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Molecular Genetics of the *Drosophila melanogaster ovo* Locus, a Gene Required for Sex Determination of Germline Cells

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ABSTRACT

The *Drosophila melanogaster ovo* gene is required for survival and differentiation of female germline cells, apparently playing a role in germline sex determination. We recovered 60 kb of genomic DNA from its genetic location at 4E1,2 on the *X* chromosome. A transcription unit coding for an apparently female-specific germline-dependent 5-kb poly(A)$^+$ RNA size class is located substantially in a 7-kb region, within which three DNA-detectable lesions for mutations that inactivate the *ovo* function are located at two sites ≈4 kb apart. The breakpoint of a deficiency that removes the neighboring lethal complementation group *shavenbaby* (*svb*) but leaves the *ovo* function intact maps ≈5 kb to the molecular left of the leftmost *ovo* mutant site. A class of mutations that inactivates both the *svb* function and the *ovo* function affects genomic DNA between the two *ovo* sites. Sequences required for the two genetic functions are partly overlapping. In spite of this overlap, *P* element-mediated gene transfer of a 10-kb genomic DNA segment containing the 5-kb poly(A)$^+$ RNA transcription unit rescues the female sterility phenotypes of *ovo* mutations, but not the *svb* lethality.

GREAT progress has been made in understanding the genetic mechanisms responsible for somatic sex determination in the laboratory fruit fly *Drosophila melanogaster*. The *X* chromosome-to-autosome balance, or *X*:A ratio, determines sex in Drosophila (Bridges 1916, 1925). Female Drosophila possess two *X* chromosomes, and males a single *X* chromosome and a *Y* chromosome; the *Y* chromosome is required only for the completion of sperm differentiation (Bridges 1916) (reviewed by Lindsley and Tokuyasu 1980). Each somatic cell measures the *X*:A ratio early in development, stably determines its sex in a cell-autonomous manner, and stably sets the appropriate level of *X* chromosome gene transcription (reviewed by Baker and Belote 1983; Baker 1989). *X*:A ratio assessment is at least partly dependent on the relative amounts of two DNA-binding proteins coded by the *X*-linked *sis-b* (*scute* T4) gene (Parkhurst, Bopp and Ish-Horowicz 1990) and the second-chromosomal *daughterless* (*da*) gene (Cline 1976; Cronmiller, Schedl and Cline 1988). Once the *X*:A ratio is assessed, the *Sex-lethal* (*Sxl*) gene is activated in 2*X*:2A female embryos, and its mRNAs are translated into a set of RNA-binding proteins (Bell *et al.* 1988; Bopp *et al.* 1991). Their function is to activate a gene regulatory cascade operating at the level of alternative RNA splicing (Baker and Wolfner 1988; McKeown, Belote and Boggs 1988; Nagoshi *et al.* 1988; Bell *et al.* 1991). The effector of the cascade is the bifunctional *doublesex* (*dsx*) gene (Baker and Ridge 1980), which codes for two related DNA-binding proteins (Burtis and Baker 1989; Burtis *et al.* 1991), one male-specific, the other female-specific, which exert opposite effects on sexual differentiation. Thus, in the somatic cells of Drosophila, regulation of transcription and of alternative RNA splicing acts to establish and to maintain sexual identity.

In contrast to this detailed knowledge of the somatic sex-determination pathway, relatively little is known about germline sex determination (reviewed by Pauli and Mahowald 1990). Pole cell transplantation experiments showed that the somatic sex-determination loci *da* (Cronmiller and Cline 1987), *transformer*, *dsx* and *intersex* are not required for germline sex determination (Marsh and Wieschaus 1978; Schüpbach 1982, 1985). *Sxl*, however, is required in female germ cells. Some *Sxl* mutations are female-sterile; females homozygous for these mutations, as well as females carrying germline cells homozygous for lethal mutations of the *Sxl* locus, fail to produce eggs (Schüpbach 1985; Salz, Cline and Schedl 1987). Measurement of the *X*:A ratio in germline cells does not result in germ-cell-autonomous sexual differentiation. Chromosomally male germ cells die in chromosomally female host embryos, and vice versa, when the host embryos possess functional germ cells (Van Deusen 1976; Steinmann-Zwicky, Schmid and Nöthiger 1989). In addition, somatic cells can alter germline

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sexual identity and differentiation through the action of a signal or signals of unknown biochemical nature (STEINMANN-ZWICKY, SCHMID and NÖTHIGER 1989).

Another participant in the sex dimorphism of germline cells is the *X* chromosome *ovo* locus (polytene bands 4E1,2). Females homozygous for apparent null mutations of *ovo* (ovo^-) are sterile because germline cell death begins during gastrulation, resulting in ovaries completely lacking germ cells (OLIVER, PERRIMON and MAHOWALD 1987). These ovo^- mutations have no detectable effect in the male germline or in somatic tissues. Females homozygous for partial loss-of-function mutations or heterozygous for dominant antimorphic mutations (ovo^D) are also sterile; the germline cells survive, however, and populate the developing ovary, only to fail in oogenesis (BUSSON *et al*.. 1983; OLIVER, PERRIMON and MAHOWALD 1987). In certain genotypes the *ovo*-defective germline cells adopt a morphology that resembles male germline cells (OLIVER, PAULI and MAHOWALD 1990). The ovarian phenotype of ovo^D heterozygotes can be modified by mutations at other loci, including several of the genes known to be involved in somatic cell sex determination (OLIVER, PAULI and MAHOWALD 1990). X-ray-induced mitotic recombination in the female germline demonstrated that the requirement for *ovo* function is germline cell autonomous (PERRIMON and GANS 1983; PERRIMON 1984). Thus, by morphological and genetic criteria, *ovo* mutations disrupt a process active only in female germline cells, and the results of the disruption include cell death during embryogenesis, failure to complete oogenesis, or a germline sex transformation.

An additional class of *ovo* mutant chromosomes exists that is homozygous- and hemizygous-lethal by virtue of the loss of the *shavenbaby* (*svb*) function. The *shavenbaby* phenotype was named because of the defect in ventral denticle belts and dorsal hairs observed in cuticle preparations of late embryos (WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984); it is a polyphasic lethal, and occasionally mutant males survive to adulthood. The *svb* escaper males develop slowly, have thin bristles, rough eyes, and dusky wings, and yet are fertile. The comutability of the *ovo* function and the *svb* function is independent of the mutagen and screen used. For example, ethyl methanesulfonate (EMS) mutagenesis of a wild-type *X* chromosome, followed by a screen for recessive lethal mutations, has yielded mutant chromosomes chosen on the basis of *svb* lethality which are female-sterile when heterozygous with viable *ovo* tester chromosomes (OLIVER, PERRIMON and MAHOWALD 1987). Likewise, in screens for loss of the dominant female sterility caused by the ovo^{D1} mutation, P-M hybrid dysgenesis, EMS, or γ-rays all yield mutant chromosomes which have become ovo^- and also possess *svb* lethal mutations (OLIVER, PERRIMON and MAHOWALD 1987). The possibility that *ovo* and *svb* are separate genes lying in close proximity on the *X* chromosome is raised by the fact that the cytologically visible deficiency, $Df(1)bi^{D2}$, removes the *svb* function but retains the *ovo* function. However, the frequency with which the svb^- ovo^- class arises, even under conditions likely to produce "point" mutations, suggests that the two genetic functions are either partially overlapping transcription units, or derive from alternative RNA splicing of a single transcription unit containing both sex-independent somatic exon sequences and female-specific, germline-specific, exon sequences.

The cloning of the *ovo* locus will provide molecular tools for the analysis of *ovo* function in the specification of germline sexual identity and oogenesis, and explain the genetic basis for the comutability of the *svb* function and the *ovo* function. In the work we report here, we have recovered nearly 60 kb of genomic DNA from the *svb-ovo* region at 4E1,2. We show that *P* element-mediated gene transfer of a 10-kb genomic DNA segment rescues the female sterility of *ovo* mutations, but not the *svb* lethality. Within this 10-kb segment, three DNA-detectable lesions for mutations that inactivate only the *ovo* function are located at two points near opposite ends of a transcription unit coding for a 5-kb poly$(A)^+$ RNA size class that appears to be female-specific and germline-dependent, properties compatible with the *ovo* female fertility function. Mutations that inactivate both the *svb* function and the *ovo* function fall into three classes: those that affect DNA between the two *ovo* sites; those that affect DNA to the molecular right of the rightmost *ovo* site; and those with no readily detectable change in DNA structure. Thus, we observe a complex interdigitation of sequences required for either or both of the genetic functions. As we neared completion of this manuscript, data on the transcription, transformation rescue and partial sequence of the *ovo* locus were independently published by MÉVEL-NINIO, TERRACOL and KAFATOS (1991).

MATERIALS AND METHODS

Fly strains, culture and methods: Drosophila was cultured on standard fly food in vials or bottles according to standard procedures at 25° unless otherwise indicated. Strains mutant for *ovo* and/or *svb* used in this study, their sources and references, are listed in Table 1. Polytene chromosome *in situ* hybridization was carried out using nick-translated biotinylated-11-dUTP-labeled probes (LANGER, WALDROP and WARD 1981). Hybridizations were visualized using the horseradish peroxidase-diaminobenzidine system (ENZO Biochemicals), and chromosome preparations were mounted in Euparal. Germline transformation was carried out essentially as described by SPRADLING and RUBIN (1982), using the host strain $y\ w^{1118}$. Test constructs were built using the w^+ vector pCaSpeR4 (see PIRROTTA 1988). They were designated pOWx, signifying a *P* element-bearing plasmid, containing an x-kilobase-long segment of the putative *ovo*

TABLE 1

***svb* and *ovo* mutant strains used in this study**

Strain	*svb-ovo* mutant properties	Origin	Source and references
ovo^{lzl}/*FM3*	*svb*$^+$ *ovo*$^-$; recessive, complete loss of function; cold-sensitive eye defect; *gypsy* element insertion	Spontaneous	M. SAMUELS and M. GANS
ovo^{D1rS1}/*FM3*	*svb*$^+$ *ovo*$^-$; recessive, complete loss of function	Spontaneous	OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{D1rP1}/*C(1)DX, y f*	*svb*$^+$ *ovo*$^-$; recessive, complete loss of function	P-M hybrid dysgenesis (π_2)	OLIVER (1989)
ovo^{7E}/*FM7*	*svb*$^+$ *ovo*$^-$; recessive, partial loss of function	EMS mutagenesis	SALZ (1992)
ovo^{8K}/*FM7*	*svb*$^+$ *ovo*$^-$; recessive partial loss of function	EMS mutagenesis	SALZ (1992)
ovo^{rM1}/*FM0*	*svb*$^-$ *ovo*$^-$; recessive, partial loss of function	EMS mutagenesis	MOHLER (1977); OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{rM2}/*FM0*	*svb*$^+$ *ovo*$^-$; recessive, partial loss of function	EMS mutagenesis	MOHLER (1977); OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{D1rG2}/*FM7*	*svb*$^-$ *ovo*$^-$	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{D1rG3}/*FM7*	*svb*$^-$ *ovo*$^-$	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{D1rG4}/*FM7*	*svb*$^-$ *ovo*$^-$	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
In(1)ovo^{D1rG5}/*FM7*	*svb*$^-$ *ovo*$^-$; cytology In(1)4E1,2; 5A1,6	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
Df(1)ovo^{D1rG6}/*FM7*	*svb*$^-$ *ovo*$^-$; cytology Df(1)4C5,6; 4E2,3	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
Df(1)ovo^{D1rG7}/*FM7*	*svb*$^-$ *ovo*$^-$; cytology Df(1)4C5,6; 4E2,3	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{D1rG8}/*FM7*	*svb*$^-$ *ovo*$^-$	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{D1rG9}/*FM7*	*svb*$^-$ *ovo*$^-$	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{D1rG10}/*FM7*	*svb*$^-$ *ovo*$^-$	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{D1rG11}/*FM7*	*svb*$^-$ *ovo*$^-$	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{D1rG23}/*FM7*	*svb*$^-$ *ovo*^{reduced}	γ-Irradiation	OLIVER, PERRIMON and MAHOWALD (1987)
ovo^{D1rOL}/*FM7*	*svb*$^-$ *ovo*$^-$	*P* element mutagenesis	This work
ovo^{D1r38D}/*FM7*	*svb*$^-$ *ovo*$^-$	*P* element mutagenesis	This work
ovo^{D1r47J}/*FM7*	*svb*$^-$ *ovo*$^-$	*P* element mutagenesis	This work
ovo^{D2}/*C(1)DX, y f*	*svb*$^+$ *ovo* dominant antimorphic	EMS mutagenesis	KOMITOPOULOU *et al.* (1983); BUSSON *et al.* (1983)
svb^{YP17B}/*FM7*	*svb*$^-$ *ovo*$^+$	EMS mutagenesis	WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS (1984); OLIVER, PERRIMON and MAHOWALD (1987)
svb^{YD39}/*FM7*	*svb*$^-$ *ovo*$^-$; isolated as lethal	EMS mutagenesis	WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS (1984); OLIVER, PERRIMON and MAHOWALD (1987)
Df(1)bi^{D2}/*FM7*	*svb*$^-$ *ovo*$^+$; cytology Df(1)4B6,C1; 4D7,E1	γ-Irradiation	BANGA *et al.* (1986); OLIVER, PERRIMON and MAHOWALD (1987)

The mutations attributed to SALZ (1992), to WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS (1984), and to BANGA *et al.* (1986) were induced on independently isogenized *white* chromosomes that were initially *svb*$^+$ *ovo*$^+$. *ovo*^{rM1} and *ovo*^{rM2} were induced by MOHLER (1977) on an isogenized *y cv v f* chromosome. *ovo*^{D2} was induced by KOMITOPOULOU *et al.* (1983) on a *vermilion*^{24} chromosome. All other mutant chromosomes were derived from *ovo*^{D1}, which was also induced on v^{24} (KOMITOPOULOU *et al.* 1983; BUSSON *et al.* 1983).

gene, and the *white*$^+$ marker gene. Test constructs were injected at 500 μg/ml, mixed with the helper plasmid phsπ (STELLER and PIRROTTA, 1986) at 100 μg/ml.

Single *P* element mobilization to obtain revertants of *ovo*^{D1}: New recessive loss-of-function *ovo*$^-$ alleles derived from *ovo*^{D1} were isolated as follows. W. GEHRING provided the stock *CyO, P*[lArB]/*b Adh cn lethal*; *ry*^{506}, containing the *P*[lArB] element described by BELLEN *et al.* (1989). In the P generation, *CyO/Sp; P*[*ry*$^+$, Δ2–3](99B) *Sb/TM6* (ROBERTSON *et al.* 1988) females were crossed to *ovo*^{D1} v^{24}/B^SY; *CyO, P*[lArB]/+ males. F_1 female progeny that were *ovo*^{D1} v^{24}/+; *CyO, P*[lArB]/*Sp; P*[*ry*$^+$, Δ2-3](99B) *Sb*/+ were crossed to *FM7a/Y* males. The F_1 females are sterile unless an intragenic loss-of-function mutation has occurred in the *ovo*^{D1} allele, or a germline exchange eliminated the *ovo*^{D1} allele from some germ cells; B^SY allowed the detection of fertile females arising from maternal nondisjunction. The mutagenized chromosomes of fertile F_2 progeny were tested for new *ovo*$^-$ mutations by complementation tests with *ovo*^{rM1} and *ovo*^{rM2}. Of 8761 *ovo*^{D1} v^{24}/+; *CyO, P*[lArB]/*Sp; P*[*ry*$^+$, Δ2-3](99B) *Sb*/+ females tested, 85 were fertile. Three carried new *ovo*$^-$ mutations, designated *ovo*^{D1r38D}, *ovo*^{D1r47J} and *ovo*^{D1r85A}, the last proving to be unstable and was lost. In a control cross using females of the genotype *ovo*^{D1} v^{24}/+; *CyO*/+; *P*[*ry*$^+$, Δ2–3](99B) *Sb*/+ (*i.e.*, producing P transposase, but lacking a mobilizable *P* element), one additional *ovo*$^-$ derivative, *ovo*^{D1rOL}, was isolated. All four *ovo*$^-$ chromosomes were lethal, producing rare escaper males with the characteristic *svb* morphology. In no case did *in situ* hybridization detect a *P*[lArB] element at the 4E1,2 polytene chromosome

site of the *ovo* locus. Previous studies have also shown the *ovo* locus to be refractory to *P* element insertion during P-M hybrid dysgenesis screens for ovo^{D1} reversion events (OLIVER, PERRIMON and MAHOWALD 1987; MÉVEL-NINIO, MARIOL and GANS 1989).

DNA gel blots, RNA gel blots and other molecular biology methods: Recombinant DNA methods–Southern blot preparation and hybridization, RNA gel blot preparation and hybridization, gel purification of DNA fragments, nick-translation labeling with [α-^{32}P]dCTP, growth and purification of bacteriophage λ and plasmid DNAs, among others–are described in DAVIS, BOTSTEIN and ROTH (1980), MANIATIS, FRITSCH and SAMBROOK (1982), SAMBROOK, FRITSCH and MANIATIS (1989), and the references cited therein. Additional details are in the figure legends.

Inverse polymerase chain reaction (PCR) of the *gypsy* junction fragments from the 4E1,2 element present in the ovo^{lzl} mutant chromosome: DNA from ovo^{lzl} adult flies was extracted by the BENDER, SPIERER and HOGNESS (1983) procedure. For each ligation and PCR amplification, DNA from three flies was digested with either *Bgl*II or *Pst*I, diluted, and ligated at room temperature for 1 hr to cyclize the DNA. Following ethanol precipitation, primers (1.5 μM each final concentration), dNTPs (0.2 mM each), and *Taq* polymerase were added (OCHMAN, GERBER and HARTL 1988). Thirty cycles of PCR were conducted with denaturation at 94° for 1 min, annealing at 55° for 1 min, and extension at 72° for 2.5 min. The *Bgl*II cyclization product, corresponding to the left junction of the *gypsy* inserted in ovo^{lzl} (based on MÉVEL-NINIO, MARIOL and GANS 1989), was amplified using primers from positions 46 to 23 and positions 392 to 415 of the *gypsy* long terminal repeat (MARLOR, PARKHURST and CORCES 1986). The *Pst*I product, corresponding to the right junction of the *gypsy* inserted in ovo^{lzl} (MÉVEL-NINIO, Mariol and GANS 1989), was amplified using primers from positions 392 to 415 and positions 2356 to 2332. Amplified DNAs were excised from a 1.4% low melting point agarose gel, labeled and used to screen a wild-type genomic DNA library. Counter-screening with a *gypsy* probe eliminated genomic DNA clones that were homologous only with the *gypsy* portion of each amplification product.

Lambda library construction and screening: DNA from ovo^{lzl} adult flies was further purified by CsCl gradient centrifugation in the presence of ethidium bromide. After complete digestion with *Eco*RI, fragments were separated by size using 10–40% sucrose gradients as described by MEYEROWITZ and MARTIN (1984). Genomic DNA fragments 7–10-kb long were pooled, ethanol-precipitated, and ligated to *Eco*RI-digested λNM1149 vector DNA (MURRAY 1983) under the conditions recommended by HUYNH, YOUNG and DAVIS (1985). Stratagene GigaPack Gold packaging extract was used to assemble infectious particles. Recombinant phage were selected for their plaque-forming ability on *Escherichia coli* C600 *hflA*150. Twenty-two thousand recombinants were screened by hybridization (BENTON and DAVIS 1977) with the 6.8-kb *Xho*I fragment containing the prototype *gypsy* element from λbx^{34e}-6a2 (BENDER *et al.* 1983; MODOLELL, BENDER and MESELSON 1983). Twenty-four strongly positive plaques were plaque-purified. Of these, one clone, designated λnDm9500, contained the *gypsy* element responsible for the ovo^{lzl} mutation and adjacent DNA unique to the *ovo* locus at 4E1,2 as determined by identity to the map published for this mutant allele, and the ability of the adjacent single-copy DNA fragment to detect several *ovo* mutant lesions in genomic DNA blotting experiments.

Cosmid library screening: Approximately 25,000 colonies of the "Iso-1" genomic DNA cosmid library constructed by J. W. TAMKUN and M. P. SCOTT (University of Colorado, Boulder) were screened and positive clones purified essentially as described by J. W. TAMKUN (personal communication). Hybond-N filters (Amersham) were used to prepare colony lifts; they were prehybridized at 65–68° in 5 × SSPE, 5 × Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 100 μg/ml denatured salmon sperm DNA, and then hybridized with nick-translation-labeled 4E1,2-specific 1.2-kb *Eco*RI-*Sal*I fragment from λnDm9500. After hybridization, filters were washed in 0.1 × SSPE, 0.1% SDS at 50° (T_m-20° conditions). Plasmid minipreps were performed according to standard procedures, and restriction digests were done in order to determine the degree of overlap of the inserts. Large-scale plasmid preparations were made using the lysozyme-Triton procedure; cosmid DNAs were purified and concentrated by two rounds of CsCl-EtBr gradient centrifugation.

RESULTS

Entry into polytene region 4E1,2: In their initial report on cloning a portion of the *ovo* locus, MÉVEL-NINIO, MARIOL and GANS (1989) demonstrated that the "lozenge-like" class of ovo^{D1} pseudorevertant arose from the insertion of the transposable element *gypsy* into a specific site at the *ovo* locus. We made use of this observation first to show that an ovo^{lzl} allele obtained from M. SAMUELS and M. GANS also possessed a *gypsy* at 4E1,2, and second to recover unique DNA sequences adjacent to the element. The unique probes were identified in two ways. In the first approach, the inverse polymerase chain reaction (OCHMAN, GERBER and HARTL 1988), using *gypsy* element-derived synthetic oligodeoxynucleotide primers, generated amplification products that corresponded in size to the predicted left junction and right junction of the *gypsy* element inserted into the *ovo* locus. The PCR products were then radioactively labeled and separately hybridized to plaque filters of a wild-type genomic DNA library. The second method made use of bacteriophage λ library construction, plaque-purification of distinct *gypsy* element-containing clones, restriction mapping, and hybridization analysis to identify the clone λnDm9500, which bears the *gypsy* element responsible for the ovo^{lzl} mutation. Both methods resulted in the recovery of DNA sequences from the *ovo* locus at 4E1,2.

Chromosome walk in 4E1,2: The starting point for our chromosome walk in 4E1,2 is the 1.2-kb *Eco*RI-*Sal*I fragment of non-*gypsy* DNA adjacent to the transposable element present in the clone λnDm9500, which was gel-isolated and radiolabeled to screen a cosmid library of genomic DNA from the whole-genome isogenic strain "Iso-1." Four overlapping positive clones, called wDm9501, wDm9502, wDm9503 and wDm9504, were identified, restriction mapped and the distribution of repetitive DNA (localized in two blocks: coordinates +2.3 to +5.5, and coordinates

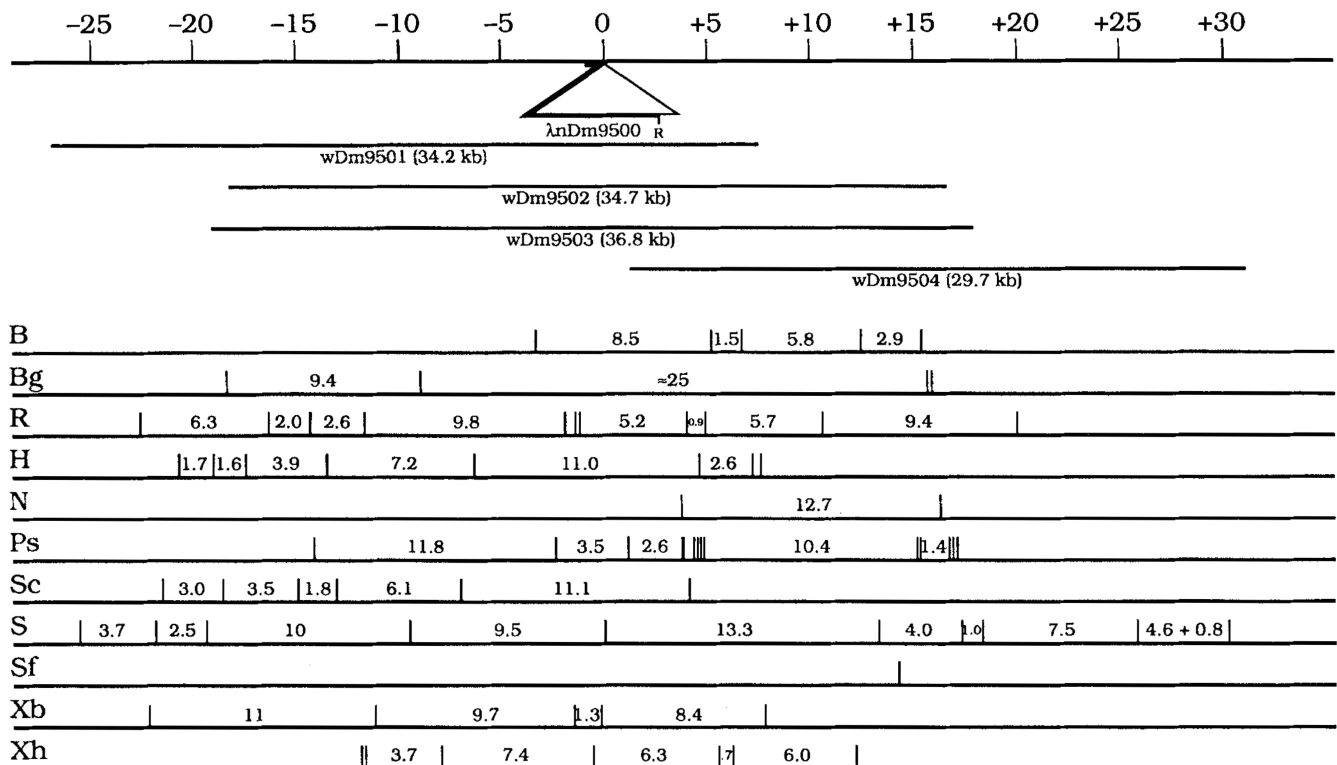

FIGURE 1.—Chromosome walk in 4E1,2. The top line represents the genomic DNA. The triangle below this line represents the *gypsy* insertion present in the ovo^{lzl} allele. The thickened part represents the *Eco*RI fragment isolated in the λnDm9500 clone. The coordinate system, in kilobase-pairs, follows from MÉVEL-NINIO, MARIOL and GANS (1989). The extents of the genomic DNA inserted in four cosmid clones, wDm9501, wDm9502, wDm9503 and wDm9504, are shown. The bottom part of the figure shows the positions of restriction sites mapped in the cosmid clones: B, *Bam*HI; Bg, *Bgl*II; R, *Eco*RI; H, *Hin*dIII; N, *Not*I; Ps, *Pst*I; Sc, *Sac*I; S, *Sal*I; Sf, *Sfi*I; Xb, *Xba*I; Xh, *Xho*I. *Kpn*I was also used and no sites were found anywhere within the walk. Ambiguity in the order of restriction fragments is indicated by the "+" symbol. Several polymorphisms distinguish the restriction map of the "Iso-1" strain from that of other *X* chromosomes used in this work. The *Xba*I site at coordinate −0.2 is absent from ovo^{D1} and pseudorevertants derived from it. An additional *Bgl*II site at coordinate −0.17 is present in ovo^{D1} and its derivatives; it is located to the left of the coordinate zero *Sal*I site. The *Bgl*II and *Xba*I site differences are not responsible for the ovo^{D1} mutation since at least one other female-sterile mutation induced by GANS, AUDIT and MASSON (1975) on the same v^{24} parental chromosome, *fs(1)A107*, which maps to polytene region 3F-4A, also shows these differences (for *Xba*I this is shown in Figure 2). An additional *Pst*I site at coordinate −6.4 was observed in the *w* parent chromosome of $Df(1)bi^{D2}$ (BANGA *et al.* 1986). A *Bam*HI site present only in wDm9503 was found at the extreme right end of the insert. Finally, we note that wDm9504 suffered a deletion of ≈0.4 kb removing the *Eco*RI site at +4.0.

+13.3 to +17.3) was determined. The repetitive DNA is high copy number but poorly conserved. A composite restriction map for these clones, which cover approximately 60 kb of the *D. melanogaster* genome, is given in Figure 1.

Molecular analysis of *ovo* mutations: We mapped three mutations that affect only the *ovo* function. They arose independently during ovo^{D1} reversion screens. The ovo^{D1} allele is not detectably different by restriction analysis from the parental ovo^{+} allele (MÉVEL-NINIO, MARIOL and GANS 1989; Figure 2). The mutations mapped are ovo^{D1rP1}, shown in Figures 2 and 3, which was recovered as a stable ovo^{-} allele following P-M hybrid dysgenesis (OLIVER 1989); ovo^{D1rS1}, described by OLIVER, PERRIMON and MAHOWALD (1987), which arose spontaneously (Figure 3); and the ovo^{lzl} allele already described as a *gypsy* element insertion at coordinate +0.9 (MÉVEL-NINIO, MARIOL and GANS 1989; Figure 3). Both ovo^{D1rS1} and ovo^{D1rP1} alter the *Eco*RI-*Hin*dIII fragment between coordinates +4.0 and +4.6, approximately 4 kb away from the site altered by the ovo^{lzl} gypsy insertion (summarized in Figure 3). ovo^{D1rS1} is most simply explained as a 5.8-kb long insertion; the apparent restriction map resembles an almost full-length copy of the retrovirus-like transposable element *H.M.S. Beagle* (SNYDER *et al.* 1982). The ovo^{D1rP1} mutation is most simply explained as a 6.7-kb insertion. In spite of the P-M hybrid-dysgenic origin of this allele, the appended DNA sequence is not a *P* element.

Molecular analysis of *svb ovo* double mutations: The most numerous class of revertants obtained following mutagen treatment of ovo^{D1} are *X* chromosomes that are both ovo^{-} and svb^{-}. We examined 14 svb^{-} ovo^{-} double mutations derived from two different ovo^{D1} reversion screens (gamma irradiation, and *P* element mutagenesis using an element marked with ry^{+} and lacZ) by whole-genome Southern blot hybrid-

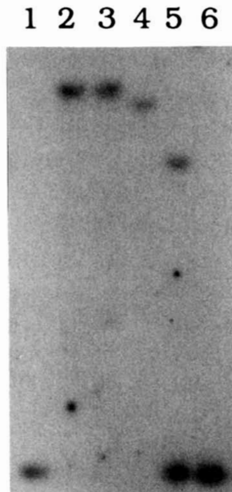

FIGURE 2.—Representative whole genome Southern of *Xba*I-digested Drosophila DNA demonstrating DNA lesions associated with *ovo* and *svb ovo* double mutations. Lane 1, "Iso-1"; lane 2, *fs(1)A107/Y* male; lane 3, ovo^{D1}/Y male; lane 4, ovo^{D1rP1}/Y (male; lane 5,) $ovo^{D1rOL}/FM7$ female; lane 6, *FM7/FM7* female. The hybridization probe is the 1.3-kb *Xba*I fragment. In v^{24}-derived chromosomes, such as ovo^{D1} and *fs(1)A107*, the *Xba*I site at −0.2 is absent, so that the DNA found in the 1.3-kb fragment is joined to DNA of the 8.4-kb fragment. The insertion present in ovo^{D1rP1}, which inactivates *ovo* function while leaving *svb* intact, is located between coordinates +4.0 and +4.6. The ovo^{D1rOL} insertion, which inactivates both *ovo* and *svb* functions, maps between coordinates 0 and +3.6, a position between the sites that affect only *ovo* function.

ization. Three of the γ-induced revertants were previously known to be cytologically abnormal. Two are deficiencies which remove the entire 60-kb cloned segment: $Df(1)ovo^{D1rG6}$ and $Df(1)ovo^{D1rG7}$ both possess breakpoints in 4C5,6 and in 4E2,3 (OLIVER, PERRIMON and MAHOWALD 1987), and lack *ovo*, *svb* and other neighboring loci. The third cytologically aberrant chromosome is the inversion chromosome, $In(1)ovo^{D1rG5}$, which is broken in 4E1,2 and 5A1,2 (OLIVER, PERRIMON and MAHOWALD 1987). The inversion fails to complement *ovo* alleles and *svb* alleles. The distal breakpoint is in the 6.3-kb *Xho*I fragment and is associated with a deletion of some 4E material as well (summarized in Figure 3).

The remaining eleven svb^- ovo^- double mutations are cytologically normal (Table 1). Nine have no readily detectable alterations within a 35-kb section of the cloned walk when analyzed using the set of restriction enzymes listed in Figure 1 (data not shown). Eight of these were induced by γ-irradiation [ovo^{D1rG2}, ovo^{D1rG3}, ovo^{D1rG4}, ovo^{D1rG8}, ovo^{D1rG9}, ovo^{D1rG10}, ovo^{D1rG11} and ovo^{D1rG23} (OLIVER PERRIMON and MAHOWALD 1987)], and the ninth by single *P* element mobilization (ovo^{D1r38D}, this work). The remaining pair, ovo^{D1r47J} and ovo^{D1rOL}, have DNA lesions detectable within the *ovo* cosmid walk. Both were recovered following exposure to *P* element transposase (ovo^{D1r47J} in the presence of the mobilizable *P*[lArB] element), yet neither possesses any *P* element sequences inserted at the *ovo* locus based on polytene *in situ* hybridization and whole-genome Southern hybridization. ovo^{D1rOL} is most simply explained as an insertion of 6.3 kb between the *Sal*I site at coordinate 0 and the *Not*I site at +3.6 (shown in Figures 2 and 3), the same region broken by $In(1)ovo^{D1rG5}$. ovo^{D1r47J} is most simply explained as an insertion of 1.5 kb into the 0.7-kb *Xho*I fragment located at coordinates +5.5 to +6.2 (shown in Figure 3).

Adult transcription units in the cloned region: Since *ovo* mutations are female-sterile, have no effect in the soma, and are germline cell-autonomous, a candidate for the *ovo* transcript should be specific to the female germline. It should overlap the DNA region where mutant lesions map; *ovo*-mutant animals heterozygous for alterations in gene structure may produce a second transcript with altered structure from the mutant allele. Finally, the candidate transcription unit possessing these properties should rescue *ovo* mutant phenotypes following DNA-mediated genetic transformation of Drosophila.

The cosmid wDm9503 detected three poly(A)$^+$ RNA classes (Figure 4). Two size classes, with apparent sizes of 5 and 6 kb, are found in adult females but not in adult males; one poly(A)$^+$ RNA size class, 0.4 kb, is found in adult males but not in adult females. All three transcript classes are germline dependent. Of the two different transcript classes possessing the properties of female specificity and germline dependence, only the 5-kb size class has the additional properties of an *ovo* candidate mRNA.

By using gel-isolated restriction fragments, we have found that the 5-kb adult female RNA size class and the 0.4-kb adult male RNA size class map within the *ovo* mutant cluster (Figure 5A), while the 6-kb adult female RNA size class is homologous with DNA several kilobases away (Figure 5B). The 6-kb RNA size class appears to be much less abundant than the 5-kb transcript size class. The 0.4-kb male RNA hybridizes with the repetitive DNA-containing 2.6-kb *Pst*I fragment (coordinates +1.1 to +3.7). The 5-kb RNA size class hybridizes with genomic DNA fragments such as the 0.7-kb *Xho*I fragment (coordinates +5.5 to +6.2) which is unique in the genome, as well as the repetitive DNA-containing 2.6-kb *Pst*I fragment (coordinates +1.1 to +3.7) and 0.9-kb *Eco*RI fragment (coordinates +4.0 to +4.9). Sequences hybridizing with the 5-kb transcript size class map within DNA fragments altered by ovo^- mutations and by svb^- ovo^- double mutations, confirming the results of MÉVEL-NINIO, TERRACOL and KAFATOS (1991).

Because some of the *ovo* mutations are due to transposable element insertions, we tested for the presence of altered RNAs using gel blots prepared from heterozygous-mutant females (homozygous-mutant females lack germline cells). In Figure 6, we show that

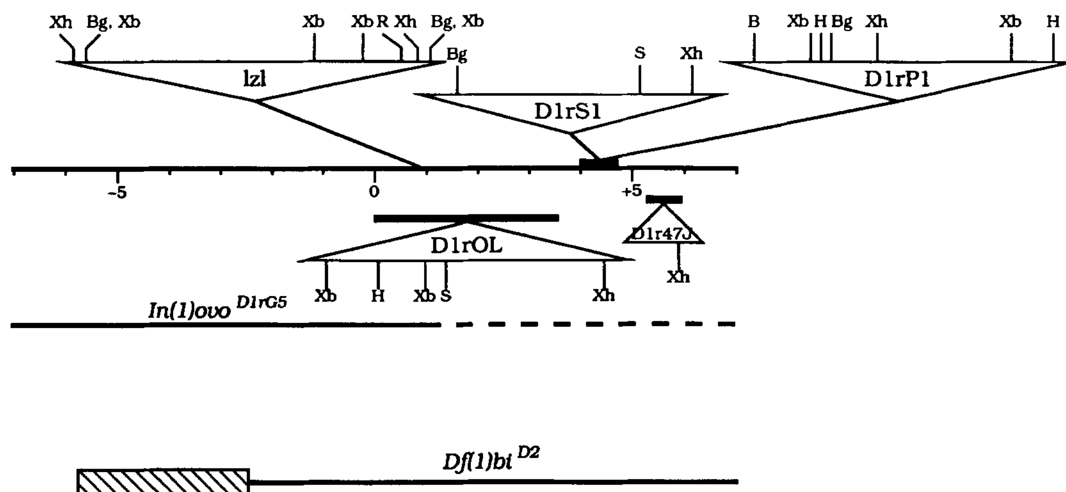

FIGURE 3.—Summary restriction map of the mutations with detectable changes in gene structure. The coordinates correspond with those in Figure 1. Above the line are restriction maps of the inserted DNA responsible for the *svb*$^+$ *ovo*$^-$ mutations *ovo*^{D1rS1}, *ovo*^{D1rP1} and *ovo*^{lzl} (our data for this last mutation confirms MÉVEL-NINIO, MARIOL and GANS 1989). Below the line are restriction maps of the inserted DNA responsible for the *svb*$^-$ *ovo*$^-$ double mutations present in *ovo*^{D1rOL} and *ovo*^{D1r47J}. The thickened lines indicate the uncertainties of the insertion points. DNA present at the 4E1,2 breakpoint of *In(1)ovo*^{D1rG5}, another *svb*$^-$ *ovo*$^-$ double mutant chromosome is also shown as a solid line, with novel DNA sequences shown by the dotted line. The DNA present at the 4E1,2, breakpoint of *Df(1)bi*^{D2}, a *svb*$^-$ *ovo*$^+$ chromosome is shown as a solid line, with the uncertainty in the endpoint indicated by a box filled with diagonal lines.

females heterozygous for the *ovo*^{D1rS1} mutation accumulate, in addition to the *ovo* candidate 5-kb RNA size class, a second transcript size class, 3.2 kb long, with homology to the *ovo* locus probe (Figure 6, lane 1). The 3.2-kb transcript size class, apparently derived from the *ovo*^{D1rS1}-bearing chromosome, is also germline dependent since females homozygous for the *ovo*^{D1rS1} mutation, and therefore lacking germline cells altogether, fail to accumulate either the 5-kb RNA size class or the 3.2-kb RNA size class (Figure 6, lane 2). We conclude that the *ovo*^{D1rS1} mutation, which affects *ovo* gene structure, apparently alters the structure of the 5-kb transcript size class too. Females heterozygous for the *ovo*^{lzl} allele accumulate only the one, 5-kb, *ovo* candidate size class. Neither of these *ovo* mutations alters the 0.4-kb male transcript (Figure 6, lanes 3 and 6).

P factor transformation of the 5-kb transcription unit rescues *ovo* but not *svb*: A 10-kb segment (located between coordinates −3.3 to +6.7), obtained by gel electrophoresis following limited digestion with *Bam*HI, was cloned into the pCaSpeR4 vector, which contains a *w*$^+$ minigene and polylinker between *P* element termini. The 10-kb *Bam*HI segment was chosen as likely to contain all of the 5-kb *ovo* candidate mRNA size class. Following microinjection of *y w*^{1118} *ovo*$^+$ embryos with a mixture of the plasmid pOW10.0 and the transposase-source plasmid, phsπ, three independent transformed lines were obtained. One insertion is on the *X* chromosome, the other two are on autosomes. The transformation events will be referred to as *Tf(1)OW10.0-17, Tf(A)OW10.0-12* and *Tf(A)OW10.0-13* (following the terminology coined by CROSBY and MEYEROWITZ 1986).

The autosomal transformant sublines were tested for their capacity to rescue the female germline defects associated with five different *ovo* mutations. These included two recessive partial loss-of-function *ovo* alleles, *ovo*^{7E} and *ovo*^{8K} (SALZ 1992), and two apparent complete loss-of-function alleles, *ovo*^{lzl} and *ovo*^{D1rS1}. Females homozygous for any of these *ovo* mutant alleles and also carrying the 10.0-kb *Bam*HI fragment transposon are fully fertile; stocks homozygous for the *ovo* mutations and carrying the autosomal transformant chromosomes have been produced.

In the case of *ovo*^{lzl}, we also noted that a single copy of the transformed 10.0-kb *Bam*HI fragment, while able to rescue the recessive female-sterility, is unable to modify the "lozenge-like" eye phenotype associated with this mutant allele. Two copies of the transformed 10.0-kb *Bam*HI fragment partially suppress the "lozenge-like" eye phenotype. This agrees with the results presented by MÉVEL-NINIO, TERRACOL and KAFATOS (1991).

The dominant female sterility of the *ovo*^{D2} mutation is suppressed by two doses of *ovo*$^+$ (BUSSON *et al.*. 1983). In agreement with this result, we have found that the *Tf(1)OW10.0–17, y w*^{1118} *ovo*$^+$ chromosome is also able to restore fertility to female flies heterozygous for *ovo*^{D2}. This supports the interpretation that *ovo*^{D2} functions as an antimorphic mutation.

Both of the autosomal transformant chromosomes were tested for their capacity to rescue the *svb* lethality. Virgin females heterozygous for the *FM7* balancer and either *svb*^{YP17B} or *svb*^{YD39} (WIESCHAUS, NÜSSLEIN-VOLHARD AND JÜRGENS 1984) were mated to *y w*^{1118} males heterozygous for either of the *Tf(A)OW10.0*

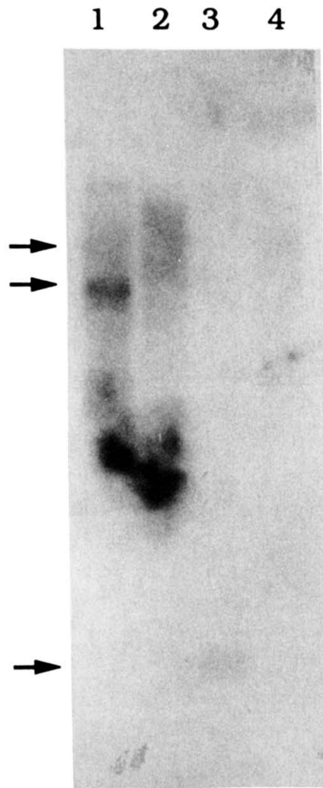

FIGURE 4.—Adult RNA gel blot hybridization. Lane 1, adult female; lane 2, daughters of *tudor*-homozygous mothers; lane 3, adult male; lane 4, sons of *tudor*-homozygous mothers. Females homozygous for *tudor* mutations lay eggs in which germplasm formation is defective; the germline precursor cells never form in the embryos, resulting in adult flies completely lacking germline tissue (BOSWELL and MAHOWALD 1985). Lanes 1 and 2 contained 10 μg poly(A)$^+$ RNA each; lanes 3 and 4 contained 3 μg poly(A)$^+$ RNA each. Relative loading was verified by hybridization with a Drosophila ribosomal protein rpA1 gene probe (D. ODOM, personal communication) and a Drosophila clathrin heavy chain gene probe (C. BAZINET, personal communication). Hybridization is shown with nick-translated cosmid wDm9503, performed as described in MATERIALS AND METHODS; the filter was washed in 0.2 × SSPE, 0.1% SDS at 65°. Two female-specific transcript size classes, length 5 and 6 kb, and one male-specific transcript, length 0.4 kb, are seen (arrows). The accumulation of each transcript type is germline dependent.

chromosomes. Progeny from the crosses were scored for the appearance of males carrying the maternally derived y^+ *w svb X* chromosome. As summarized in Table 2, these males appeared in the svb^{YP17B} crosses at a low frequency. The y^+ *w svb X* chromosome-bearing males were slow to develop and possessed the morphological characteristics of *svb* escaper males, independent of the presence of the paternally derived *Tf(A)OW10.0* chromosome bearing the w^+-marked 10.0-kb *ovo* gene segment (OLIVER, PERRIMON and MAHOWALD 1987).Thus, the 10.0-kb *Bam*HI fragment, while able to complement the *ovo* female sterile phenotypes, fails to complement the *svb* lethal phenotype.

Repetitive DNA in the cloned region and evidence for an ancient gene duplication: Within the 60-kb long segment of genomic DNA from the *svb-ovo* region there are two regions that score as repetitive when cosmid clone blot filters, hybridized with ^{32}P-labeled Drosophila DNA, are washed under T_m-20° conditions. When the filters are rewashed at T_m-10° conditions, most of the hybridized signal is released from the repetitive restriction fragments. Thus, these regions contain DNA sequences that are apparently high copy number within the *D. melanogaster* genome, but poorly conserved. The repetitive regions are localized to the section between coordinates +2.3 and +5.5, from within the *ovo* mutant cluster that is transcribed into the 5-kb *ovo* mRNA, and the section between coordinates +13.3 and +17.3, from the region transcribed into the 6-kb female-specific mRNA. Both of the repeated sequences are divided by the two genomic *Not*I restriction sites. The two *Not*I sites are found 12.7 kb apart and each is 4.6 kb to the right of a *Xho*I site. The two 4.6-kb long *Not*I-*Xho*I double-digest fragments comigrate during agarose gel electrophoresis. The evidence–two adjacent regions containing transcription units producing several kilobase long female-specific germline-dependent RNA, possessing repetitive DNA with similar properties and which cross-hybridize to each other–is suggestive of an ancient gene duplication. Mutations in the 6-kb female-specific germline-dependent transcription unit are not yet known.

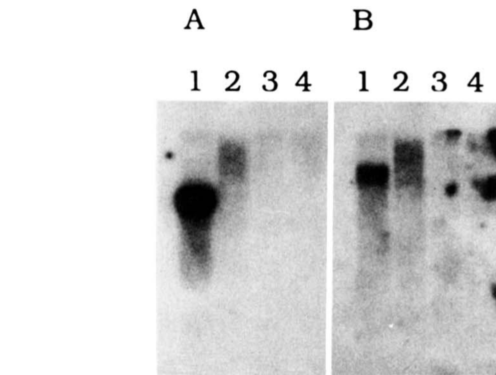

FIGURE 5.—Localization of adult female RNAs. The filter shown in Figure 4 was stripped of hybridization signal, and subjected to further cycles of hybridization, washing, autoradiography and stripping. Lane 1, adult female; lane 2, daughters of *tudor*-homozygous mothers; lane 3, adult male; lane 4, sons of *tudor*-homozygous mothers. Panel A, hybridization with the 6.3-kb *Xho*I fragment. Panel B, hybridization with the 9.4-kb *Eco*RI fragment. Conditions of filter hybridization and washing as in Figure 4. The autoradiograph in panel A was exposed for one-third the exposure time used for panel B.

DISCUSSION

We have cloned the *ovo* gene region of *D. melanogaster*. Within a 60-kb segment of the genome, we have identified the DNA lesions responsible for three *ovo* loss-of-function mutations and three svb^- ovo^-

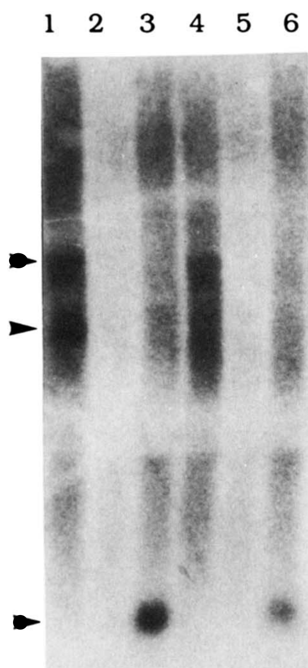

FIGURE 6.—Adult RNA gel blot hybridization of animals carrying DNA lesions demonstrating abnormal RNA structure. 25 μg total RNA were loaded in each lane. Lane 1, $ovo^{D1rS1}/FM3$ female; lane 2, ovo^{D1rS1}/ovo^{D1rS1} female; lane 3, ovo^{D1rS1}/Y male; lane 4, $ovo^{lzl}/FM3$ female; lane 5, ovo^{lzl}/ovo^{lzl} female; lane 6, ovo^{lzl}/Y male. Gel blot filter hybridized with the 8.4-kb *Xba*I fragment and washed under the same conditions as in Figure 4. Arrowheads indicate the positions of transcripts. Note that, in addition to the 5-kb female transcript, ovo^{D1rS1}-heterozygous females produce a second 3.2-kb RNA, and that neither transcript is present in the germline-deprived ovo^{D1rS1}-homozygous female RNA preparation. Note also that the 0.4-kb RNA is present in both mutant male RNA preparations.

double mutations at the locus. The mutations cluster within a 7-kb region. Homologous with this 7-kb region is the female-specific, germline-dependent, transcription unit for a 5-kb poly(A)$^+$ RNA size class. At least one mutation that alters the structure of the template DNA for this transcript also alters the apparent RNA structure. Finally, a cloned 10-kb segment, which includes the mutant cluster and the transcription unit for the 5-kb RNA size class but no part of the neighboring 6-kb RNA transcription unit, when reintroduced into the *D. melanogaster* genome by *P* factor-mediated transformation is capable of rescuing the defects in germline survival and oogenesis caused by one dominant and four recessive *ovo* mutations, but not the somatic defects caused by either of two *svb* mutations. In accord with this molecular and genetic evidence, we will henceforth refer to the 5-kb poly(A)$^+$ female-specific, germline-dependent, transcript size class we find in adults as the *ovo* mRNA, and the corresponding transcription unit and regulatory sequences contained within the 10-kb complementing genomic DNA fragment as the *ovo* gene.

The relationship between *svb* mutations and the *ovo* gene is complex. The two genetic functions are separable. Both the svb^{YP17B} allele (WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984) and $Df(1)bi^{D2}$ eliminate *svb* without interfering with *ovo* function. Moreover, the germline transformant strains carrying the genomic DNA fragment located between coordinates −3.3 and +6.7 are able to rescue *ovo* mutations but not *svb* mutations. These results clearly show that sequences required for the *ovo* function can be separated from those required for the *svb* function, and that the sequences within the 10-kb fragment that contribute to the 5-kb female-specific germline-dependent RNA size class are not sufficient to account also for the *svb* function, corroborating the results of MÉVEL-NINIO, TERRACOL and KAFATOS (1991). Other mutations, however, suggest that the two functions are interdigitated. The alternating locations of the mutant sites we observe–*svb* to the left of coordinate −3.3, *ovo* at coordinate +0.9, two svb^- ovo^- double mutations between coordinates 0 and +3.6, two svb^+ ovo^- mutations between coordinates +4.0 through +4.6, and a third svb^- ovo^- double mutation to the region between +5.5 and +6.2–is consistent with an overlapping arrangement of the two genetic functions, corroborating and extending the results of MÉVEL-NINIO, MARIOL and GANS (1989).

We have not yet detected a candidate for the *svb* transcript using RNA gel blots. The embryonic anatomy, the polyphasic lethality and the adult escaper phenotype of *svb* mutations taken together predict that a *svb* transcript will be found during embryonic through pupal development. Failure to detect a transcript may not be surprising if the *svb* transcript does not persist into adulthood, the stage used in our RNA gel blot hybridizations. We know that *svb* mutations exert no effects on oogenesis when made homozygous in female germline clones (PERRIMON, ENGSTROM and MAHOWALD 1989), which is consistent with an absence of *svb* transcripts in adults.

MÉVEL-NINIO, TERRACOL and KAFATOS (1991) reported that their *ovo* gene probes, which are substantially similar to the ones we used, detected a doublet of transcripts 5.0 and 4.8 kb in length. We did not detect two size classes in our experiments, probably due to the electrophoresis conditions used. They also reported that these transcripts are neither sex specific nor germline specific, since they were weakly detected in female carcass RNA and male RNA. MÉVEL-NINIO, TERRACOL and KAFATOS (1991) suggested that the more abundant 5.0-kb form, which they found to be greatly enriched in ovaries, corresponds to the *ovo* function, while the rarer 4.8-kb form may correspond to the *svb* function. We do not detect hybridization signals in the germline-deprived female RNA and the male RNA preparations even on long exposures of the blots used for our experiments. Experiments using

TABLE 2

Transformants carrying the 10.0-kb *BamHI* fragment cannot rescue the *svb* lethality

Cross	♀♀ Progeny			♂♂ Progeny				
	w svb/y w	*w svb/y w; Tf*	*FM7; ±Tf*	*w svb/Y*	*w svb/Y; Tf*	*FM7/Y; ±Tf*	*y w/O*	*y w/O; Tf*
YP17B × *Tf12*	132	156	228	13	18	162	10	13
YP17B × *Tf13*	79	95	148	9	2	101	5	6
YD39 × *Tf12*	33	62	106	0	0	86	0	0
YD39 × *Tf13*	27	34	63	0	0	63	0	0

Crosses were of *w svb/FM7* virgin females mated with $y\ w^{1118}/Y$; *Tf(A)OW10.0/+* males. Transformant strains and *svb* allele numbers are given in the leftmost column. Progeny genotypes are given in the remaining column heads. Replica crosses using two or three females mated with four to six males were conducted in shell vials at 25°C. Parents were transferred to fresh vials every four days. The $w\ svb^{YD39}/FM7$ stock is less vigorous than the $w\ svb^{YP17B}/FM7$ stock. The presence of w^a and *B* on the *FM7* balancer chromosome complicated scoring for the presence of the *Tf(A)OW10.0* chromosomes. Male progeny described as *y w/O* are presumed to be descended from *X* chromosome nondisjunction in the $w\ svb^{YP17B}/FM7$ mothers.

nuclease protection assays and RNA-directed PCR will provide quantitative values for the relative abundances of the different *ovo*-derived RNA products expressed during each phase of development. Complementary DNA clones isolated from various sources indicate that there are alternatively spliced RNAs from the *ovo* region (J. WANG, M. D. GARFINKEL and A. P. MAHOWALD, unpublished results). *In situ* hybridization experiments using embryos, third instar larvae and pupae should allow better identification of transcribed regions responsible for the *svb* function. Given the overlapping sites mutable to the *ovo* and *svb* phenotypes, such experiments may reveal a somatic-specific promoter or somatic-specific exons joined onto at least some exons that are also required for *ovo* function in the female germline. Alternatively, expression of the transcript responsible for *svb* function may be regulated by sequences lying within the *ovo* gene.

The fact that none of the eight cytologically normal svb^- ovo^- double mutations obtained as γ-ray-induced revertants of ovo^{D1} possesses changes in DNA structure detectable by Southern blotting is not unusual. At the rosy locus, 7 out of 41 radiation-induced *ry* mutations had alterations greater than 0.6 kb in size detectable by whole-genome Southern blot hybridization (COTÉ *et al.* 1986), while five more were deletions or substitutions involving net fragment size changes of as few as a single base pair (GRAY *et al.* 1991). At the *white* locus, 2 out of 11 mutations were deletions of several hundred base pairs detected using Southern blots (PASTINK *et al.* 1987), while four others proved to have net deletions between 6 and 29 bp in size (PASTINK *et al.* 1988). Thus, among cytologically normal radiation-induced *w* and *ry* mutations, only 17% possess alterations easily detected by Southern blotting of genomic DNA. Methods such as denaturing gradient gel electrophoresis (SHEFFIELD, COX and MYERS 1990; GRAY *et al.* 1991) should detect the small deletions, insertions, substitutions or inversions likely to be responsible for the other eight intragenic svb^- ovo^- double mutations.

We propose that the "lozenge-like" eye phenotype is the result of a neomorphic, gain-of-function mutational event, rather than as evidence of an additional gene at 4E1,2 for several reasons. The "lozenge-like" phenotype appears only among revertants of the ovo^{D1} allele, and involves only one orientation of gypsy retrotransposon insertion at coordinate +0.9 (MÉVEL-NINIO, MARIOL and GANS 1989). Mapping of the *ovo* mRNA places this insertion site within the *ovo* transcription unit proper (MÉVEL-NINIO, TERRACOL and KAFATOS 1991; this work). Whether it is within an intervening sequence or an exon is not yet known. The apparently null state of the ovo^{lzl} allele for female germline function could be caused by the insertional inactivation of an essential sequence element in the *ovo* gene. The "lozenge-like" eye phenotype would depend upon *cis* interaction of the *gypsy* element and the surrounding *ovo* gene sequences. Supporting this proposal are the observations that one dose of the transformed 10-kb fragment conferring ovo^+ function does not modify the eye phenotype of ovo^{lzl} but does rescue the ovo^{lzl} female sterility phenotype (MÉVEL-NINIO, TERRACOL and KAFATOS 1991; this work), and that the cold-sensitive period for the "lozenge-like" phenotype of ovo^{lzl} ends during the middle of pupal development (M. D. GARFINKEL, unpublished observation), at which time the abundance of the full-length *gypsy* transcript is maximal (PARKHURST and CORCES 1985, 1986). If the *ovo* gene is not normally expressed in the developing eye, then the effect of the *gypsy* element may be to promote ectopic, inappropriate, expression. A *gypsy*-ovo^{D1} hybrid transcript or hybrid protein would then interfere with normal eye development; cold-sensitivity would be attributed to aberrant protein-protein interactions (*e.g.*, SCHATZ, SOLOMON and BOTSTEIN 1988). Insertions of *Saccharomyces cerevisiae* and *D. melanogaster* retrovirus-like elements in or near transcription units often cause orientation-dependent mutant phenotypes involving misregulation of gene expression, production of hybrid tran-

scripts, or quantitative changes in gene function (reviewed by BOEKE and CORCES 1989; CORCES and GEYER 1991). In any event, a *gypsy-ovo*^{D1} hybrid transcript does not persist into adulthood or is not easily distinguished from the normal *ovo* mRNA, even though *gypsy* RNA is present in adult flies (PARKHURST and CORCES 1985, 1986). The search for a novel transcript produced by the *ovo*^{lzl} mutant allele in third instar eye-antennal disks or pupal eye tissue is an area of future experimentation.

Perhaps half the length of the *ovo* mRNA is accountable by the high copy number, low fidelity repetitive DNA block. That the repeated DNA block is homologous with a much smaller, 0.4-kb, male transcript which is also germline dependent raises several interesting questions. First and most important, do the male transcripts actually originate from the *ovo* gene at 4E1,2, with base-for-base correspondence in DNA sequences? If so, what is the base-for-base correspondence between the 0.4-kb male RNA and the 5-kb female RNA size class? Sex-regulated alternative splicing in the germline, as observed for the somatic sex-determination genes, is an intriguing possibility, as is the possibility of germline-specific splicing as observed in the regulation of the *P* element transposase.

We suspect that the *ovo* gene is related by an ancient gene duplication event to the second transcription unit at 4E1,2, and is likely to be a member of a gene family. Both the functional *ovo* gene between coordinates −3.3 and +6.7 and the region between +10.6 and +20.0 are transcribed into several kilobase long female-specific, germline-dependent, poly(A)$^+$ RNA size classes. Both transcribed regions contain similarly sized blocks of repetitive DNA sequences, with similar hybridization properties. In clone blot hybridization experiments the two regions cross-hybridize with each other. The neighboring 6-kb RNA transcription unit may be a weakly expressed pseudogene or possess a distinct genetic function as yet unidentified by mutation.

P element-mediated gene transfer of the wild-type *ovo* gene to autosomal locations demonstrates the practicality of using the cloned *ovo*^{D1} mutant allele to generate transformant strains carrying the dominant female sterile allele on each autosomal arm. The molecular analysis of the *ovo*^{D} alleles (MÉVEL-NINIO, MARIOL and GANS 1989; this work, and data not shown) indicates that these mutations are not due to gross alteration in *ovo* gene structure, such as transposable element insertions, and therefore predicts that the functional *ovo*^{D1} gene segment will be the same size as the *ovo*$^{+}$ gene segment. The earlier work of PERRIMON and MAHOWALD (1986, and references cited therein) on the female germline effects of *X*-linked zygotic lethal mutations surveyed around 20% of Drosophila lethal mutations. Use of the germline clonal analysis technique to test for maternal functioning of the autosomal complement will undoubtedly provide new insights into the genes required for oogenesis, embryonic pattern formation, and neurogenesis, among other processes.

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