<th>Genotype</th>
<th>Total gonads</th>
<th>No SXL*</th>
<th>Cytoplasmic SXL†</th>
<th>Reduced cytoplasmic with nuclear SXL‡</th>
<th>P§</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. <em>tra</em>2*/tra* 2 pseudomales:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td><em>Sxl</em> +/<em>otu</em>*</td>
<td>30</td>
<td>0.40 (12)</td>
<td>0.57 (17)</td>
<td>0.03 (1)</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td><em>Sxl</em>+/<em>otu</em></td>
<td>20</td>
<td>0.35 (7)</td>
<td>0.50 (10)</td>
<td>0.15 (3)</td>
<td>0.90–0.10</td>
</tr>
<tr>
<td>B2</td>
<td><em>Sxl</em>+/<em>hs-otu</em></td>
<td>25</td>
<td>0.52 (13)</td>
<td>0.28 (7)</td>
<td>0.20 (5)</td>
<td>0.90–0.10</td>
</tr>
<tr>
<td>B3</td>
<td><em>Sxl</em>+/<em>hs-otu</em></td>
<td>20</td>
<td>0.05 (1)</td>
<td>0.25 (5)</td>
<td>0.70 (14)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>II. <em>tra</em>/tra*3 pseudomales:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td><em>Sxl</em>+/<em>otu</em></td>
<td>25</td>
<td>0.64 (16)</td>
<td>0.24 (6)</td>
<td>0.12 (3)</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td>C2</td>
<td><em>Sxl</em>+/<em>hs-otu</em></td>
<td>25</td>
<td>0</td>
<td>0.16 (4)</td>
<td>0.84 (21)</td>
<td>&lt; 0.005‡</td>
</tr>
</tbody>
</table>

Cross A: +/+; *tra-2*/*CyO* X +/+ Y; cn *tra-2*/*bw/CyO*. Cross B: y cm *Sxl*+/*otu*† v f /FM6; *tra-2*/*CyO*; *hs-otu*/TM3 *Ser X* +B/Y; cn *tra-2*/*bw/CyO*; +/+. Cross C: *otu*†/v f /FM6; *tra-2*/*CyO* X *otu*×/v f *B/Y; cn *tra-2*/*bw/CyO*. Cross C: +/+; *tra-1*/*TM6* X FM6/Y; *tra-1*/*Kar* Y² red /TM6.

*No germ cells with detectable SXL.*
†Contains at least one cluster of germ cells with predominantly nuclear SXL localization.
‡The same *x*² probability was found for the comparison with Cross C1.
§*x*² probability using Cross A as the expected.
polyploid chromosomes derived from salivary glands (data not shown), suggesting that this phenotype is germline-specific. How these SXL-associated chromosome sites might relate to the known functions of Sxl as a regulator of both RNA splicing and transcriptional efficiency is unclear. Furthermore, because the germline polyploid chromosomes are produced in an otu mutant background, it is not known whether the same binding patterns occur in wild-type germ cells. Nevertheless, the fact that SXL can associate with chromosomes in a dynamic and developmentally specific manner provides circumstantial evidence that the nuclear localization is critical for Sxl germline function.

In addition to SXL protein, otu function is also required for the correct localization of several RNAs, including its own transcripts, during later stages of oogenesis (Tirronen et al., 1995). In particular, it was noted that oskar RNA was dependent on wild-type levels of otu for its translocation to the posterior pole of the oocyte, a process requiring microtubules (Pokrywka & Stephenson, 1995; Theurkauf et al., 1993). These observations are interesting, given other indications for an association between otu and cytoskeletal elements. For example, otu mutations affect the distribution of actin filaments during the maturation of fusomes and ring canals, and the formation of an actin cytoskeletal network necessary to anchor the nucleus of stage 10 nurse cells (Rodesch et al., 1997; Storto & King, 1988). It may be that the intracellular localization of SXL involves some aspect of the germline cytoskeleton specifically controlled by otu.

Experimental procedures

Fly strains
Flies were raised on standard cornmeal, molasses, yeast and agar media containing propionic acid as a mould inhibitor and supplemented with live yeast. Unless otherwise noted, alleles and chromosomes used are as previously described (Lindsley & Zimm, 1992). The hs-otu construct places otu function under the control of the Drosophila hsp70 promoter and has previously been described (Nagoshi et al., 1995). The rye transgene is inserted into a third chromosome that is ry and e.

otu\textsuperscript{RM1} is a deletion of the entire otu coding region (Geyer et al., 1993; Sass et al., 1993). Females that are homozygous mutant for this allele are completely sterile with a tumorous or quiescent ovary phenotype. The chromosome is marked with v and f. otu\textsuperscript{RM6} and otu\textsuperscript{RM2} are two different deletions in the otu promoter that reduce transcription levels to different degrees (Geyer et al., 1993; Sass et al., 1993). otu\textsuperscript{I} is a severe allele that produces primarily quiescent ovaries when homozygous (King & Riley, 1982; Storto & King, 1988).

Immunohistochemistry
Adults were aged 4–7 days after eclosion unless otherwise noted. Gonads were dissected in PBT (0.1% Triton X-100, 0.05% Tween 80 in PBS) for 15 min, then permeabilized overnight in blocking buffer (PBT + 1 mg/ml crystalline bovine serum albumin, Sigma) at room temperature.

All antibodies were diluted to the appropriate concentration in blocking buffer. Incubations with primary antibodies were performed overnight at 4 °C. Primaries included the following monoclonals: antixr-spectrin (3A9, 1 : 200 dilution; from the laboratory of D. Branton), anti-SXL (1 : 50 dilution; American Type Culture Collection and University of Iowa Developmental Studies Hybridoma Bank), and a polyclonal VASA antibody preparation (1 : 1000) from the laboratory of L. & Y. Jan. Primaries were removed with three 15-min washes in PBT, followed by incubation with secondary antibodies (diluted 1 : 200) for 3 h at room temperature. Secondaries used were Oregon Green or Texas Red-conjugated anti-mouse or anti-rabbit IgG (Molecular Probes). When necessary, nuclei were labelled with propidium iodide. In this case, 125 µg/ml RNase (Boehringer Mannheim) was added to the secondary antibody incubation solution. Preparations were mounted in Vectashield containing 1.5 µg/ml propidium iodide (Vector Laboratories).

STAU-specific antibodies were a gift from the laboratory of D. St. Johnston (1 : 200). Secondary antibodies used were alkaline phosphatase-conjugated anti-mouse IgG (1 : 200; Molecular Probes). Peroxidase activity was detected using a diaminobenzidine-based detection kit (Sigma). The preparation was mounted in 50% glycerol/PBS.

Microscopy and image analysis
Confocal images were obtained on a Nikon Optiphot using a Bio-Rad MRC 1024 confocal laser apparatus. Sections were manipulated using Bio-Rad LASersharp image analysis software. Other microscopy was performed on an Olympus Vanox AHB3 microscope using an Optronics LX450A camera for image capture. Figures were produced by transferring the captured images to Adobe Photoshop. All processing was performed simultaneously over the entire image. Therefore, within each plate, the relative signal intensities between different areas are as originally captured.

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