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The ovo locus is required for sex-specific germ line maintenance in Drosophila

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Mutations at the ovo locus result in a defective female germ line. The male germ line is not affected. Adult females homozygous for loss-of-function alleles have no germ line stem cells. The sex-specific phenotype is evident at late blastoderm and early gastrula stages when the pole cells of embryos homozygous for a loss-of-function allele begin to die. This is the only zygotically acting gene known that is required specifically for embryonic germ line survival. Females heterozygous for dominant alleles or homozygous for alleles reducing gene activity exhibit a range of defects in oogenesis. We have mapped the ovo locus to position 4E1-2 of the salivary gland X chromosome by using a set of cytologically visible deletions.

[Key Words: ovo; oogenesis; germ line; sexual dimorphism; Drosophila]

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The maternal-effect grandchildless mutations block pole cell formation [reviewed by Boswell and Mahowald 1985a]. These mutations are also associated with somatic defects, including fragile blastoderms, delayed migration of nuclei to the germ plasm, and lethality. Only one mutation exists for each of these loci [two alleles of paralog exist, but these may be reisolates [Thierry-Mieg 1982]] and they are not fully penetrant, suggesting that the grandchildless mutations are reduced-function alleles of genes required for viability [cf. Perrimon et al. 1986]. Pole cell formation may be more sensitive to depressed levels of gene products required for cellularization. The tudor-like class includes the maternal effect loci vasa, valois, staufen, oskar, and tudor [Boswell and Mahowald 1985b; Lehmann and Nüsslein-Volhard 1986, Schüpbach and Wieschaus 1986]. Embryos derived from females homozygous for mutations at these loci fail to form pole cells, show deletions of a variable number of abdominal segments, and may exhibit head defects, depending on the locus. The absence of pole cells in tudor-like embryos correlates with a lack of polar granules [Boswell and Mahowald 1985b; Lehmann and Nüsslein-Volhard 1986, Schüpbach and Wieschaus 1986], a prominent feature of normal germ plasm [Mahowald 1962]. Some gene products derived from these loci may be required specifically for germ line initiation because embryonic lethal and grandchildless phenotypes are separable in the cases of oskar and tudor [Boswell and Mahowald 1985b; Lehmann and Nüsslein-Volhard 1986]. However, in the case of oskar, the effect on the establishment of the germ line may be secondary to a more general effect on the anterior—posterior axis of the embryo [Schüpbach and Wieschaus 1986], suggesting that the tudor-like loci may be important for the localization

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of both somatic and germ line determinants in the posterior of the egg.

Whereas the grandchildless and tudor-like loci are required for pole cell formation, the germ line maintenance loci are required for the survival of the germ line following its establishment. The first described germ line maintenance locus, agametic, exhibits a grandchildless phenotype, but this is not due to failure to form pole cells [Engstrom et al. 1982]. The agametic maternal effect results in the selective death of the germ line in embryos after the pole cells migrate to the lateral mesoderm. We present evidence that ovo is a zygotically acting germ line maintenance locus. The ovo locus is unique among loci affecting either the establishment or maintenance of the germ line by virtue of this early gastrulation zygotic requirement. Additionally, ovo+ is required only in females. No other zygotically acting genes are known to disrupt the embryonic germ line, and ovo mutations provide evidence for germ line sexual dimorphism in the first 4 hr of embryonic development.

Results

Reversion of ovoD1

The three original ovo alleles are dominant mutations [Komitopoulos et al. 1983] that act against the ovo+ gene product [Busson et al. 1983]. Because ovo alleles are dominant and because females heterozygous for deletions of the ovo region are viable and fertile, it is possible to isolate recessive ovo alleles by screening for loss of ovoP. Previous studies have shown that spontaneous reversion of ovoD occurs in females in a background-specific manner [Busson et al. 1983]. The result of reversion was always a loss-of-function ovo allele. Loss of ovo function results in the absence of eggs. These revertants often had new mutations elsewhere on the X chromosome, suggesting that spontaneous reversion may be due to mobilization of transposable elements. Reversion to loss of ovo function frequently resulted in mutations closely linked to the ovo locus. These were typified by polyplastic recessive lethality or a lozenge-like [Izl] eye phenotype. These two phenotypes have not been separated from the ovo locus [Busson et al. 1983].

We failed to recover any spontaneous revertants of ovoD in the y f mal background that previously reverted ovoD alleles at a frequency of up to 6% [Busson et al. 1983]. One spontaneous revertant, ovoD1st, was recovered in a different background [FM3]. ovoD1st has excellent hemizygous and homozygous viability and shows no visible phenotype. Homozygous ovoD1st females never lay eggs, and ovoD1st fails to complement the female sterility of the loss-of-function ovo allele, I216C. Additionally, ovoD1st/ovoD4 females show an ovarian phenotype more extreme than that seen in ovoD4/+ females and similar to the phenotype seen in hemizygous ovoD4 females. The ovoD1st allele in these tests behaves as a deletion of the ovo locus. Based on these characteristics, ovoD1st is likely to be null.

We screened for gamma-ray-induced reversion of ovoD1 to generate new alleles and rearrangements. Seventeen distinct revertants, derived from different lines of gamma-irradiated males, were isolated. Of these, 16 are sterile when heterozygous with ovoD11st and/or I216C. All but one of the revertants, ovoD1g2, failing to complement ovoD11st and/or I216C are probably null for ovo; no eggs are laid, and the ovaries are extremely atrophic. ovoD11st/ovoD1g2 and ovoD1g2/I216C females lay defective eggs, suggesting that ovoD1g2 retains partial ovo function. One revertant, ovoD1g+G14, complements ovoD11st and the female sterility of I216C, suggesting that the reversion event restored ovo+ function. This chromosome was isolated many times in one line of mutagenized males, raising the possibility that this mutation arose spontaneously prior to mutagenesis. All of the gamma-ray-induced revertants, including ovoD1g+G14, are hemizygous lethal. This lethality is polyphasic with the exceptions of Df(1)ovoD1g2 and Df(1)ovoD1g7 males, which always die as embryos. These two chromosomes carry cytologically visible deletions [see Materials and methods] and remove hindl, a known embryonic lethal locus [Wieschaus et al. 1984]. Male revertant embryos and/or larvae exhibit the same reduction in the number of denticle belt setae shown by hemizygous shavenbaby [svb] embryos and larvae [Wieschaus et al. 1984].

Because the association of ovoD1g alleles with svb might be dependent on the mutagen utilized in the reversion screen, we screened for reversion of ovoD1 following ethylmethanesulfonate (EMS) mutagenesis. In addition, we examined EMS-induced svb alleles for ovo function. Five EMS revertants were recovered. These alleles are null for ovo, based on complementation tests with ovoD11st and I216C. Of these alleles, all but ovoD16es exhibit the svb cuticular pattern and polyphasic lethality. Male ovoD16es flies die as larvae, and ovoD16es complements svb (rarely enclosing svb males were mated to ovoD16es heterozygous females). The duplication T[1;2]rb+718, which includes ovo+ [Busson et al. 1983], does not complement the lethality of ovoD16es at 18°C [this test was done at 18°C because the T[1;2]rb+718 duplication causes temperature-sensitive male diplolethality [B. Oliver, N. Perrimon, and A. Mahowald, unpubl.]]. ovoD16es lethality is probably due to a second mutation far removed from the ovo locus.

The occurrence of svb mutations is characteristic of ovoD1 reversion. Of 24 revertants recovered, 22 exhibit a svb phenotype. We also examined three EMS-induced svb alleles generated in screens for lethal mutations [Wieschaus et al. 1984; D. Eberl and A. Hilliker, unpubl.]. Two fail to complement ovoD11st and I216C. Two female sterile mutants [fs(1)M1 and fs(1)M38 [Mohler 1977], renamed ovoDfM1 and ovoDfM2, respectively] map to the ovo region, and females heteroallelic for either of these mutations and either ovoD11st or I216C are sterile. Both of these mutations result in partial loss of ovo function, because homozygous females and hemizygous females lay defective eggs. ovoDfM2 is not associated with any visible phenotype. ovoDfM1 male and homozygous female larvae exhibit a weak svb phenotype, and adults exhibit a weak rough eye phenotype similar to either I216C or the closely linked rugose [rg] locus. Therefore, the
The ovo locus

### Table 1. Classes of ovo and svb alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>Screen</th>
<th>ovo genetics*</th>
<th>Lethal phase</th>
<th>Male germ line</th>
<th>Female germ line*</th>
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<tr>
<td><strong>Class 1. ovo− svb+</strong></td>
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<td>loss</td>
<td>larv&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>atroph</td>
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<tr>
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<td>rev</td>
<td>loss</td>
<td>via</td>
<td>fert</td>
<td>atroph</td>
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</table>

| **Class 2. ovo+ svb−**|        |               |              |                |                   |
| ovo<sup>DT+ tC</sup> | rev    | wt            | pol          | wt<sup>1</sup> | wt<sup>1</sup>    |
| svb<sup>T</sup><sup>21B</sup> | leth  | wt            | pol          | ND             |                   |

| **Class 3. ovo− svb−**|        |               |              |                |                   |
| ovo<sup>DM1</sup>    | fs     | red           | via          | fert           | perm              |
| ovo<sup>DMG2</sup>   | rev    | loss          | pol          | ND             | atroph            |
| ovo<sup>DMG3</sup>   | rev    | loss          | pol          | ND             | atroph            |
| ovo<sup>DMG4</sup>   | rev    | loss          | pol          | ND             | atroph            |
| In(1)ovo<sup>DMG5</sup> | rev  | loss          | pol          | ND             | atroph            |
| Df(1)ovo<sup>DMG6</sup> | rev | loss          | emb<sup>b</sup> | ND       | atroph            |
| Df(1)ovo<sup>DMG7</sup> | rev | loss          | emb<sup>b</sup> | ND       | atroph            |
| ovo<sup>DC31</sup>   | rev    | loss          | pol          | fert           | atroph            |
| ovo<sup>DC32</sup>   | rev    | loss          | pol          | fert           | atroph            |
| ovo<sup>DC33</sup>   | rev    | loss          | pol          | fert           | atroph            |
| ovo<sup>DC34</sup>   | rev    | loss          | pol          | fert           | atroph            |
| ovo<sup>DC35</sup>   | rev    | loss          | pol          | fert           | atroph            |
| ovo<sup>DC36</sup>   | rev    | loss          | pol          | fert           | atroph            |
| ovo<sup>DC37</sup>   | rev    | loss          | emb<sup>b</sup> | ND       | atroph            |
| svb<sup>DC38</sup>   | leth   | loss          | emb<sup>b</sup> | ND       | atroph            |
| svb<sup>DC39</sup>   | leth   | loss          | emb<sup>b</sup> | ND       | atroph            |

* Screens for: Dominant female sterile mutations [Fs], recessive female sterile mutations [fs], reversion of Fs(1)ovo<sup>DA</sup> [rev]. ovo genetics: Mutant gene activity acts in opposition to the wild-type gene activity [opp], gene activity is reduced [red], gene activity is not detectable [loss], wild type [wt]. Lethal phase: die as embryos [emb], die as larvae [larv], die as embryos, larvae, pupae or adults [pol], viable [via]. Male germ line: Fertile [fert], spermatocytes visible in third instar larvae [wt<sup>1</sup>], not determined (ND). Female germ line: No egg chambers [atrophi], no vitellogenic egg chambers [arrest], eggs are permeable [perm], wild type [wt].

* Genetics and ovarian phenotypes of lethal alleles are based on the phenotype over ovo<sup>DA</sup> and/or Iz<sup>L</sup>.

* No germ line cells visible in histological sections.

* Lethality is probably due to a second mutation elsewhere on the X chromosome (see text).

* Viable in germ line clones.

* Embryonic lethality is due to the removal of a large block of the X chromosome (see text).

* Shows a weak hind<sup>sight</sup> phenotype, probably a second mutation or deletion.

* Lethal in germ line clones.

dual isolation of ovo and svb mutations is not restricted to reversion.

The alleles of ovo and svb have been arranged into the following classes: (1) ovo− svb+, (2) ovo+ svb−, and (3) ovo− svb− [Table 1]. Class 1 alleles include the original three dominant mutations, a recessive ovo allele isolated in a screen for female sterile mutations, and three loss-of-function revertants of ovo<sup>DA</sup>. We have included only Iz<sup>L</sup> in class 1; however, other ovo− Iz<sup>L</sup> alleles have been reported [Busson et al. 1983]. These alleles may represent another class. Class 2 alleles include the revertant of ovo<sup>DA</sup> with restored ovo<sup>+</sup> function associated with loss of svb function and one of the three svb alleles isolated as lethal mutations. Class 3 alleles include nearly all of the reversion events, two alleles originally isolated as lethals, and one mutant recovered in a screen for female sterility. All but two of the class 3 alleles are null with respect to ovo.

**Morphology of ovo ovaries**

The ovaries of Drosophila are composed of a number of tubes known as ovarioles, which connect with the ovi-
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Oviducts (King 1970; Mahowald and Kambsellis 1980). Both of these structures are derived from somatic cell lines. During normal oogenesis, the germ line stem cells are located in the anterior tip of the ovarioles. These divide to generate new stem cells and a multicellular cyst. Fifteen of the germ line cells in each cyst become polyploid and serve as nurse cells for the sixteenth cell, which goes on to form the oocyte. A major oocyte growth phase occurs as a result of yolk protein uptake and transfer of the nurse cell cytoplasm. Nurse cells, oocyte, and the surrounding layer of follicle cells constitute an egg chamber. Follicle cells deposit the vitelline and chorionic layers of the egg.

All ovo mutations have effects on the adult female germ line. An extreme phenotype is seen in ovo^{D1S1}/ovo^{D1S1} females [Fig. 1a] and in females heterozygous for ovo^{D1S1} and other loss-of-function alleles. The somatically derived ovarian structures appear normal, but no egg chambers are visible in the adult ovary. An intermediate phenotype is seen in the ovaries of ovo^{D1+} females [Fig. 1b] and in many of the ovarioles of ovo^{D1+} [Fig. 1c] and ovo^{M1}/ovo^{M1} females [Fig. 1d]. A few normal-appearing early egg chambers are formed, but they degenerate prior to vitellogenesis. Vitellogenic eggs are never seen in ovo^{D1+} females, but a few egg chambers in ovo^{D2+} and ovo^{M1}/ovo^{M1} females proceed into vitellogenesis. These eggs are laid but have gross abnormalities in the anterior chorion. Occasionally, more than 15 nurse cells are seen in ovo^{D2+} females [Busson et al. 1984]. In females heterozygous for ovo^{D2} or ovo^{D3} and reduced-function ovo alleles (ovo^{D1S2}, ovo^{M1}, and ovo^{M2}), oogenesis is blocked [not shown].

Figure 1. Ovarian phenotypes of ovo females. Phase-contrast micrographs of ovaries dissected from 6-day-old ovo females. (a) ovo^{D1S1}/ovo^{D1S1}: The ovarioles of these females are atrophic. The diameters of the ovarioles are slightly larger than the oviducts [d] at the posterior end of the ovaries. No egg chambers are visible in the ovarioles [o], and germ line cells are not visible in the germarium [g] at the anterior end of the ovary. The threadlike structures are trachea [t]. (b) ovo^{D1+}: These ovarioles are reduced in size relative to wild type. Egg chambers [ec] form but degenerate in the posterior regions of the ovarioles [region below the arrows]. No vitellogenic eggs are seen. (c) ovo^{D2+}; (d) ovo^{M1}/ovo^{M1}: The ovarioles are reduced in size relative to wild type. The egg chambers usually degenerate, but vitellogenic eggs [v] are seen in a few ovarioles. The external egg layers surrounding the oocytes [ö] often have gross abnormalities, such as open-ended chorion [arrow in 1c]. (e) ovo^{D2+}; (f) ovo^{M2}/ovo^{M2}. These ovarioles overlap wild type in size.
These females have egg chambers with more than 15 nuclei showing nurse cell nuclear morphology and/or tumorous egg chambers. The weakest phenotype results in completed, but defective, oogenesis. The ovaries of ovoD3/+ [Fig. 1e] and ovoM2/ovoM2 [Fig. 1f] females overlap those of wild-type females in appearance but lay a reduced number of eggs. These eggs are permeable to neutral red, which is taken up primarily through the anterior pole. ovoD1r23/ovoD1r31 females have the same phenotype as ovoD3/+ and ovoM2/ovoM2 females [not shown].

In summary, the severity of the recessive ovo mutant phenotypes from most to least extreme can be ranked as follows: ovoD1r31/deletion = ovoD1r31/ovoD1r31 = ovoD1r31/class 3 loss-of-function alleles >ovoM1/ovoM1 >ovoM2/ovoM2 = ovoD1r31/ovoD1r23.

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Figure 2. The germ cells of svb males appear normal. We were unable to test many of the ovo alleles for male fertility because of the lethal effect of svb. Males from some of these stocks were dissected, and the gonads were examined while living by phase-contrast microscopy. Germ line cells were found in all males examined [at least five males for each stock]. Whole mounts are shown in the top panels: [a] Ore R/Y; [b] ovoD1+G12/Y. Squashes are shown in lower panels: [c] Ore R/Y; [d] ovoD1r23/Y; [e] ovoD1r31/Y.
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The ovo male germ line is normal

Occasional adult males were recovered from class 3 lethal stocks, and these flies were fertile, provided they survived long enough to mate. Because we were unable to recover males from many of the ovo mutant stocks that were also svb−, it is possible that these alleles disrupt the male germ line. The svb− male third instar larvae were identified by the cuticle phenotype and dissected. Although the overall size of the svb− testis is consistently smaller (Fig. 2b) than those of wild-type males (Fig. 2a), for every genotype examined, normal appearing germ line cells were found in the male gonad (Fig. 2b,d,e). The slight reduction in size of the svb− male gonads may reflect the "poor health" of such individuals and not a specific effect of ovo mutations.

Female ovo activity is required during gastrulation

Because ovoD1st1/ovoD1st1 females show no signs of germ line derivatives in the adult gonad, we examined embryos for germ line inactivation. Embryos from a cross of ovoD1st1/FM3 females and ovoD1st1/Y males were examined while living. For an X-linked sex-specific mutation, 25% of the embryos should have the mutant phenotype. During early stages of gastrulation when pole cells are visible above the presumptive anterior midgut invagination, 32 of 156 embryos [21%] had either no pole cells or a reduced number of pole cells. To confirm that embryos showing a reduction in pole cell number were of the presumed genotype, living embryos were examined, sorted, allowed to develop, and scored as adults in a separate experiment. A total of 95 embryos were examined during early gastrulation. Of these, four [4%] had no pole cells and developed into sterile ovoD1st1 homozygotes; 18 [19%] had fewer than 10 pole cells, 12 developed into sterile ovoD1st1 homozygous females [2 died]. The remaining embryos were scored incorrectly as either ovoD1st1 males or heterozygous females. The efficiency of picking the mutant females was 73% [16/22].

Late blastoderm to early gastrula embryos from crosses generating ovoD1st1/ovoD1st1 females were examined by scanning electron microscopy to determine whether the reduction in the number of pole cells in ovoD1st1 homozygotes was due to the failure to form pole cells or pole cell loss. Pole cells form in all embryos. However, during blastoderm and early gastrulation, pole cells of some embryos begin to exhibit the surface "blebbing" characteristic of cell death (Fig. 3a). During early gastrulation, some embryos had a few [Fig. 3b] or no pole cells [Fig. 3c].

The data shown in Figure 3 are summarized in Figure 4. The number of pole cells per embryo in wild-type embryos, Ore R strain, varies from 23 to 52, with a mean of 37 [± 15] [Turner and Mahowald 1976]. Because the number of pole cells per embryo from the cross of ovoD1st1/FM3 females to ovoD1st1/Y was 23.6 ± 7.0 at cellular blastoderm [Fig. 4a], we examined the embryos derived from the cross of ovoD1st1/Ore R females to ovoD1st1/Y males. The mean number of pole cells per embryo at cellular blastoderm was 37.4 ± 7.7 [Fig. 4c]. At early gastrulation, the mean pole cell number per embryo was decreased for both crosses, and the standard deviation increased [Fig. 4b,d]. This was due to a decrease in the number of pole cells in an embryo subpopulation, comprising approximately 25% of the total.

The pole cells of homozygous ovo females continue to die during embryogenesis. Late embryos (16–18 hr) from the cross of ovoD1st1/Ore R females to ovoD1st1/Y males

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Figure 3. The ovo− female germ line is lost during gastrulation. Scanning electron micrographs of early gastrulation embryos derived from heterozygous ovoD1st1 females mated with ovoD1st1 males. (a) Pole cells of a presumptive ovoD1st1 homozygous female. Some pole cells appear normal [n] but one pole cell shows the surface blebbing characteristic of cell death [d]. Bar represents 10 μM. (b) A presumptive ovoD1st1 homozygous female embryo with only 13 pole cells. The pole cells have normal morphology and are located in the region destined to form the posterior mid-gut invagination [arrow]. Bar represents 100 μM (b,c).
were serially sectioned (not shown). Of 20 embryos examined, 75% (15/20) had normal-appearing embryonic gonads complete with germ line cells. The remaining 25% of the embryos had either no germ line cells (2/20) or gonads that included dead polar cells (3/20).

**Genetics of the ovo region**

The three *ovo* alleles have been previously mapped to the 10-band region deleted by both *Df(1)Rc40* and *Df(1)C70* [Busson et al. 1983]. By utilizing a set of preexisting deletions and three rearranged *ovo* revertants, we have mapped *ovo*Δ11, *ovo*Δ1, *ovo*Δ2, and *ovo*Δ3 more precisely. Five deletions and the inversion *In(1)ovo*Δ457 breaking in or near the 4E region are shown in Figure 5. All of these rearrangements result in a *svb* phenotype in hemizygous embryos. Two deletions, *Df(1)bi*Δ2 and *Df(1)bi*Δ3, retain *ovo* function and fail to complement *svb* (using the rare *svb* males that reach adulthood), indicating that *ovo* and *svb* functions can be separated genetically. Interestingly, the reduced-function allele *ovo*Δ11Δ23 is not complemented by *Df(1)bi*Δ2, suggesting that this *ovo* function is in the same region as the *svb* function. We did not recover enough *ovo*Δ11Δ23 males to test all of the rearrangements. The *rg* locus is removed by all the deletions of the *ovo* locus but not by *In(1)ovo*Δ457. The rearrangements, *Df(1)ovo*Δ65, *Df(1)ovo*Δ176, and *In(1)ovo*Δ457 have break points within the 4E region of the salivary gland X chromosome. *Df(1)bi*Δ2 and *Df(1)bi*Δ3 also break in 4E or just distal to 4E. The 4E region contains only three bands. The first two appear as a doublet, and the third, as a faint band. *Df(1)ovo*Δ65 and *Df(1)ovo*Δ176 remove the doublet and leave the heavy 4F1 band intact, and *In(1)ovo*Δ457 breaks in the doublet. These data indicate that the *ovo* locus is probably located in 4E1-2.

Because of the complex association of *ovo* with *svb* and *lz1* phenotypes, we tested for interallelic complementation between the viable alleles, *ovo*Δ11, *lzl*Δ1, *ovo*Δ1, and *ovo*Δ2, and the lethal alleles. *ovo*Δ1 complements the remaining two partial loss-of-function alleles, *ovo*Δ2 and *ovo*Δ3. It is not clear whether this interallelic complementation defines different *ovo* functions, based on the limited number of alleles where complementation testing was possible. However, this example of interallelic complementation and the different location of *ovo*Δ1 in revealed by deletion mapping suggests that *ovo* may code for more than one gene product. Un-
Figure 5. Genetic map of the ovo region. Rearrangements breaking in the 4E1–4F1 region of the salivary gland X chromosome are shown in the upper portion. This region contains four bands. Deletions are shown as bold horizontal lines. Vertical bars at the ends of deletion lines indicate that the extent of the deletion does not remove the next proximal complementation group tested. Arrows at the ends of the deficiency lines indicate that the deletion extends into other complementation groups. The inversion In(1)ovoD12S is shown as a triangle. Relevant break points are also given [see Materials and methods for other break points]. In the lower portion of the diagram, tested complementation groups are shown. Male viable ovo alleles and r9 were tested by complementation. ovo complementation refers only to the female sterility phenotype, not the svb phenotype. svb was tested by examination of hemizygous embryos, except for Df(1)ovoD12S and Df(1)b2D, which were tested by examination of male embryos and by complementation. Failed complementation is indicated by the junction of the vertical lines from the complementation groups and the rearrangements.

fortunately, further analysis of the majority of the ovo alleles (e.g., lethal class 2 and 3 alleles) was hindered by lack of a suitable duplication.

Discussion

The germ plasm of Drosophila is characterized by a putative localized cytoplasmic determinant (Mahowald 1962). Polar granules and associated RNAs may be the active substance in specifying germ line determination. Germ plasm containing these structures is capable of both rescuing UV-irradiated germ plasm and the alteration of nuclear fates at ectopic locations [Illmensee and Mahowald 1974]. Two lines of evidence suggest that the formation of pole cells, the first overt commitment to the germ line, is not sufficient for long-term germ line stability. First, although modified in ultrastructural appearance, the germ line-specific polar granules are present following pole cell formation, during migration, during proliferation, and up to the onset of terminal differentiation of the germ cells [Mahowald 1971]. Additionally, RNAs that are able to rescue pole cell formation in UV-irradiated embryos are insufficient for fertility. These germ line cells die [Togashi et al. 1986]. Second, mutations at either the agametic or ovo locus result in death of the germ line, following the initial segregation of those cells from the soma. It seems likely that germ line maintenance requires the action of genes responding to an initial determinant.

ovo is required early in development

Newly formed ovo− pole cells are lost progressively beginning at early gastrulation, as viewed either by scanning electron or light microscopy. Interestingly, pole cell death begins prior to the general activation of the pole cell genome [Zalokar 1976; Allis et al. 1979]. The early phenotype seen in the absence of zygotic ovo+ activity suggests that ovo+ may be one of the first genes activated in the zygotic germ line. Lack of a punctual time for the death of all the pole cells in ovo− females may be due to residual gene activity from the ovoD1s5 allele or depletion of a maternally derived product.

Disruption of terminal differentiation of oocytes due to dominant ovoD product acting against ovo+ or partial loss of ovo function indicates that ovo+ is also required for proper oogenesis. The ovo+ gene product could have two distinct periods of action. However, clonal analysis of ovoD alleles has shown that removal of the dominant mutation at any time in development rescues the mutant phenotype, suggesting that ovo+ expression is constitutive in the female germ line [Perrimon 1984].

Because ovo+ is required for maintenance of the female germ line, it is difficult to determine whether ovo+ is required maternally for the formation of pole cells in the progeny. In the typical genetic screen for mutations affecting pole cell formation, homozygous mutant females must be fertile. If components of the germ plasm required for pole cell formation are also required for the maintenance of the homozygous female germ line, then many mutations affecting pole cell establishment could have been overlooked. We are examining other mutations that result in agametic females to determine whether other members of the germ line maintenance class of loci affect pole cell establishment or viability. This class of mutations may be the best place to look for genes affecting the germ plasm.

ovo may be genetically complex

The association of ovo and svb mutations is striking. The simultaneous appearance of these two phenotypes occurs independently of the mutagen or screening protocol. For example, two of three svb− alleles generated in EMS-induced lethal screens are ovo− and svb−ovo− chromosomes have been isolated by gamma-ray and EMS-induced reversion of ovoD1. Previously isolated spontaneous revertants of ovoD alleles have also been associated with closely linked, fully or partially penetrant lethality in males, or a lzl phenotype. In the lethal revertant lines that yielded some males, the phenotype was characterized by lightly curved bristles and unsuprad wings [Busson et al. 1983]. These males may have been svb− because this is similar to the phenotype we have
observed in the few class 2 and 3 males that eclosed.
Further suggestion of complexity within the ovo locus is provided by the interallelic complementation between the partial loss-of-function allele ovo~M2 and the other two partial loss-of-function alleles, ovo~P15G23 and ovo~M2, and the more distal location of ovo~P15G23 compared with the remaining mapped alleles. If the ovo locus codes for a complex array of gene products, svb~ and ovo~ may be more related biochemically than indicated by the phenotypes. Although these data suggest that ovo is a complex locus, a number of other causes may be postulated. For example, (1) preferential induction of small deletions in this region, consistent with the imprecise excision of transposable element, could be removing both ovo and svb; (2) position effects at one locus could be influencing the expression of the other; (3) overlapping ovo and svb transcriptional units and/or regulatory sequences could both be removed by point mutations. Further molecular and genetic analysis of ovo and svb should enable us to distinguish between these models.

The ovo requirement is female specific

Mutant alleles of ovo result in female germ line defects, and no alleles affect male fertility. This includes the few males that have been recovered from the ovo~ svb~ stocks. The germ line function of ovo~ is probably exclusively female. This suggests that ovo~ may be involved in germ line processes analogous to early somatic sex-specific functions. A clear hierarchy has been established for somatic sex determination, in which Sex-lethal (Sxl) responds to the chromosomal sex to regulate the branch point of the sex determination and dosage compensation pathways [Cline 1984; Maine et al. 1985]. Sex determination in germ cells is different.

Female or male pole cells transplanted into hosts of the opposite sex do not function [Van Deusen 1976]. In the case of male pole cells transplanted into females, this is likely to be due to germ cell death [Schüpbach 1985]. Even though the germ line chromosomal sex must match the chromosomal sex of the soma (X/X germ line in an X soma or an X germ line in a X soma), loss of most sex determination gene functions downstream from Sxl has no effect on the germ line [Marsh and Wieschaus 1978; Schüpbach 1982; Belote and Baker 1983]. Homozygous Sxl pole cells transplanted to female hosts fail to complete oogenesis; ovarian tumors are produced instead of 15 nurse cells and 1 oocyte. Triploid intersex germ lines yield a similar phenotype [Schüpbach 1985]. Neither of these genetic constitutions appear to affect the viability of embryonic germ line cells, only the differentiation of stem cells appears to be altered.

Because removal of any of the known sex determination genes is less deleterious to the germ line than having the wrong chromosomal sex, it seems likely that genes exist that play an early and perhaps primary role in germ line sex determination. The pole cells of ovo~ females begin to die at about the same time that somatic sex becomes determined [Sanchez and Notherger 1982]. Are ovo~ pole cells more “male-like,” and if so, why

should they die in female embryos? Transplantation data indicate that germ line survival is nonautonomous. Germ line cells must be provided with the appropriate sexual environment. Loss of ovo~ activity may result in germ line/somatic line incompatibility. The female somatic cells may be actively killing the more “male-like” pole cells of ovo~ females, or the ovo~ pole cells may not be capable of receiving sexually appropriate signals. It is also possible that ovo suppresses germ line dosage compensation. In Drosophila, the expression of at least some X-linked genes is up-regulated in males such that the levels are equivalent in X and XX individuals. If ovo affects germ line dosage compensation, then X-linked expression in X/X pole cells could be boosted. Overexpression of X-linked genes could result in germ line cell lethality, as is the case for somatic cells [Lucchesi and Stripsky 1981]; however, it is unclear whether dosage compensation is required in the early germ line [Schüpbach 1985].

Conclusions

The ovo locus is required for early female germ line maintenance but does not appear to be required for male germ line stability. A number of new ovo alleles have been identified from previous female sterile screens, lethal screens, and a reversion screen for ovo~. These include the first examples of partial loss-of-function ovo mutations. The first manifestation of the female sterile phenotype, in case of a loss-of-function allele, is seen shortly after pole cell formation, making ovo the only zygotically acting gene known to be involved in maintenance of the embryonic germ line. Further, ovo may be required throughout female germ line development. This may include maternal ovo expression required for zygotic germ line determination. Study of the ovo locus and other loci affecting germ line maintenance may provide a tool for working back toward the primary signal for the segregation of the germ and somatic lines, and the study of the ovo locus may lead to a better understanding of sex-specific germ line requirements.

Materials and methods

Stocks

Visible mutants and balancer stocks are described in Lindesley and Grell [1968]. vg was obtained from the Bowling Green stock center. The dominant female sterile mutations F5(1)ovoD1 (= F5(1)K1237), F5(1)ovoD2 (= F5(1)K1103), and F5(1)ovoD3 (= F5(1)K155) are marked with vg and were maintained in C(1)DXy f Y stocks [Komitopoulou et al. 1983]. The recessive female sterile mutations F5(1)M1 (= F5(1)ovoM1) and F5(1)M2 (= F5(1)ovoM2) are marked with y v f and balanced with FM0 [Mohler 1977]. Revertants of F5(1)ovoD2 were maintained in FM7a stocks, with the exception of F5(1)ovoD1st, which was maintained in a FM3 or C(1)DXy f Y stock. l(1)svb7D39 and l(1)svb7D39 are marked with w and balanced with FM7 [Wieschaus et al. 1984]. l(1)svb7D39 was obtained from E. Gehr and A. Hilliker. IzP1, a spontaneous revertant of ovoD1, was obtained from M. Samuels and M. Gans. The w v l(1)44P/FM3/BfY stock was used for collecting virgins [Komitopoulou et al. 1983]. Rearrangements, cytology, and references are as fol-
Both spontaneous and induced recessive alleles of ovo were isolated by screening for the loss of the dominant sterility phenotype associated with ovo<sup>PI</sup>. Two protocols were used to screen for reversion. In the first protocol, ovo<sup>PI</sup>/EM5/Y males were mated to virgin y v f mal / y f mal females. The progeny were mated, and the gravid females were recovered. These potential revertant females could be fertile for three reasons: (1) loss of ovo<sup>PI</sup>; (2) spontaneous mitotic recombination between ovo<sup>PI</sup> and the centromere; or (3) maternal nondisjunction. To distinguish between these possibilities, the phenotypes of the progeny of potential revertants were examined, and only females producing both y<sup>+</sup> and y<sup>+</sup> progeny were saved. In the second protocol, ovo<sup>PI</sup>/EM5/Y males were mated to virgin w v l(1)4A4/FM3 females. Because FM3/Y males die, only females eclosed. These virgin females were mated to FM7a/Y males. As in the first protocol, the progeny of gravid females were examined. Only females whose progeny included y<sup>+</sup> flies were saved. Both protocols were used in screens for spontaneous reversion; the second protocol was used for mutagenesis screens.

New ovo alleles were induced by either gamma-rays or EMS mutagenesis. Gamma-irradiation (4000–5000 rad) was delivered using a 60Co source. EMS mutagenesis was performed according to Lewis and Bacher [1968]. The EMS doses used resulted in a 40–60% lethal X-linked mutation frequency, as assayed by mating a subset of mutagenized and nonmutagenized males to sets of C1/1DX; y f females and comparing the sex ratio of the progeny derived from mutagenized versus nonmutagenized male parents. The parental and mutagenic origin of each revertant is indicated in the superscript of each mutant name: D1rS = spontaneous revertant of ovo<sup>PI</sup>, D1rG = gamma-ray-induced revertant of ovo<sup>PI</sup>, D1r + G = gamma-ray-induced ovo<sup>+</sup> revertant of ovo<sup>PI</sup>, and D1rE = EMS-induced revertant of ovo<sup>PI</sup>. The number of chromosomes screened in each mutagenesis is as follows: spontaneous (y v f mal background) = 1500, spontaneous (FM3 background) = 30,000, gamma-ray (FM3 background) = 28,000, EMS (FM3 background) = 3500. The salivary gland polytene chromosomes of revertants numbered ovo<sup>PI</sup>/ovo<sup>PI</sup> were examined for visible rearrangements.

**Genetic analysis**

Each recessive female sterile or visible mutation was tested for complementation with deficiencies or other mutations. Although complementation testing is not possible for the dominant alleles of ovo [ovo<sup>PI</sup>], these mutations can be mapped by taking advantage of the more extreme phenotype observed when ovo<sup>PI</sup> is trans to other ovo alleles or deficiencies uncovering the ovo locus [Busson et al. 1983]. Deletions were tested for by scoring hindgut and/or sub functions by examining the embryonic cuticles of hemizygous males. Germ line clones were produced by gamma-irradiation of ovo<sup>PI</sup> heterozygotes, as described by Perrimon [1984].

**Phenotypic examination**

Cuticle phenotypes were evaluated by collecting and mounting embryos according to van der Meer [1977]. These embryo whole mounts were examined microscopically under phase-contrast and dark-field illumination. For all deletion mapping experiments involving the ovo locus, ovaries were dissected into phosphate-buffered saline (PBS) solution and examined under a dissecting microscope. Adult ovaries and third instar larval testes, again dissected in PBS, were also examined by phase-contrast microscopy. Ovaries from females heterozygous for dominant alleles, homozygous for partial-loss-of-function alleles, or homozygous for the loss-of-function allele ovo<sup>PI</sup> were fixed overnight in 2% glutaraldehyde at 4°C, embedded in plastic, and sectioned. The ovaries of females heterozygous for dominant and partial-loss-of-function ovo alleles were either sectioned as above or stained with Hoechst 33258 (1 μg/ml in PBS) and examined by epifluorescence microscopy. Blastoderm and gastrula embryos were prepared for scanning electron microscopy, as described by Turner and Mahowald [1976]. Living embryos were examined and sorted in fluorocarbon oil, as described by Wieschaus and Nüsslein-Volhard [1986].

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