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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 commitment of blastomeres to germ cell lineages in many organisms is associated with the segregation of a distinct germ plasm into presumptive germ line blastomeres (reviewed by Beams and Kessel 1974; Boswell and Mahowald 1985a). In insects, UV-irradiation of germ plasm results in sterility (Okada et al. 1974; von Brunn and Kalthoff 1983). The germ plasm is both necessary and sufficient for the initiation of complete *Drosophila* germ line development. UV-irradiated *Drosophila* germ plasm can be functionally rescued by the transfer of nonirradiated germ plasm (Okada et al. 1974), and transfer of germ plasm to an ectopic site results in the production of functional germ line cells at the new location (Illmensee and Mahowald 1974; Niki 1986).

Karyokinesis is not accomplished by cytokinesis during early *Drosophila* development (Zalokar and Erk 1976; Foe and Alberts 1983). The germ plasm, at the posterior tip of the embryo, is the first cytoplasm to become cellularized to produce pole cells (Rabinowitz 1941). Pole cells migrate into the mesoderm after gastrulation to form, by association with somatic cells, the embryonic gonads. The germ line gonadal cells proliferate during larval development and establish a stem cell population during the pupal stage (King 1970; Wieschaus and Szabad 1979). There are a large number of mutations that result in male or female sterility, but most of these affect the terminal differentiation of spermatocytes or oocytes. Only a few mutations are known that prevent or disrupt early germ line development. These mutations can be grouped into three classes; grandchildless, tudor-like, and germ line maintenance.

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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

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 ovo^{D1}

The three original *ovo* alleles are dominant mutations (Komitopoulou et al. 1983) that act against the ovo^+ gene product (Busson et al. 1983). Because ovo^D 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 ovo^D. Previous studies have shown that spontaneous reversion of ovo^D 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 polyphasic recessive lethality or a *lozenge-like* (*lzl*) 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 ovo^D in the *y v f mal* background that previously reverted ovo^D alleles at a frequency of up to 6% (Busson et al. 1983). One spontaneous revertant, ovo^{D1rS1}, was recovered in a different background (FM3). ovo^{D1rS1} has excellent hemizygous and homozygous viability and shows no visible phenotype. Homozygous ovo^{D1rS1} females never lay eggs, and ovo^{D1rS1} fails to complement the female sterility of the loss-of-function *ovo* allele, lzl^G. Additionally, ovo^{D1rS1}/ovo^{D3} females show an ovarian phenotype more extreme than that seen in $ovo^{D3}/+$ females and similar to the phenotype seen in hemizygous ovo^{D3} females. The ovo^{D1rS1} allele in these tests behaves as a deletion of the *ovo* locus. Based on these characteristics, ovo^{D1rS1} is likely to be null.

We screened for gamma-ray-induced reversion of ovo^{D1} 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 ovo^{D1rS1} and/or lzl^G. All but one of the revertants, ovo^{D1rG23}, failing to complement ovo^{D1rS1} and/or lzl^G are probably null for *ovo*; no eggs are laid, and the ovaries are extremely atrophic. ovo^{D1rG23}/ovo^{D1rS1} and ovo^{D1rG23}/lzl^G females lay defective eggs, suggesting that ovo^{D1rG23} retains partial *ovo* function. One revertant, ovo^{D1+G14}, complements ovo^{D1rS1} and the female sterility of lzl^G, 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 ovo^{D1+G14}, are hemizygous lethal. This lethality is polyphasic with the exceptions of $Df(1)ovo^{D1rG6}$ and $Df(1)ovo^{D1rG7}$ males, which always die as embryos. These two chromosomes carry cytologically visible deletions (see Materials and methods) and remove *hindsight*, 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 ovo^{D1rG} alleles with *svb* might be dependent on the mutagen utilized in the reversion screen, we screened for reversion of ovo^{D1} 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 ovo^{D1rS1} and lzl^G. Of these alleles, all but ovo^{D1rE5}, exhibit the *svb* cuticular pattern and polyphasic lethality. Male ovo^{D1rE5} flies die as larvae, and ovo^{D1rE5} complements *svb* (rarely enclosing *svb* males were mated to ovo^{D1rE5} heterozygous females). The duplication $T(1;2)rb^{+71g}$, which includes ovo^+ (Busson et al. 1983), does not complement the lethality of ovo^{D1rE5} at 18°C [this test was done at 18°C because the $T(1;2)rb^{+71g}$ duplication causes temperature-sensitive male diplolethality (B. Oliver, N. Perrimon, and A. Mahowald, unpubl.)]. ovo^{D1rE5} lethality is probably due to a second mutation far removed from the *ovo* locus.

The occurrence of *svb* mutations is characteristic of ovo^{D1} 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 ovo^{D1rS1} and lzl^G. Two female sterile mutants [*fs(1)M1* and *fs(1)M38* (Mohler 1977), renamed ovo^{rM1} and ovo^{rM2}, respectively] map to the *ovo* region, and females heteroallelic for either of these mutations and either ovo^{D1rS1} or lzl^G are sterile. Both of these mutations result in partial loss of *ovo* function, because homozygous females and hemizygous females lay defective eggs. ovo^{rM2} is not associated with any visible phenotype. ovo^{rM1} male and homozygous female larvae exhibit a weak *svb* phenotype, and adults exhibit a weak rough eye phenotype similar to either lzl^G or the closely linked *rugose* (*rg*) locus. Therefore, the

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

Allele	Screen	*ovo* genetics[a]	Lethal phase	Male germ line	Female germ line[a]
Class 1. *ovo*$^-$ *svb*$^+$					
ovo^{D1}	Fs	opp	via	fert	arrest
ovo^{D2}	Fs	opp	via	fert	perm
ovo^{D3}	Fs	opp	via	fert	perm
ovo^{rM2}	fs	red	via	fert	perm
ovo^{D1rS1}	rev	loss	via	fert	atroph[b]
ovo^{D1rE5}	rev	loss	larv[c]	ND	atroph
lzl^{G}	rev	loss	via	fert	atroph
Class 2. *ovo*$^+$ *svb*$^-$					
ovo^{D1+G14}	rev	wt	pol	wt?	wt
svb^{YP17b}	leth	wt	pol	ND	wt[d]
Class 3. *ovo*$^-$ *svb*$^-$					
ovo^{rM1}	fs	red	via	fert	perm
ovo^{D1rG2}	rev	loss	pol	ND	atroph
ovo^{D1rG3}	rev	loss	pol	ND	atroph
ovo^{D1rG4}	rev	loss	pol	ND	atroph
$In(1)ovo^{D1rG5}$	rev	loss	pol	ND	atroph
$Df(1)ovo^{D1rG6}$	rev	loss	emb[e]	ND	atroph
$Df(1)ovo^{D1rG7}$	rev	loss	emb[e]	ND	atroph
ovo^{D1rG8}	rev	loss	pol	fert	atroph
ovo^{D1rG9}	rev	loss	pol	ND	atroph
ovo^{D1rG10}	rev	loss	pol	ND	atroph
ovo^{D1rG11}	rev	loss	pol	wt?	atroph
ovo^{D1rG12}	rev	loss	pol	ND	atroph
ovo^{D1rG19}	rev	loss	pol	fert	atroph
ovo^{D1rG20}	rev	loss	pol	fert	atroph
ovo^{D1rG21}	rev	loss	pol	fert	atroph
ovo^{D1rG23}	rev	red	pol	fert	perm
ovo^{D1rG25}	rev	loss	pol	wt?	atroph
ovo^{D1rE1}	rev	loss	pol	ND	atroph
ovo^{D1rE2}	rev	loss	pol	ND	atroph
ovo^{D1rE3}	rev	loss	pol	ND	atroph
ovo^{D1rE4}	rev	loss	pol	fert	atroph
ovo^{D1rE6}	rev	loss	pol	wt?	atroph
svb^{EH587}	leth	loss	emb[f]	ND	atroph
svb^{YD39}	leth	loss	pol	ND	atroph[g]

Screens for: Dominant female sterile mutations (Fs), recessive female sterile mutations (fs), reversion of $Fs(1)ovo^{D1}$ (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?), not determined (ND). Female germ line: No egg chambers (atroph), no vitellogenic egg chambers (arrest), eggs are permeable (perm), wild type (wt).

[a] Genetics and ovarian phenotypes of lethal alleles are based on the phenotype over ovo^{D1rS1} and/or lzl^{G}.

[b] No germ line cells visible in histological sections.

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

[d] Viable in germ line clones.

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

[f] Shows a weak *hindsight* phenotype, probably a second mutation or deletion.

[g] 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^{D1}. We have included only lzl^{G} in class 1; however, other *ovo*$^-$ *lzl*$^-$ alleles have been reported (Busson et al. 1983). These alleles may represent another class. Class 2 alleles include the revertant of ovo^{D1} with restored *ovo*$^+$ 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-

ducts (King 1970; Mahowald and Kambysellis 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^{D1rS1}/ovo^{D1rS1} females (Fig. 1a) and in females heterozygous for ovo^{D1rS1} 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 $ovo^{D2}/+$ (Fig. 1c) and ovo^{rM1}/ovo^{rM1} 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^{rM1}/ovo^{rM1} 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^{D1rG23}, ovo^{rM1}, and ovo^{rM2}), oogenesis is blocked (not shown).

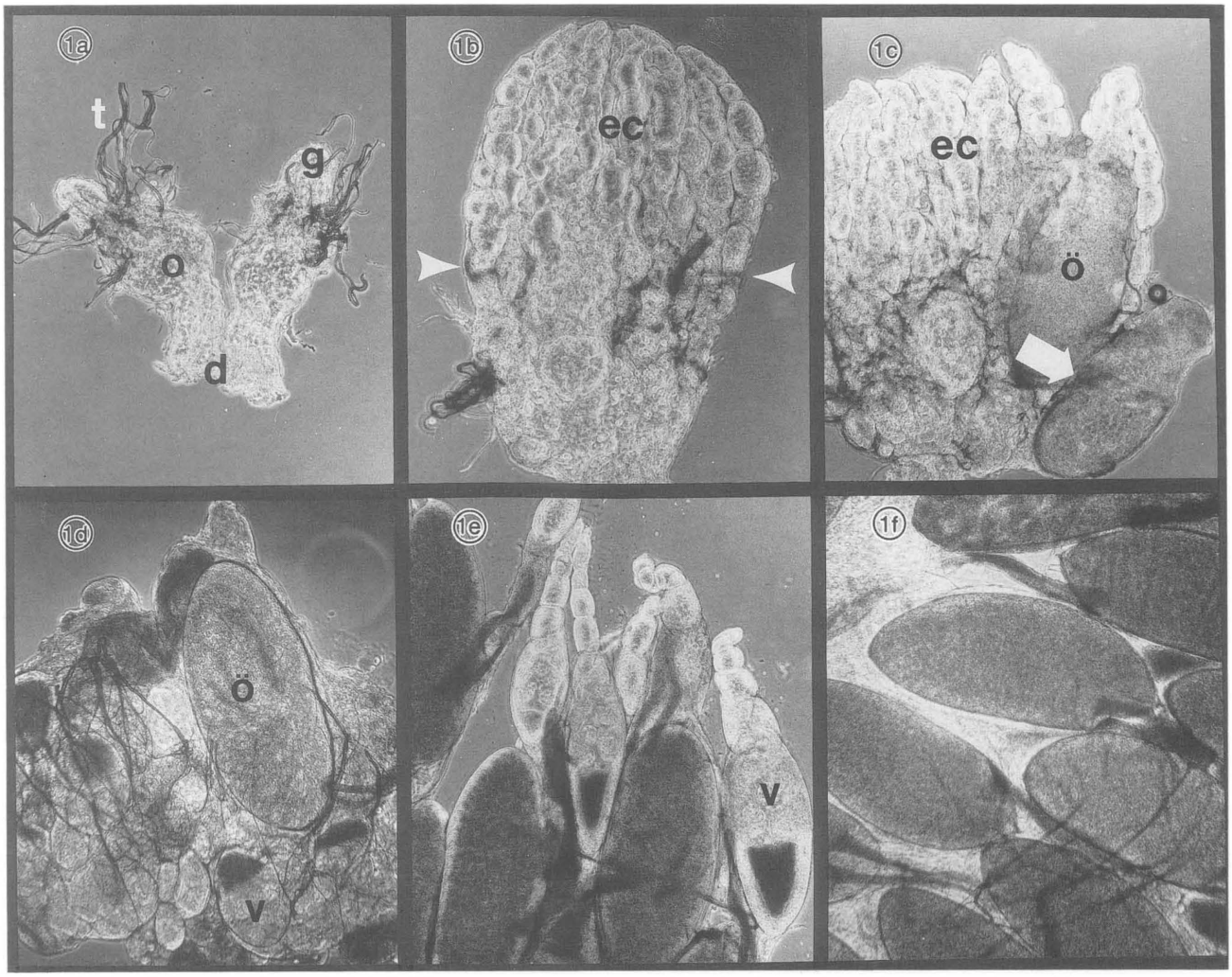

Figure 1. Ovarian phenotypes of *ovo* females. Phase-contrast micrographs of ovaries dissected from 6-day-old *ovo* females. (*a*) ovo^{D1rS1}/ovo^{D1rS1}: The ovaries of these females are atrophic. The diameters of the ovaries are slightly larger than the oviducts (d) at the posterior end of the ovaries. 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 ovaries are reduced in size relative to wild type. Egg chambers (ec) form but degenerate in the posterior regions of the ovaries (region below the arrows). No vitellogenic eggs are seen. (*c*) $ovo^{D2}/+$; (*d*) ovo^{rM1}/ovo^{rM1}: The ovaries 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*) $ovo^{D3}/+$; (*f*) ovo^{rM2}/ovo^{rM2}. These ovaries overlap wild type in size.

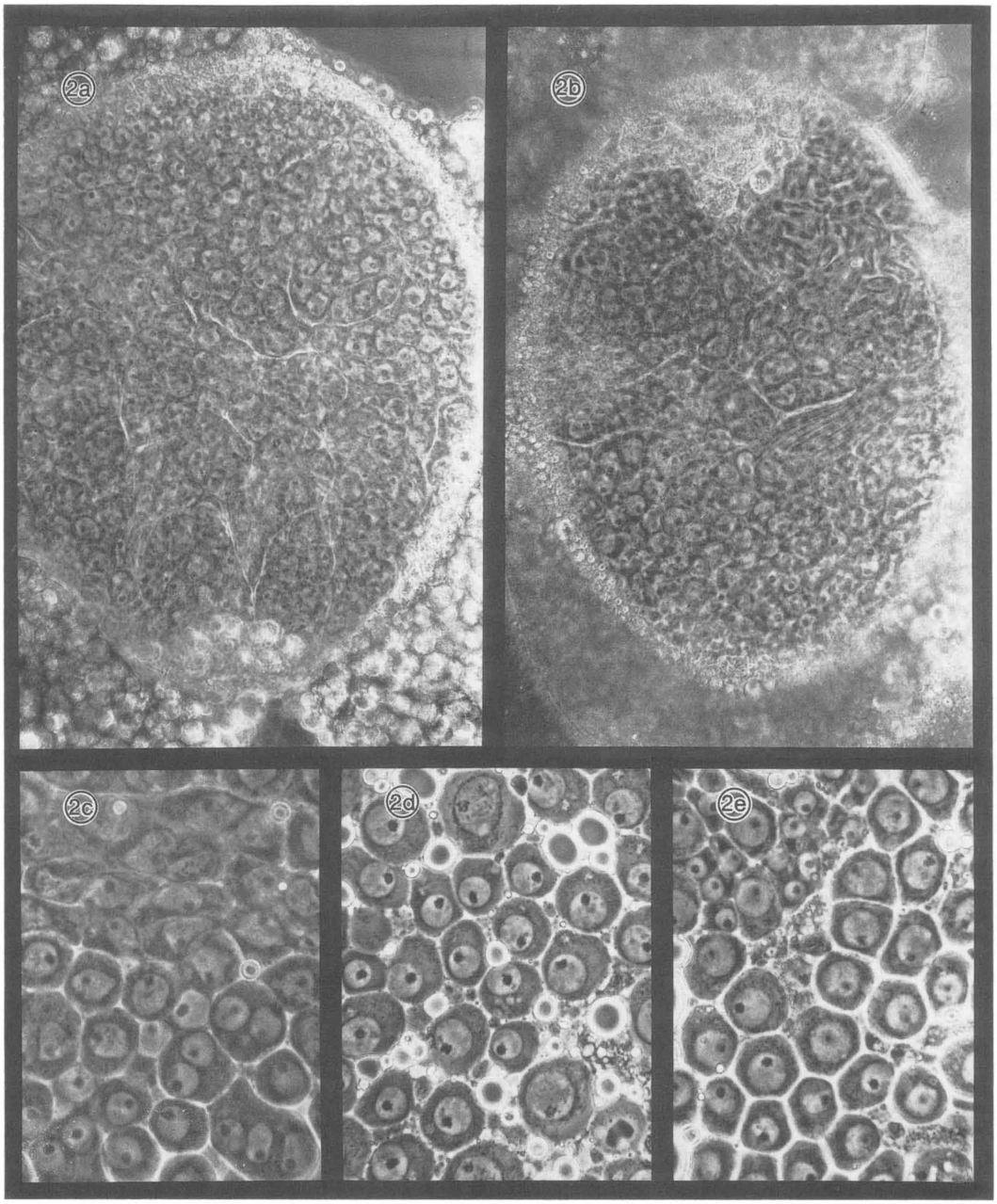

Figure 2. The germ cells of *svb* males appear normal. We were unable to test many of the *ovo* alleles for male fertility because of the lethal effect of *svb*. Males from some of these stocks were dissected, and the gonads were examined while living by phase-constrast microscopy. Germ line cells were 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*) ovo^{D1+G14}/Y. Squashes are shown in lower panels: (*c*) Ore R/Y; (*d*) ovo^{D1rG25}/Y; (*e*) ovo^{D1rE6}/Y.

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 $ovo^{D3}/+$ (Fig. 1e) and ovo^{rM2}/ovo^{rM2} (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. ovo^{D1rG23}/ovo^{D1rS1} females have the same phenotype as $ovo^{D3}/+$ and ovo^{rM2}/ovo^{rM2} females (not shown).

In summary, the severity of the recessive *ovo* mutant phenotypes from most to least extreme can be ranked as follows: ovo^{D1rS1}/deletion = ovo^{D1rS1}/ovo^{D1rS1} = ovo^{D1rS1}/class 3 loss-of-function alleles $>ovo^{rM1}/ovo^{rM1}>ovo^{rM2}/ovo^{rM2} = ovo^{D1rS1}/ovo^{D1rG23}$.

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 ovo^{D1rS1}/ovo^{D1rS1} females show no signs of germ line derivatives in the adult gonad, we examined embryos for germ line defects. Embryos from a cross of $ovo^{D1rS1}/FM3$ females and ovo^{D1S1}/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 posterior 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 ovo^{D1rS1} homozygotes; 18 (19%) had fewer than 10 pole cells, 12 developed into sterile ovo^{D1rS1} homozygous females (2 died). The remaining embryos were scored incorrectly as either ovo^{D1rS1} males or heterozygous females. The efficiency of picking the mutant females was 73% (16/22).

Late blastoderm to early gastrula embryos from crosses generating ovo^{D1rS1}/ovo^{D1rS1} females were examined by scanning electron microscopy to determine whether the reduction in the number of pole cells in ovo^{D1rS1} 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 (n = 15) (Turner and Mahowald 1976). Because the number of pole cells per embryo from the cross of $ovo^{D1rS1}/FM3$ females to ovo^{D1rS1}/Y was 23.6 ± 7.0 at cellular blastoderm (Fig. 4a), we examined the embryos derived from the cross of $ovo^{D1rS1}/Ore\ R$ females to ovo^{D1rS1}/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 ovo^{D1rS1}/Ore R females to ovo^{D1rS1}/Y males

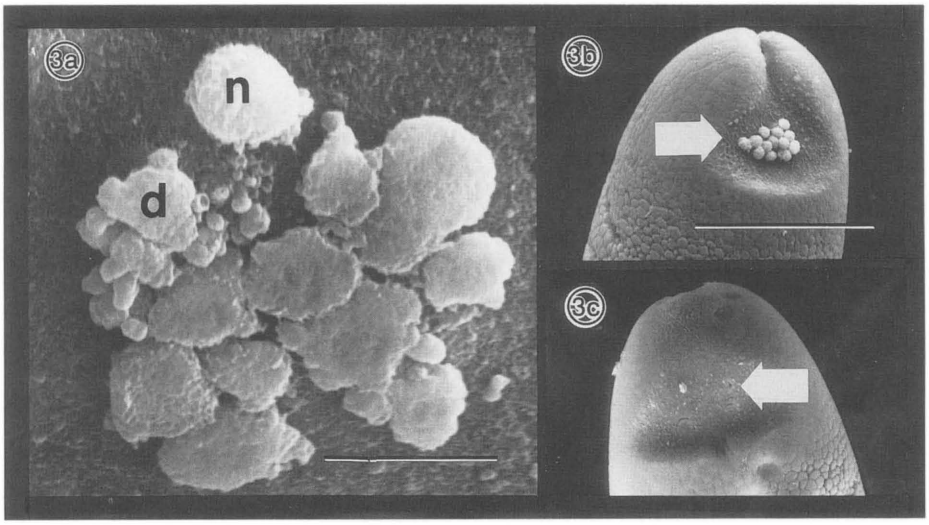

Figure 3. The ovo^- female germ line is lost during gastrulation. Scanning electron micrographs of early gastrulation embryos derived from heterozygous ovo^{D1rS1} females mated with ovo^{D1rS1} males. (*a*) Pole cells of a presumptive ovo^{D1rS1} homozygous female. Some pole cells appear normal (n) but one pole cell shows the surface blebbing characteristic of cell death (d). Bar represents 10 μM. (*b*) A presumptive ovo^{D1rS1} 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). (*c*) A presumptive ovo^{D1rS1} homozygous female embryo with no pole cells in the region destined to form the posterior mid-gut invagination (arrow). Bar represents 100 μM (*b,c*).

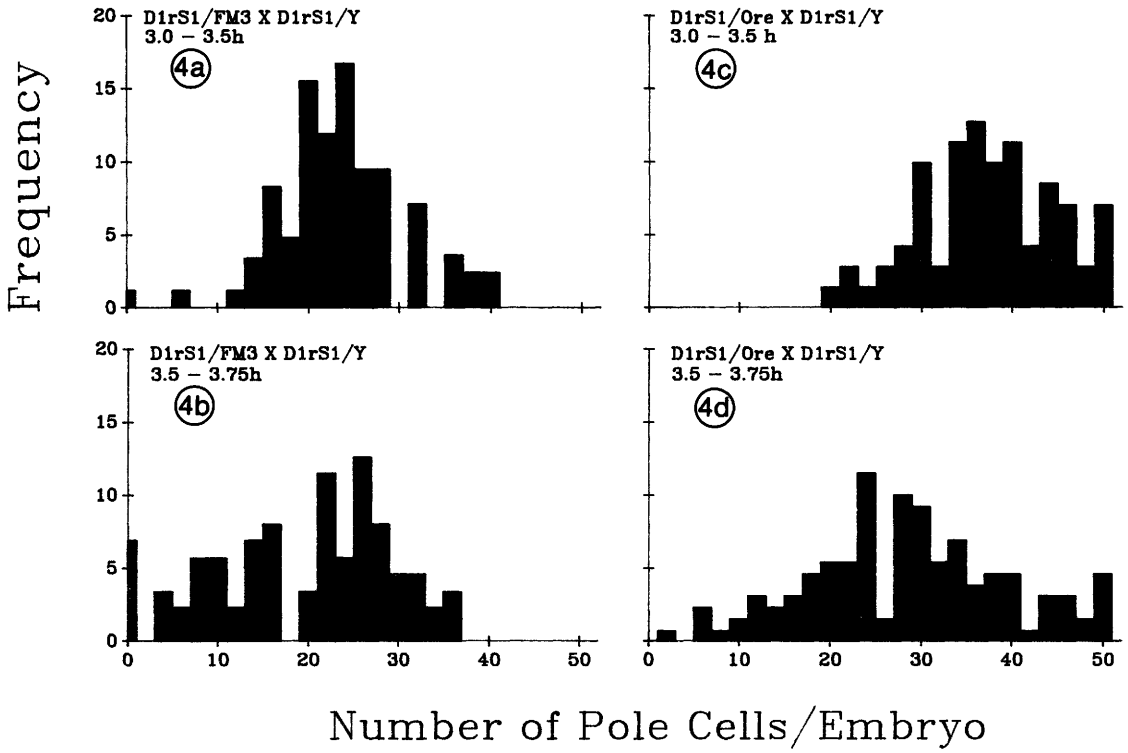

Figure 4. *ovo* is required prior to completion of gastrulation. Embryos were collected and prepared for scanning electron microscopy, as described in Materials and methods. The number of pole cells per embryo has been plotted against the frequency members of that class where seen. The number of pole cells per embryo has been rounded to the next even number. The crosses generating the scored embryos and the age of the embryos scored are shown in the upper left corner of each panel. For each panel, 80–150 embryos were scored. The number of pole cells in crosses generating homozygous ovo^{D1rS1} females is normal during cellular blastoderm (3.0–3.5 hr), but during early gastrulation (3.5–3.75 hr), a subpopulation of embryos have a reduced number of pole cells. (*a*) ovo^{D1rS1} v^{24}/*FM3* × ovo^{D1rS1} v^{24}/*Y* at cellular blastoderm; (*b*) ovo^{D1rS1} v^{24}/*FM3* × ovo^{D1rS1} v^{24}/*Y* at early gastrulation; (*c*) ovo^{D1rS1} v^{24}/*Y* at cellular blastoderm; (*d*) ovo^{D1rS1} v^{24}/Ore R × ovo^{D1rS1} v^{24}/*Y* at early gastrulation.

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 pole cells (3/20).

Genetics of the ovo *region*

The three ovo^{D} alleles have been previously mapped to the 10-band region deleted by both *Df(1)RC40* and *Df(1)JC70* (Busson et al. 1983). By utilizing a set of preexisting deletions and three rearranged ovo^{D1} revertants, we have mapped ovo^{D1rS1}, ovo^{rM1}, ovo^{rM2}, and ovo^{D3} more precisely. Five deletions and the inversion $In(1)ovo^{D1rG5}$ 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, $Df(1)bi^{D2}$ and $Df(1)bi^{DL5}$, 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^{D1rG23} is not complemented by $Df(1)bi^{D2}$, suggesting that this *ovo* function is in the same region as the *svb* function. We did not recover enough ovo^{D1rG23} males to test all of the rearrangements. The *rg* locus is removed by all the deletions of the *ovo* locus but not by $In(1)ovo^{D1rG5}$. The rearrangements, $Df(1)ovo^{D1rG6}$, $Df(1)ovo^{D1rG7}$, and $In(1)ovo^{D1rG5}$ have break points within the 4E region of the salivary gland X chromosome. $Df(1)bi^{DL5}$ and $Df(1)bi^{D2}$ 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. $Df(1)ovo^{D1rG6}$ and $Df(1)ovo^{D1rG7}$ remove the doublet and leave the heavy 4F1 band intact, and $In(1)ovo^{D1rG5}$ 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 *lzl* phenotypes, we tested for interallelic complementation between the viable alleles, ovo^{D1rS1}, lzl^{G}, ovo^{rM1}, and ovo^{rM2}, and the lethal alleles. ovo^{rM1} complements the remaining two partial loss-of-function alleles, ovo^{rM2} and ovo^{D1rG23}. 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^{D1rG23} revealed by deletion mapping suggests that *ovo* may code for more than one gene product. Un-

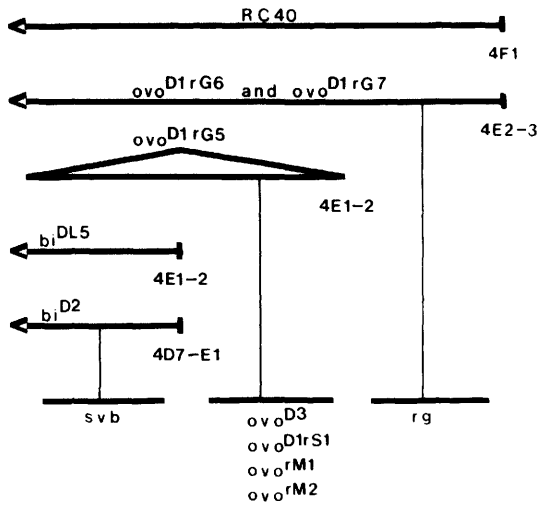

Figure 5. Genetic map of the *ovo* region. Rearrangements breaking in the 4E1–4F1 region of the salivary gland X chromosome are shown in the upper portion. This region contains four bands. Deletions are shown as bold horizontal lines. Vertical bars at the ends of deletion lines indicate that the extent 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)ovo^{D1rG5}* 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 *rg* 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)ovo^{D1rG6}* and *Df(1)bi^{D2}*, 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 *ovo^{D1rS1}* allele or depletion of a maternally derived product.

Disruption of terminal differentiation of oocytes due to dominant *ovo^D* 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 *ovo^D* 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 effecting 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 *ovo^{D1}*. Previously isolated spontaneous revertants of *ovo^D* 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 unspread 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^{rM1} and the other two partial loss-of- function alleles, ovo^{D1rG23} and ovo^{rM2}, and the more distal location of ovo^{D1rG23}, 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 a X/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 Nothiger 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 incompatability. 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^{D1}. 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 Lindsley and Grell (1968). *rg* was obtained from the Bowling Green stock center. The dominant female sterile mutations $Fs(1)ovo^{D1}$ (= *Fs(1)K1237*), $Fs(1)ovo^{D2}$ (= *Fs(1)K1103*), and $Fs(1)ovo^{D3}$ (= *Fs(1)K155*) are marked with v^{24} and were maintained in *C(1)DX;y f/ Y* stocks (Komitopoulou et al. 1983). The recessive female sterile mutations *fs(1)M1* (= $fs(1)ovo^{M1}$) and *fs(1)M2* (= $fs(1)ovo^{M2}$) are marked with *y cv v f* and balanced with *FM0* (Mohler 1977). Revertants of $Fs(1)ovo^{D1}$ were maintained in *FM7a* stocks, with the exception of $fs(1)ovo^{D1rS1}$, which was maintained in a *FM3* or *C(1)DX;y f/ Y* stock. $l(1)svb^{YD39}$ and $l(1)svb^{YP17b}$ are marked with *w* and balanced with *FM7* (Wieschaus et al. 1984). $l(1)svb^{EH587}$ was obtained from D. Eberl and A. Hilliker. lzl^G, a spontaneous revertant of ovo^{D1}, was obtained from M. Samuels and M. Gans. The *w v* $l(1)44^{ts}$/*FM3*/B^sY stock was used for collecting virgins (Komitopoulou et al. 1983). Rearrangements, cytology, and references are as fol-

lows: *Df(1)bi^{DL5}* = Df(1)3C7-12;4E1-2, *Df(1)bi^{D2}* = Df(1)4B6; 4D7-E1 (Banga et al. 1986); *Df(1)RC40* = Df(1)4B1;4F1, *T(1;2)rb^{+71g}* = T(1;2)3F3;5E8;23A15 (Craymer and Roy 1980); *Df(1)ovo^{D1rG6}* = Df(1)4C5-6;4E2-3, *Df(1)ovo^{D1rG7}* = Df(1)4C5-6;4E2-3, *In(1)ovo^{D1rG5}* = In(1)4E1-2;5A1-6 (this study). Flies were grown on standard *Drosophila* medium. Egg, embryo, and larvae collections were made on agar molasses media supplemented with yeast. Except where indicated, flies were grown at 25 ± 0.5°C.

Reversion of ovo^{D1}

Both spontaneous and induced recessive alleles of *ovo* were isolated by screening for the loss of the dominant sterility phenotype associated with *ovo^{D1}*. Two protocols were used to screen for reversion. In the first protocol, *ovo^{D1}v^{24}*/Y males were mated to virgin *y v f mal*/*y v 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^{D1}*; (2) spontaneous mitotic recombination between *ovo^{D1}* 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^- and y^+ progeny were saved. In the second protocol, *ovo^{D1}*v^{24}/Y males were mated to virgin *w v l(1)44^{ts}*/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 v^- 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 ^{60}Co 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 *C(1)DX; 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^{D1}*, D1rG = gamma-ray-induced revertant of *ovo^{D1}*, D1r + G = gamma-ray-induced *ovo$^+$* revertant of *ovo^{D1}*, and D1rE = EMS-induced revertant of *ovo^{D1}*. 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^{D1rG2}*–*ovo^{D1rG11}* 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^D*), these mutations can be mapped by taking advantage of the more extreme phenotype observed when *ovo^D* is *trans* to other *ovo* alleles or deficiences uncovering the *ovo* locus (Busson et al. 1983). Deletions were tested for loss of *hindsight* and/or *svb* functions by examining the embryonic cuticles of hemizygous males. Germ line clones were produced by gamma-irradiation of *ovo^{D1}* 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^{D1rS1}* 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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