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Developmental Genetical Analysis and Molecular Cloning of the *abnormal oocyte* Gene of *Drosophila melanogaster*

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ABSTRACT

Studies of the *abnormal oocyte* (*abo*) gene of *Drosophila melanogaster* have previously been limited to the analysis of a single mutant allele, *abnormal oocyte*^{1} (*abo*^{1}). The *abo*^{1} mutation causes a maternal-effect lethality that can be partially rescued zygotically by the *abo*$^{+}$ allele and by increasing the dosage of specific regions of heterochromatin denoted *ABO*. This report describes the properties of *abo*^{2}, a new *P*-element-induced allele that allowed us to reexamine the nature of maternal-effect defect. Comparisons of the phenotype of progeny of *abo*^{1}/*abo*^{1} and *abo*^{1}/*abo*^{2} females show that the preblastoderm lethality previously described as a component of the *abo* mutant maternal effect results from a recessive fertilization defect associated with the *abo*^{1} chromosome. We demonstrate here that the *abo*-induced maternal effect lethality occurs predominately late in embryogenesis after cuticle deposition but before hatching. The phenocritical period for zygotic rescue by heterochromatin coincides with this period of late embryogenesis. We have used the *abo*^{2} mutation to map and molecularly clone the gene. We show that the *abo* gene is located in the 32C cytogenetic interval and identify the putative *abo* transcript from mRNA isolated from adult females. Using germline transformation, we show that a 9-kb genomic fragment to which the transcript maps, partially fulfills requirement for maternal and zygotic *abo*$^{+}$ function.

THE heterochromatin of *Drosophila melanogaster* is the best genetically and molecularly characterized heterochromatin of all organisms studied to date. A wide variety of genetic functions are known to exist within Drosophila heterochromatin, including those encoded by repetitive DNA sequences (reviewed by GATTI and PIMPINELLI 1992). These include the 18 + 28S rDNA, the *Rsp* locus, and the *ABO* and *DAL* elements. The *ABO* and *DAL* elements are unusual in that they are dispersed heterochromatic loci defined by their ability to interact with specific maternal-effect mutations that map to the euchromatin on the left arm of chromosome *2*. The euchromatic mutations were identified by SANDLER (1977) as a set of five closely linked recessive, maternal-effect mutations that includes: *abnormal oocyte*^{1}, *hold up, wavoid-like, daughterless* and *daughterless-abnormal oocyte-like*. These mutations form a unique group among maternal-effect mutations because the maternal defect can be partially compensated by a paternal copy of the wild-type gene in the zygote or by increasing the dosage of heterochromatin that the zygotes receive. Specific regions of heterochromatin interact with both *abo* and *dal* (PIMPINELLI *et al.* 1985).

The heterochromatic regions that interact with *abo* have been best characterized. These regions, designated *ABO* have been mapped by comparing the effect of cytologically defined duplications or deficiencies of heterochromatin on the survival rates of progeny of *abo*^{1} females. These studies have identified *ABO* loci in the distal heterochromatin of the *X*, on the long and short arms of the *Y*, and the right arm of the second chromosome (SANDLER 1970, 1977; PARRY and SANDLER 1974; HAEMER 1978; YEDBOVNICK *et al.* 1980; PIMPINELLI *et al.* 1985; L. FANTI and S. PIMPINELLI, unpublished results).

Recently, TOMKIEL *et al.* (1991) demonstrated that an increase in the dosage of *ABO* heterochromatin in the mother can partially rescue the maternal-effect lethality caused by *abo*^{1}. The *ABO* elements appear to function very early in embryogenesis (PIMPINELLI *et al.* 1985; TOMKIEL *et al.* 1991), at a time when genetic, molecular, and biochemical studies all suggest that the zygotic genome is transcriptionally silent (LAMB and LAIRD 1976; MCKNIGHT and MILLER 1976; ZALOKAR 1976; ANDERSON and LENGYEL 1979; EDGAR and SCHUBIGER 1987). Examination of the effects of various doses of *ABO* in *abo*^{1} mothers led to the conclusion that maternal *ABO* may function either in oogenesis or in the egg before completion of meiosis I (TOMKIEL *et al.* 1991). Analysis of

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survival of embryos mosaic for various amounts of *ABO* suggests that the developmental timing of zygotic rescue by *ABO* is before completion of the first zygotic division. Thus, both maternally and zygotically supplied *ABO* appear to exert their rescuing effect between fertilization and the first zygotic division.

Several models have been proposed to explain the rescue of the *abo* maternal-effect defect by *ABO* heterochromatin. One model postulates that *abo* and *ABO* carry out similar functions, perhaps by encoding related products. The location of loci in heterochromatin may allow expression of a necessary product during times when the euchromatin is obligately silent (PIMPINELLI *et al.* 1985). A second class of models proposes that *abo* and *ABO* loci have different developmental functions with some interaction required, either at the level of their gene products or via regulatory interactions. For instance, the product of one gene may be required to activate the expression of the other. Alternatively, the heterochromatin may exert its effect directly or indirectly on the expression of the euchromatic *abo* locus by titration of regulatory factors or by influencing chromatin structure (TOMKIEL 1990). Finally, a third model proposes that the observed interaction of *ABO* with *abo* reflects the specific nature of the abo^1 allele, rather than a requirement necessary for normal embryogenesis. LAVORGNA *et al.* (1989) have suggested that the abo^1 allele is associated with a copia-like transposable element inserted in or near the gene. They attribute the mutant phenotype to the disruptive effect of the insertion on gene expression and postulate that the effect of *ABO* is due to the suppressive influence of heterochromatin on the transposable element.

The above models are not easily distinguished by genetic means. A clearer understanding of the mechanism of interaction between the euchromatic mutation and the heterochromatic elements requires a molecular analysis of both *abo* and *ABO*. Toward this end, we have isolated new allele of *abo*. Using abo^2, a *P*-element-induced allele, we have reexamined and clarified certain aspects of the *abo* maternal-effect lethality, cloned the *abo* gene and identified a candidate *abo* mRNA.

MATERIALS AND METHODS

Drosophila stocks: All cultures were raised at 25° on cornmeal-molasses yeast media with 0.2% propionic acid added as a mold inhibitor. Unless otherwise noted, mutations and chromosomal aberrations used in this study are described in LINDSLEY and ZIMM (1992).

***P*-element mutagenesis:** The goal of the mutagenesis was to obtain cytogenetically or molecularly mappable *abo* mutations, including deficiencies or *P*-element transpositions that might be helpful to refine the localization of the *abo* gene. We took advantage of four marked *P*-element transposons inserted within the 31–32 cytogenetic interval. To increase the efficiency of recovering lesions in region 31–32, we selected for *P*-element-induced male recombination events. *P*-element transposition was induced by introducing the *Δ2-3(99B)* transposon source (LASKI *et al.* 1986; ROBERTSON *et al.* 1988) into males that were also heterozygous for two of the *P* elements and heterozygous for the wild-type and dominant mutant alleles of the flanking markers *Jammed* (*J*) and *Tufted* (*Tft*).

The four different *P* elements, each marked with the *ry+* gene, were as follows: *CHBΔ-89* $P[ry^+]$ and *Cp70ΔB* $P[ry^+]$ isolated by SIMON *et al.* (1985), $fs(2)ry^5$ $P[ry^+]$ provided by D. MCKEARIN and *g65* $P[ry^+]$ isolated by BOUROUIS and RICHARDS (1985). These *P[ry+]* elements are located at 31B, 32C/D, 32E and 32F, respectively. The *fs(2)ry5* chromosome that we received also contained a second *P*-element insertion that we could detect by Southern blotting. With the exception of the $fs(2)ry^5$ insertion that results in a recessive female sterility that fully complements abo^1, these *P*-element insertions do not cause mutant phenotypes. The cross used to obtain males carrying *P* elements on both chromosome *2* homologues is described in Figure 1. It was necessary to repress *P*-element transposition in the preceding generation. This was accomplished by introducing the *X*-linked I35 element kindly provided by R.W. PHYLLIS. I35 is a mutated *P* element that effectively represses transposition because it encodes a truncated transposase protein.

From among the progeny of males that were *J Cp70ΔB* $P[ry^+]$ *Tft*/J^+ $P[ry^+]$ Tft^+, we recovered chromosomes with nonparental combinations of the flanking markers (*J* Tft^+ or J^+ *Tft*). A total of 52 recombinant chromosomes were tested for complementation of the abo^1 maternal effect lethality. Twelve were from *J Cp70ΔB Tft*/*Cp70ΔB* fathers, six were from *J Cp70ΔB Tft*/*g65* fathers, eight were from *J Cp70ΔB Tft*/*CHBΔ89* fathers and 26 were from *J Cp70ΔB Tft*/*fs(2)* ry^5 fathers. One of these was a noncomplementing *J* ry^+ Tft^+ chromosome recovered from a *J Cp70ΔB Tft*/*fs(2)* ry^5;*Δ2-3(99B)* father. This new mutation will be referred to as abo^2. (This same mutation was described as abo^3 by TOMKIEL (1990) but is renamed here).

Recombination mapping of abo^2: The abo^2 mutation was mapped by recombination relative to *Jammed* (2–41.0), the ry^+ on the abo^2 chromosome, and *Scutoid* (2–51.0). The distance between *J* and *Sco* in this experiment was 12.2 map units (131 recombination events/1070), compared to the reported distance of 10.0 map units (LINDSLEY and ZIMM 1992). Forty-three *J-Sco* recombinants were tested for the *ry* and *abo* phenotypes. These data map abo^2 ~2.1 map units proximal to *J* and 9.3 map units distal to *Sco*. This places abo^2 between 41.7 and 43.1 map units, in close proximity to the previously reported map position of abo^1 (44.0). In this mapping experiment, a single chromosome that was apparently abo^2 (*ry*-) was recovered but was not further characterized. Scoring of *abo* was based on the sex-ratio among progeny from *X*/*X*; *recombinant*/abo^1 mothers and *In (1)EN YS.X.YL,y*/O fathers. (From here on we will refer to the attached *X-Y* chromosome *In (1)EN YS.X.YL,y* as simply "*X-Y*"). Rarely an abo^+ mother will also produce progeny with a skewed sex ratio, perhaps due to the occurence of a spontaneous lethal mutation on one maternal *X* chromosome. We are uncertain if this one chromosome was truly a recombinant between abo^2 and ry^+, or was merely a false positive.

The abo^2 chromosome fails to complement the *fs(2)ry5* mutation on one of the parental chromosomes, but unlike either parent, it is homozygous lethal. We have been unable to separate the lethal mutation from the abo^2 maternal effect mutation by recombination.

Complementation tests: Before the genetic characterization of the *abo* alleles, the *X* chromosomes in all stocks were replaced with wild-type (Canton-S) *X* chromosomes from the same stock to ensure that the heterochromatic contribution was similar in the complementation tests. Single pair crosses in vial cultures were used for the data reported in Table 1.

Mass matings of at least 10 pairs of flies in bottle cultures were used for the data reported in the other tables.

For assays of the maternal-effect lethality, females homozygous or heterozygous for one of the *abo* alleles were mated to *X-Y/O* or *X-Y/Y* males, and the relative viability of their sons and their *X-Y/X* daughters was compared. For test of zygotic rescue, *chromosome A/Sco* males were mated to *chromosome B/SM5, Cy* females, and the viability of *A/B* progeny was compared to the *Sco/Cy* progeny (with over 200 progeny scored per cross). For a comparison of recovery values, the Mantel-Haenzel estimate of odds ratio was used to generate expected matrix values (BISHOP *et al.* 1975; SULLIVAN and PIMPINELLI 1986). These were then used to calculate chi-square values.

Analysis of the *abo*-induced maternal-effect lethal phenotype: For determining the proportion of lethal progeny produced by *abo* mothers, eggs were collected from females within 30 min of deposition, counted and incubated on fresh media at 25°. Cultures were inspected 36 hr later to determine the number of hatched individuals. The cultures were inspected again on day 15 to determine the number of pupal cases and eclosing adults.

The phenotype of eggs and early embryos 30 ± 10 min after egg deposition was also examined. These were fixed overnight at 4°C in methanol by the method of MITCHISON and SEDAT (1985). Nuclei were stained with 1 μg/ml of the DNA dye, DAPI (4',6-diamidine-2-phenylindole) in phosphate-buffered saline (PBS, pH 7.0) for 10 min. Tubulin was stained with mouse anti-tubulin monoclonal IgG antibody (Amersham, Arlington Heights, IL). Sperm tails were stained with the AXD5 mouse monoclonal antibody (KARR 1991). Rhodamine-conjugated goat-anti-mouse monoclonal IgG (Cappel, West Chester, PA) was used as the secondary antibody. Embryos were mounted in 20% glycerol in PBS and viewed using epifluorescent optics on a Nikon Microphot-FX microscope. To examine cuticle morphology of late embryos, embryos that failed to hatch 36 hr after egg deposition were treated with 50% commercial bleach, followed by heptane and methanol to remove the chorion and vitelline membranes, respectively (MITCHISON and SEDAT 1983). The embryos were mounted on a slide in a drop of 9:1 lactic acid-95% ethanol. Internal tissues were cleared by heat treating the slides at 60° for 24 hr (LEWIS 1978). These cuticle preparations were examined and photographed using dark field optics on a Nikon Microphot FX microscope.

Reversion of abo^2 to abo^+: To obtain abo^2 revertants, we introduced transposase into abo^2-bearing flies to remobilize the *P* element associated with the mutation. Males bearing the transposase source *Δ2-3* were mated to $abo^2/Cy;\ ry^{506}$ females to generate sons of the genotype $abo^2\ [ry^+]/CY;\ \Delta 2\text{-}3[ry^+],\ Sb,\ ry/ry$. These sons were mated to $abo^1/Cy;\ ry^{506}$ females, and the $Cy^+\ ry\ Sb^+$ daughters from this cross were tested for the *abo* maternal effect. One abo^2 revertant was identified out of 24 chromosomes tested in this manner.

Molecular analyses: DNA fragments used for probing Southern blots, Northern blots or plaque lifts were radiolabelled with ^{32}P by nick translation or random priming using standard procedures (SAMBROOK *et al.* 1989). These fragments included the *Hin*dIII genomic fragment containing the ry^+ gene (LEE *et al.* 1987), the 0.8-kb *Hin*dIII fragment containing the *P*-element end (O'HARE and RUBIN 1983) or genomic fragments cloned from the abo^+ locus.

For genomic Southern blotting, Drosophila DNA was prepared as described by BENDER *et al.* (1989). Restriction enzyme digestion and gel electrophoresis was performed as described by SAMBROOK *et al.* (1989). Enzymes were obtained from Boehringer-Mannheim (Indianapolis, IN) or Promega (Madison, WI). Hybond-N nylon filters (Amersham, Arlington Heights, IL) were used according to the manufacturer's specifications. Southern blots were washed at high stringency using four 15-min washes in 0.1% SDS at 68°, and the filter was exposed to XAR-5 X-ray film for autoradiography.

A genomic DNA library was constructed from abo^2/CyO adults. High molecular weight DNA was extracted as described by SAMBROOK *et al.* (1989) and was partially digested with *Sau*3A to obtain fragments of an average length of 20 kb. After phenol-chloroform extraction and ethanol precipitation, fragment ends were partially filled in by Klenow polymerase, dATP and dGTP to obtain *Xho*I-compatible ends. One microgram of such DNA was ligated with 0.5 μg of *Xho*I-cut LambdaGEM 11 and packaged with Packagene as per the manufacturers instructions (Promega, Madison WI). This lambda library yielded 2.5×10^5 primary plaques that were screened with a ^{32}P-labeled probe containing the 0.8-kb *Hin*dIII fragment of the *P* element. A library constructed by PIRROTA (1988) and containing Oregon R wild-type genomic sequences was also screened. Positive plaques from both libraries were isolated, mapped and subcloned using standard protocols (SAMBROOK *et al.* 1989).

For RNA analysis, total RNA was extracted from whole adult female flies according to LAUGHON *et al.* (1986). RNAs were separated on 1% agarose-3-(*N*-morphalino)propanesulfonic acid (MOPS)-formaldehyde gel, transferred to a nylon membrane and hybridized with radiolabeled DNA probes as described in SAMBROOK *et al.* (1989).

***P*-element transformation:** A 9-kb BamHI genomic fragment was isolated from an Oregon R lambda phage and cloned into the CaSpeR transformation vector that contains the mini*white* marker gene (PIRROTTA 1988). This construct was injected into a *white*– host strain and *w*+ germline transformants were obtained as described by SPRADLING (1986).

Polytene chromosome cytology and *in situ* hybridizations: Salivary gland chromosomes from $abo^2/+$ larvae were dissected, then stained with lacto-aceto-orcein and examined for chromosome aberrations. There were no cytological defects observed.

In situ hybridization to polytene chromosomes was performed essentially as described by PARDUE (1986) except nonradioactive biotinylated probes were used. Chromosomes were stained with DAPI and hybridization sites were detected using fluorescein isothiocyanate (FITC)-conjugated avidin. Digital images were obtained using a computer-controlled Zeiss Axioscope epifluorescence microscope equipped with a cooled charge coupled device camera (CCD, Photometrics). FITC and DAPI fluorescence, detected by specific filter combinations, was recorded separately as gray scale images and then pseudocolored and merged for the final image shown in Figure 4.

RESULTS

Isolation of a new mutant allele of the *abnormal oocyte* (*abo*) gene: All previous conclusions about the *abnormal oocyte* gene were based on studies of one naturally occuring mutation, abo^1, and its interaction with heterochromatin. To obtain new alleles for cytogenetic and molecular mapping of the gene, we screened for *P*-element-induced mutations that failed to complement the abo^1 maternal-effect lethality. Putative lesions were recovered as *P*-element transposase induced male recombination events between two dominant markers (*J* and *Tft*) that flank the *abo* region (see MATERIALS AND METHODS, Figure 1). The parental chromosomes each

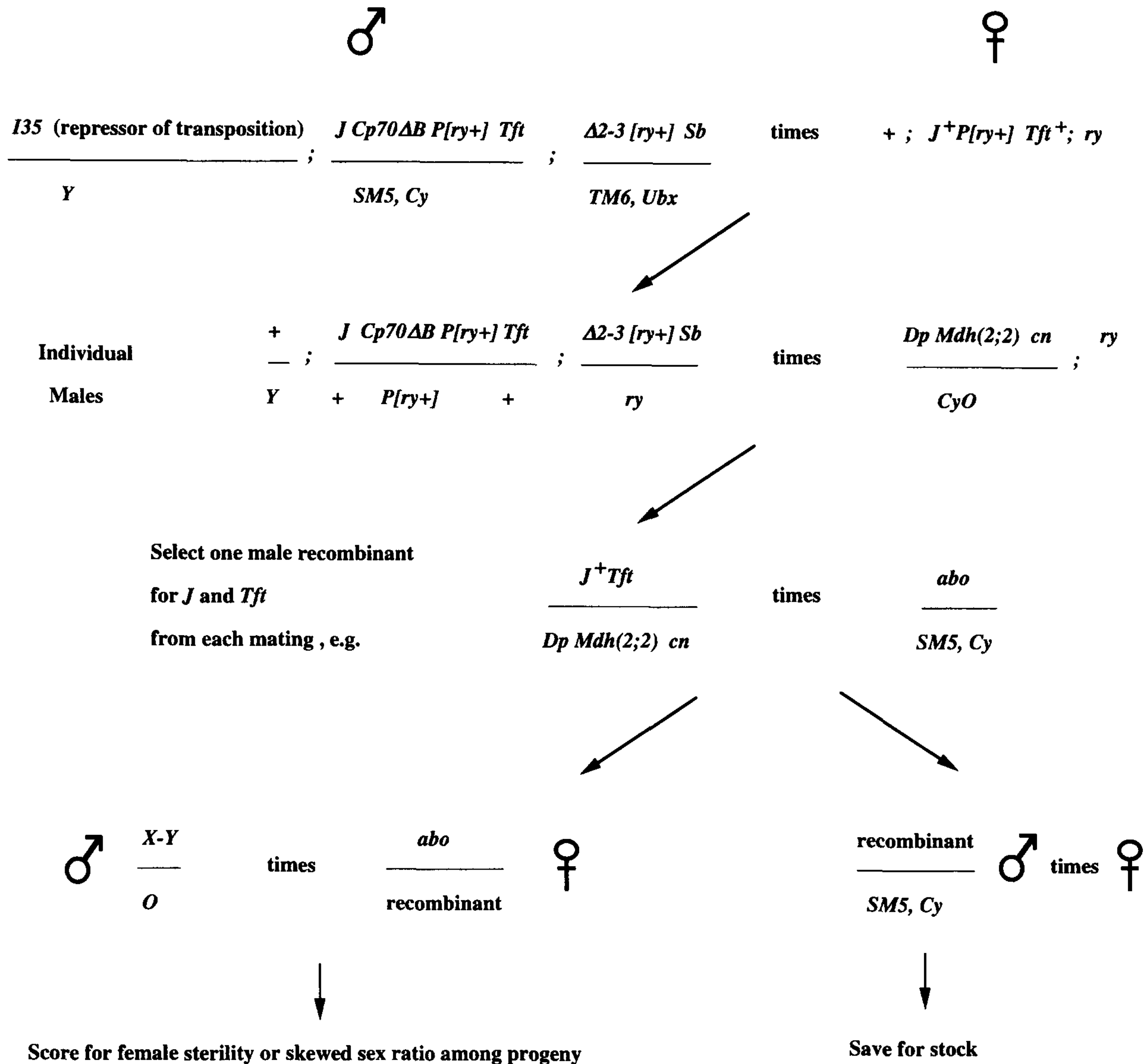

FIGURE 1.—The scheme used to isolate the *P*-element-induced abo^2 allele. See MATERIALS AND METHODS for details of the markers and stocks used. The *I35 X* chromosome was used to repress transposition of the *Cp 70 ΔB P[ry+]* element in Cross 1. In Cross 2, males heterozygous for two transposons and flanking markers (wild-type or mutant alleles of the dominant *Jammed* and *Tufted* alleles) were expected to undergo *P*-element transposition and possibly male recombination. In Cross 3, sons that carried chromosomes that were recombinant for the *J* and *Tft* markers were retained for further testing of *abo* function. New *abo* alleles that failed to complement abo^1 were expected to result in female sterility or the differential recovery of progeny that varied in the amount of heterochromatin.

carried a *P* element marked with the *ry+* gene. The *J Cp70ΔB P[ry⁺] Tft* chromosome carried a *P[ry+] element inserted at 32C/D. The J^+ fs(2)ry5 Tft^+* chromosome contained a *P[ry⁺]* insertion at 32E that causes the female sterile mutation, *fs(2)ry5* (D. MCKEARIN, personal communication) that fully complements abo^1. Putative recombinant chromosomes were recovered from the progeny of *J Cp70ΔB P[ry⁺] Tft/J^+ fs(2)ry5 Tft^+* males. One *J ry^+ Tft^+* chromosome carried a new mutation that was fully viable over abo^1 but failed to complement its maternal-effect lethality. This new mutation was mapped by recombination to the *abo* region and designated abo^2 (for details, see MATERIALS AND METHODS).

The abo^2 chromosome retained at least one functional copy of the ry^+ gene capable of conferring a wild-type eye color. To determine if abo^+ function could be restored by a change in the *P* element(s) on the abo^2 chromosome, we introduced the Δ *2-3(99B)* genomic source of *P*-element transposase (LASKI *et al.* 1986; ROBERTSON *et al.* 1988) into an abo^2 strain and selected for *ry−* mutant phenotypes. Twenty *ry−* chromosomes were recovered and tested for complementation of the abo^1 maternal-effect lethality. One restored abo^+ function and was designated abo^{2rev}.

Both the abo^2 chromosome and its derivative abo^{2rev} are homozygous lethal. All of our attempts to separate

TABLE 1

Effect of the *Y* chromosome on zygotic rescue from the *abo* maternal effect

Maternal genotype	Paternal genotype	No. of mothers		Progeny per mother ± SE: Males	Progeny per mother ± SE: Females	Relative progeny per mother[a]: Male	Relative progeny per mother[a]: Female	Male recovery[b]
abo^1/*abo*^{1}	*X-Y/O*	67	+	2.2 ± 0.25	23.1 ± 1.40	0.10[c]	0.79[c]	0.13
abo^1/*J abo*^{2}		80	+	0.7 ± 0.11	15.0 ± 1.18	0.07	1.03	0.07
			J	0.6 ± 0.13	17.7 ± 1.15	0.06	1.28	0.05
abo^1/*Cy*		37	Cy	11.5 ± 0.82	15.0 ± 0.93			
			+	10.5 ± 0.75	14.6 ± 1.05			
J abo^2/*Cy*		38	Cy	12.8 ± 1.08	16.5 ± 8.6			
			J	9.4 ± 0.85	13.8 ± 1.17			
abo^1/*abo*^{1}	*X-Y/Y*	32	+	12.8 ± 1.11	23.1 ± 1.87	0.48[c]	0.90[c]	0.53
abo^1/*J abo*^{2}		56	+	7.3 ± 0.62	16.8 ± 1.09	0.55	1.31	0.42
			J	6.6 ± 0.74	14.8 ± 0.95	0.46	0.96	0.48
abo^1/*Cy*		17	Cy	13.7 ± 0.85	12.6 ± 1.79			
			+	13.2 ± 1.28	12.8 ± 0.89			
J abo^2/*Cy*		36	Cy	12.7 ± 0.65	13.8 ± 0.92			
			J	14.3 ± 0.85	15.4 ± 0.82			

The effect of *Y* heterochromatin was monitored by measuring the viability of *X/O* and *X/Y* sons relative to their *X-Y/X* sisters. This was assayed by counting the number of male and female progeny produced per mother. The metric of progeny per mother allowed direct comparison of the viability of each progeny class to the same class from control *abo/Cy* mothers. Male recovery, which represents the survival of sons relative to daughters, was calculated by dividing the ratio of sons to daughters among *abo*-derived progeny by the same ratio among progeny from *abo/+* mothers.

[a] Progeny per *abo* mother divided by same progeny type per *abo/Cy* mother.

[b] (Relative males per mother) ÷ (relative females per mother).

[c] Comparison is made to + progeny from *abo/Cy* mothers and divided by 2 to account for *Cy* class.

the lethal mutation from the maternal effect by recombination have been unsuccessful. Consequently, tests of *abo* mutant effects were limited to comparisons of the *abo*^{1} homozygotes and *abo*^{1}/*abo*^{2} heterozygotes.

The interaction of *abo*^{1}/*abo*^{2} with *Y* heterochromatin: One of the hallmarks of the *abo*^{1}-induced maternal-effect lethality is its interaction with the heterochromatic *ABO* loci. This interaction can be assayed by comparing the sex ratio of the progeny of two crosses: *X/X; abo*^{1}/*abo*^{1} females mated to *X-Y/O* males and *X/X; abo*^{1}/*abo*^{1} females mated to *X-Y/Y* males. Relative to *X-Y/X* daughters, *X/Y* sons with two doses of sex chromosome heterochromatin survive better than *X/O* sons that have only one dose (SANDLER 1970). This differential survival reflects the ability of *ABO* heterochromatin on the *Y* chromosome to zygotically rescue progeny from the *abo* maternal-effect lethality.

We tested the ability of heterochromatin to rescue progeny of *abo*^{1}/*abo*^{2} mothers. The data, summarized in Table 1, show that the addition of a *Y* chromosome increased the viability of sons from *abo*^{1}/*abo*^{2} mothers. The recovery of *X/O* sons from *X-Y/O* fathers was <10% that of *X-Y/X* daughters, whereas the recovery of *X/Y* sons from *X-Y/Y* fathers was ~50%. In these crosses, the *abo*^{2} chromosome was marked with the closely linked dominant wing mutation *J*. Since ~98% of the progeny were expected to be nonrecombinant for *J* and *abo*^{2}, this allowed us to distinguish between *abo*^{1}- and *abo*^{2}-bearing progeny. The recovery of both *abo*^{1} and *abo*^{2} sons was increased by the presence of an additional *Y* chromosome in the zygote. Hence *abo*^{2} acts similarly to *abo*^{1} with respect to zygotic rescue by heterochromatin. However, since these tests necessarily involved *abo*^{1} in the maternal genotype, we cannot eliminate the possibility that the ability of heterochromatin to rescue might be dependent on a unique property of the *abo*^{1} maternal defect.

Phenotypic studies of the *abo*-induced maternal-effect lethality: Previous studies suggested that *abo*^{1} mothers produced embryos with two distinct lethal phenotypes (PIMPINELLI *et al.* 1985; SULLIVAN 1985). One class appeared to arrest at a stage before nuclear cycle 6, with little or no evidence of development. A second class of embryos completed blastoderm formation and formed cuticle but failed to hatch. Because the number of progeny that arrested at the preblastoderm stage showed a decrease with an increase in the zygotic dose of heterochromatin, it was suggested that *ABO* heterochromatin rescues progeny from the preblastoderm death.

We examined the progeny of *abo*^{1}/*abo*^{2} mothers to determine if they showed a biphasic lethal phase and rescue by heterochromatin similar to that reported for the progeny of *abo*^{1} homozygotes. Eggs from *abo*^{1}/*abo*^{2} females mated to either *X-Y/O* or *X-Y/Y* males were collected, allowed to develop for 4 hr, then staged after fixation and staining with the nuclear dye DAPI. Surprisingly, over 96% of the eggs developed past blasto-

TABLE 2

Paternally derived heterochromatin does not affect the survival to blastoderm of embryos from *abo* mothers

Maternal genotype		Paternal genotype	No. embryos examined	% surviving to blastoderm
abo^1/abo^1	A	X-Y/O	609	74.5
abo^1/Cy			645	90.5
abo^1/abo^1	B		217	57.1
abo^1/Cy			284	91.9
abo^1/abo^1	C		659	76.8
abo^1/Cy			685	94.2
abo^1/abo^2			205	96.6
abo^2/Cy			390	98.2
abo^1/abo^1	A	X-Y/Y	508	72.4
abo^1/C			341	95.6
abo^1/abo^1	B		177	71.8
abo^1/Cy			278	93.2
abo^1/abo^1	C		205	72.7
abo^1/Cy			340	94.4
abo^1/abo^2			112	98.2
abo^2/Cy			86	97.8

The results of crosses of females carrying normal *X* chromosomes either homozygous or heterozygous for *abo* by abo^+ males of the indicated sex chromosome constitution. A, B and C are results of three independent experiments.

derm, a percentage similar to control values (Table 2). We next repeated this experiment using homozygous abo^1 mothers and found that an average of 71% of their eggs developed past blastoderm (range 57.1–76.8%, 6 separate trials, a total of 2375 eggs examined). Unlike the previous study (PIMPINELLI *et al.* 1985), we did not observe a consistent difference in these percentages when abo^1 mothers were mated to *X-Y/Y* fathers *vs.* *X-Y/O* fathers. Thus, the amount of paternally contributed *ABO* heterochromatin had no effect on the early arrest class in these experiments.

The data above prompted to us to reexamine the phenotype of embryos of abo^1 mothers that failed to progress to blastoderm. The phenotype of the early arrest class was described by SULLIVAN (1985) as indistinguishable from that of an unfertilized egg. At the time of this earlier study, it could not readily be determined if the early phenotype resulted from an early embryonic arrest or a lack of fertilization. In the present study, eggs were assayed for fertilization by staining with the sperm tail antibody, AXD-5 (KARR 1991). In *D. melanogaster*, the entire sperm enters the egg at fertilization; the axoneme remains intact through the gastrula stage and can be easily visualized after AXD-5 staining (KARR 1991). The efficiency of fertilization of eggs produced by abo^1 females was variable among trials, with from 10 to 25% lower efficiencies when compared to that of control abo^1/Cy females (data not shown). The frequencies were not consistently influenced by the genotype of the father. From abo^1 and abo^1/Cy females mated to *X-Y/O* males, 82% (442/539) and 92.3% (670/726), respectively, contained a sperm tail, compared to 82.8% (284/343) and 94.6% (660/698) when the same classes of females were mated to *X-Y/Y* males, and 88.3% (249/282) and 100% (461/461) when mated to males of a standard wild-type strain (Oregon R). Since the proportion of unfertilized eggs produced by homozygous abo^1 females is similar to the proportion (18–29%) reported to show preblastoderm arrest by PIMPINELLI *et al.* (1985), we conclude that abo^1 early arrest class can be largely accounted for by unfertilized eggs. The variability in the frequency of fertilization observed among individual collections may account for the previous report of apparent rescue of the early arrest class by heterochromatin. The fertilization defect associated with the abo^1 chromosome is recessive and either allele specific or due to a closely linked second mutation. Greater than 96% of eggs from abo^1/abo^2 mothers were fertilized and developed normally through the cellular blastoderm stage.

***ABO* heterochromatin and the euchromatic abo^+ allele rescue a late embryonic lethality:** Table 3 summarizes the results of studies to determine the effect of paternally contributed *ABO* elements and the abo^+ allele on the viability of embryos produced by abo^1/abo^1 or abo^1/abo^2 females. In these studies, ~16% of the eggs produced by abo^1/abo^1 females were not fertilized. Fertilized eggs produced by abo^1/abo^1 or abo^1/abo^2 females failed to hatch because of an arrest late in embryogenesis after cuticle deposition. The percentage of these late embryonic lethals was lower among the progeny *X-Y/Y* fathers when compared to the progeny of *X-Y/O* fathers. This suggests that *ABO* heterochromatin on the *Y* rescues zygotes from a late embryonic *abo* maternal-effect lethality.

Previous work suggested that a paternally contributed copy an abo^+ allele was capable of rescuing a late embryonic lethality (PIMPINELLI *et al.* 1985). Similar results were obtained in our assays among the progeny of abo^1 homozygous or abo^1/abo^2 heterozygous mothers mated to *X/Y* males homozygous for abo^1 or abo^+ (Table 3).

The phenotype we observed in the lethal embryos was consistent with the late embryonic arrest of abo^1-derived embryos by SULLIVAN (1985). Some of these unhatched embryos appeared phenotypically normal. Others showed multiple defects, including failure of head involution, abnormal formation of the cephalopharyngeal apparatus, fusion of ventral denticle belts, holes in the dorsal and ventral cuticle, and in extreme cases, lack of most identifiable cuticular markers. Embryos were classified on the basis of the severity of the cuticular defects (Table 4, Figure 2). The distribution of embryos among these classes was similar among progeny of abo^1/abo^1 and abo^1/abo^2 mothers. In both cases, we found that the progeny of *X-Y/Y* fathers were on average less defective than those of *X-Y/O* fathers (Table 4). This observation supports a role of heterochromatin in ameliorating a defect that affects late embryonic development.

TABLE 3

Rescue of a late embryonic *abo* maternal-effect lethality by paternally derived heterochromatin and abo^+

Maternal genotype	Paternal genotype	No. embryos	% unhatched	No. embryos examined	% arrested after cuticle formation
abo^1/abo^1	*X-Y/O*	1350	41.7	556	25.9
abo^1/Cy		1400	8.4	100	1.5
abo^1/abo^2		630	43.0	164	37.8
abo^2/Cy		380	7.6	29	5.2
abo^1/abo^1	*X-Y/Y*	1100	34.1	145	17.0***
abo^1/Cy		1075	6.8	53	2.1
abo^1/abo^2		1000	19.3	189	16.4*
abo^2/Cy		900	9.9	54	3.7
abo^1/abo^1	*Y/Y;*	923	57.8	535	18.9
abo^1/Cy	abo^1/abo^1	1363	11.5	157	4.3
abo^1/abo^2		720	27.1	195	21.1
abo^2/Cy		590	11.4	67	2.2
abo^1/abo^1	*X/Y;+/+*	934	38.3	358	8.2***
abo^1/Cy		1250	8.4	100	1.5
abo^1/abo^2		700	15.1	106	5.6***
abo^2/Cy		450	13.6	61	3.5

The results of crosses of females carrying normal *X* chromosomes either homozygous or heterozygous for *abo* by abo^+ males of the indicated sex and second chromosome constitution.

* $P < 0.05$; ***$P < 0.001$ for difference in percentage arrested with cuticle among *abo*-derived progeny from *X-Y/O vs. X-Y/Y* or abo^1 *vs.* abo^+ fathers.

To determine if the *abo* mutations caused any maternal-effect lethality after hatching, the survival of larvae to adults was monitored from the same crosses as in Table 3. The data shown in Table 5 shows that the *abo* maternal-effect lethality is not completely restricted to embryogenesis. Of those embryos that hatch, ~22% of those produced by abo^1/abo^1 and 10% of those produced by abo^1/abo^2 mothers died before eclosion. No significant difference in the amount of larval lethality was observed in progeny from *X-Y/O* compared to those of *X-Y/Y* fathers, indicating that additional *ABO* heterochromatin does not rescue the larval lethality. However, a paternally derived abo^+ allele significantly increases larval viability. Thus, the rescue by *ABO* heterochromatin is not identical to rescue by the euchromatic abo^+ allele.

Zygotic effects of the abo^1 and abo^2 alleles: The paternal contribution of an abo^+ allele increases the viability of progeny from abo^1 mothers, presumably by complementing a maternal deficiency of abo^+ product. The viability of zygotes receiving the abo^1 allele paternally is about half that of those that receive the wild-type allele (SANDLER 1970; PIMPINELLI *et al.* 1985), indicating that abo^1 mutation alters both the maternal and zygotic function of the gene. To determine if abo^2 also affected the zygotic function of the gene, we compared the zygotic rescue by the abo^2, abo^1, and the wild-type Canton-S allele. In addition, we tested the *Dp Mdh (2;2)* chromosome that is known to be duplicated for the abo^+ region. Males heterozygous for the test chromosome and a control abo^+ *Sco* chromosome were crossed to abo^1 and abo^1/Cy females, and the survival of zygotes bearing different paternally inherited *abo* alleles were compared. Each second chromosome tested had the same Canton S sec-

TABLE 4

Classification of the defects in lethal embryos of *abo* females

Maternal genotype	Paternal genotype	No. examined[a]	Percentage of progeny in each class[b]					
			I	II	III	IV	V	VI
abo^1/abo^1	*X-Y/O*	345	40.2	20.9	10.4	0	17.1	11.0
	X-Y/Y	71	62.0	8.5	2.8	16.9	1.4	8.5
abo^1/abo^2	*X-Y/O*	154	31.2	31.8	6.5	2.6	11.0	16.2
	X-Y/Y	102	52.9	7.8	4.9	3.7	4.9	14.7

[a] Unhatched embryos were obtained from the same crosses as in Table 3.

[b] Cuticle phenotypes were classified into six groups based on the appearance of the following specific defects: class I, no defect; II, defects in head involution; III, abdominal denticle belt fusions; IV, dorsal holes; V, ventral holes; VI, rudimentary structures only. Classes II–VI are assumed to represent increasingly severe mutant phenotypes. Hence each successive class includes defects from less severe classes.

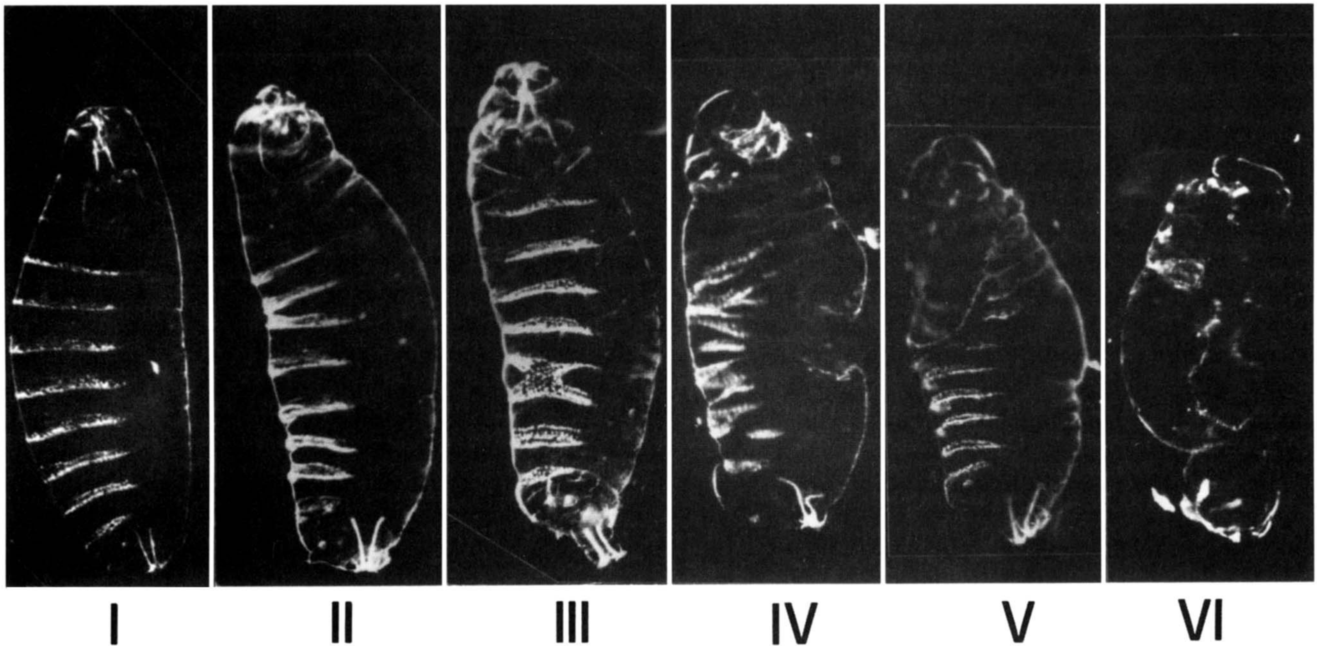

FIGURE 2.—The range of phenotypes observed among the late embryonic lethal progeny of *abo*-derived embryos. Phenotypes were classified into six groups by scoring for the following properties: I, wild type; II, failure of head involution; III, fused denticle belts; IV, holes in dorsal regions; V, holes in ventral regions; and VI, rudimentary cuticular structures. Embryos in the more severe classes contained some of the defects characteristic of the milder classes.

ond chromosome heterochromatin, so any differences could be attributed to euchromatic effects. As shown in Table 6, the amount zygotic rescue contributed by *abo*^{2} was decreased compared to wild type, which indicates that the *abo*^{2} mutation, like *abo*^{1}, also affects the zygotic function of the *abo* locus. The duplication rescued more efficiently than a single *abo*$^+$ allele. This suggests that in one dose, the zygotic *abo*$^+$ function may be a limiting component in determining survival from the maternal defect.

Molecular mapping of the *abo*^{2} and its revertant, *abo*^{2rev}: We began a molecular analysis of the *abo*^{2} allele to gain insight into the nature of the defect. Genomic DNA was isolated from a wild-type strain, from *abo*^{2}, the two parental strains and the *abo*^{2rev} strain and analyzed on Southern blots after probing with labeled *P*-element or *ry* probes (Figure 3). The *abo*^{2} stock shared some hybridizing bands with the *fs(2)ry5* parent stock, but there was no overlap with *Cp70*Δ*B* parent chromosome. This suggests that the *abo*^{2} mutation did not result from

TABLE 5

Larval-to-adult survival among progeny of *abo* mothers

Maternal genotype	Paternal genotype	No. larvae	No. adults	% larval lethality	E − C[a]
abo^1/*abo*^{1}	*X-Y/O*	444	310	30.2	22.8
abo^1/*Cy*		500	463	7.4	
abo^1/*abo*^{2}		359	267	25.6	10.2
abo^2/*Cy*		351	297	15.4	
abo^1/*abo*^{1}	*X-Y/Y*	500	373	25.4	21.7
abo^1/*Cy*		350	337	3.7	
abo^1/*abo*^{2}		797	537	32.6	8.9
abo^2/*Cy*		683	535	21.7	
abo^1/*abo*^{1}	*X/Y; abo*^{1}/*abo*^{1}	388	42	89.2	79.2
abo^1/*Cy*		1206	1085	10.0	
abo^1/*abo*^{1}	*X/Y*; +/+	476	191	59.9	50.5***
abo^1/*Cy*		1144	1036	9.4	

The results of crosses of females carrying normal *X* chromosomes either homozygous or heterozygous for *abo* by males of the indicated sex and second chromosome constitution.

[a] Percentage larval lethality among progeny of homozygous *abo* mothers (E) minus percentage larval lethality among progeny of control *abo/Cy* mothers (C).

*** $P < 0.001$.

TABLE 6

Zygotic rescue from the *abo*[1] maternal effect by different *abo* alleles

Second chromosome	Cross	Phenotype of progeny				Recovery of second chromosome[a]
		+	Sco	Cy	Sco,Cy	
+	E	1313	1207			
	C	1970	1678	2186	1670	0.90
abo[1]	E	408	1078			
	C	1559	1983	2447	1947	0.48***
abo[2]	E	284	904			
	C	399	499	451	544	0.39***
Dp Mdh (2;2)	E	593	283			
	C	716	713	774	687	2.09***

The results of crosses of males bearing the indicated second chromosome and a homologue marked with Sco by *abo*[1]/*abo*[1] (E) or sister *abo*[1]/*Cy* (C) females. The *Dp Mdh (2;2)* chromosome carries two copies of *abo*$^+$.
[a] The recovery of + progeny bearing the indicated second chromosome relative to their Sco siblings. Calculated as ((E)+/(E)Sco)*((c)Sco/(C)+).
*** $P < 0.001$ compared with +.

a simple recombination event between the parental *P* elements. We observed two novel *Bam*HI bands of 8 and 11 kb that hybridized to the *ry*$^+$ probe in *abo*^{2} flies. The *abo*^{2rev} reversion event that restores *abo*$^+$ activity is associated with the loss of the 11-kb band, suggesting that the *abo*^{2} allele was caused by a *P*-element-mediated event. We conclude that the 11-kb *Bam*HI band in the *abo*^{2}-bearing flies results from the association of a *P* element containing at least a partial *ry* gene with the *abo*^{2} mutation.

Localization of the *abo* gene in the 32C polytene interval: We probed salivary gland polytene chromosomes from *abo*^{2}/*Cy* and *abo*^{2rev}/*Cy* larvae with a labeled fragment of the *ry* gene and the *P* element. A single band of hybridization in region 32C of the salivary gland map was observed on the *abo*^{2} chromosome; no signal was detected on the *abo*^{2rev} chromosome. This position is consistent with previous localization of the *abo* locus by recombination and deficiency mapping (SANDLER 1970; MANGE and SANDLER 1972) and further refines the localization of the gene to the 32C cytogenetic interval.

Cloning of the *abo* locus: We constructed a genomic phage library from *abo*^{2}/*Cy* flies and screened this library by plaque hybridization using *ry* and *P*-element probes. Four clones were isolated that shared homology outside the *P* or *ry* sequences and hybridized *in situ* to 32C on wild-type salivary gland polytene chromosomes. Fragments flanking the *P*-element sequences were used to screen a wild-type genomic phage library. One phage, which contained ~17 kb of DNA that spanned the *P*-element site of insertion in *abo*^{2}, gave a single band of hybridization at 32C (Figure 4). A restriction enzyme map of this clone is compared to the *abo*^{2} clone in Figure 5.

Identification of a putative *abo* transcript: Total RNA was isolated from wild-type, *abo*^{1}, and *abo*^{2}/*Cy* adult females and probed with genomic clones on Northern blots to identify the *abo* transcript. When the 2.3-kb *Xho*I-*Eco*RI subclone of the wild-type phage (Figure 5) was used as a probe, two transcripts of 1.8 and 0.8 kb were identified (Figure 6). The 1.8-kb transcript was absent in *abo*^{1} strain and replaced with a larger transcript, suggesting that the 1.8-kb transcript may be the *abo* transcript. We did not observe any variation from wild type in the *abo*^{2}/*Cy* strain.

Rescue of the *abo* defect by germline transformation: To prove that the region we cloned corresponded to the *abo* locus, we used germline transformation to rescue the *abo*^{1} maternal effect lethality. A 9-kb *Bam*HI fragment that was disrupted in the *abo*^{2} allele was isolated from a phage library containing wild-type Oregon R genomic DNA. This fragment was cloned adjacent to the mini-*white* marker gene in the CaSpeR plasmid vector (PIRROTTA 1988). The plasmid was injected into *w*$^-$ flies and five independent transformed lines were obtained. One line expressed nearly wild-type levels of the *w*$^+$ marker gene carried an insertion on the third chromosome. This insertion, denoted *p*[*w*$^+$, 32C], was chosen for further analysis.

We characterized the *P*[*w*$^+$, 32C] insert in the *abo*^{1} background by Southern blotting. Figure 7 shows *Xho*I-*Eco*RI digests of genomic DNAs probed with the 2.3-kb *Xho*I-*Eco*RI fragment of the wild-type phage clone (Figure 5). *P*[*w*$^+$, 32C] contains a 2.3-kb fragment present on several wild-type *abo*$^+$ chromosomes. However, the 2.3-kb fragment is replaced by two other fragments of 1.9 and 1.4 kb on the *abo*^{1} chromosome and by a 3-kb fragment on the *abo*^{2} chromosome.

We tested the ability of the third chromosome insert to complement the *abo*^{1} maternal defect. Females that were *abo*^{1}/*abo*^{1} or *abo*^{1}/*Cy* and carrying no copies, one copy or two copies of the *P*[*w*$^+$, 32C] insertion were crossed to *X-Y*/O or *X-Y*/*Y* males. The data summarized in Table 7 show that one copy of *P*[*w*$^+$, 32C] significantly increases the recovery of both *X*/O and *X*/*Y* sons from *abo*^{1}/*abo*^{1} mothers. However, one copy

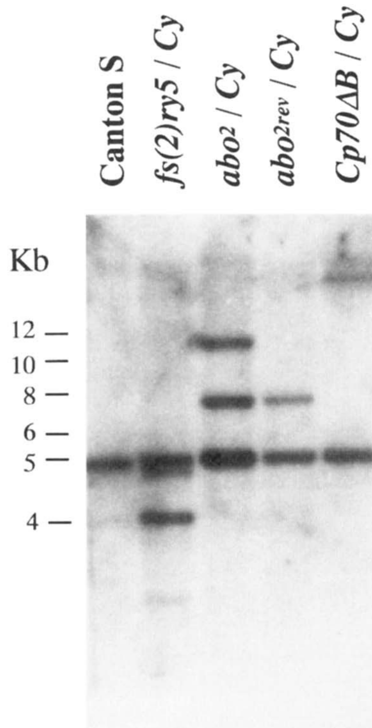

FIGURE 3.—Molecular characterization of the abo^2 lesion. Autoradiograph of a Southern blot containing *Bam*HI digests of genomic DNA isolated from adults of the indicated genotypes. The blot is probed with the radiolabeled *Hin*dIII-*Xho*I fragment of the *ry* + gene. All lanes show a 4.8-kb band of hybridization that corresponds to the endogenous ry^{506} allele present in all of the strains. Two bands of 4 and 4.7 kb were present in the *fs*(2)*ry5* flies, indicating that two ry^+ *P* elements reside on the *ry5* chromosome. The *Cp70ΔB*; ry^{506} flies contained only one band in addition to the ry^{506} band, which corresponds to the single ry^+ *P* element inserted at 32C/D. Note the two novel 8 and 11-kb bands the abo^2/Cy lane, one of which is missing in the abo^{2rev}/Cy lane.

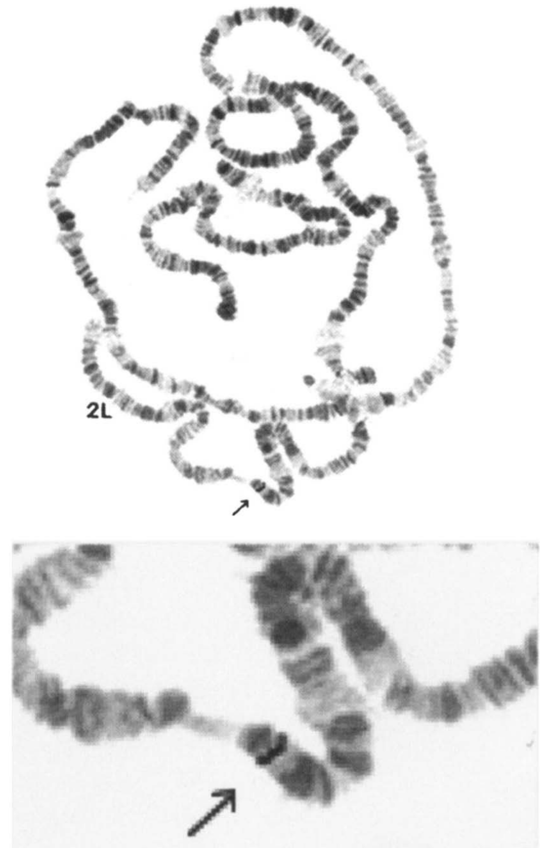

FIGURE 4.—Cytogenetic localization of the abo^+ gene on the salivary gland chromosomes. Chromosomes were obtained from a wild-type larva, hybridized *in situ* with a phage clone containing genomic sequences that flank the abo^2 insertion site. The band that stains brightly on the left arm of chromosome *2* (2L) is located within region 32 C (arrow).

of the transposon does not completely rescue the maternal defect. Two maternal copies of the insert, however, further enhanced this partial rescue. From these results, we conclude that the 9-kb *Bam*HI genomic fragment contains the abo^+ gene. The ability of the *P*[w^+, 32C] insert to complement abo^1 appears to be dosage dependent, suggesting that levels of expression from the insert may be lower than from an endogenous abo^+ gene.

We also tested the ability of this insert to rescue zygotes from the *abo* maternal effect lethality. Homozygous *w*; abo^1 females and *w*; abo^1/Cy control females were mated to *w*; *P*[w^+, 32C]/+ males, and the recovery of progeny that received the *P*[w^+, 32C]-bearing third chromosome was compared to those that received the unmarked third chromosome. The *P*[w^+, 32C] chromosome was recovered at a frequency approximately equivalent to its homologue from the progeny of the control cross (w^+:w^- recovery ratio = 1.04, n = 1460 progeny scored). However, when the progeny of abo^1 mutant mothers were scored, the recovery of *P*[w^+, 32C] was significantly greater than *w* progeny (w^+:w^- recovery ratio = 2.47, n = 718). Thus, the presence of the *P*[w^+, 32C] insertion in zygotes increases their survival from the *abo* maternal-effect defect. We conclude that the 9-kb *Bam*HI fragment also provides zygotic abo^+ function.

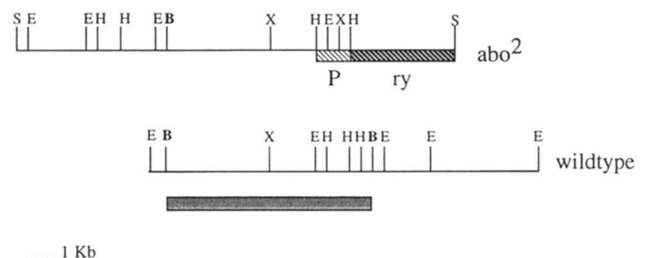

FIGURE 5.—A comparison of the restriction maps of the sequences isolated from the abo^2 and wild-type genomic phage libraries. The position of the P[*ry*+] insertion in the abo^2 strain is indicated. The wild-type 17-kb DNA clone from a wild-type genomic phage spans the abo^2 insertion site. The cross-hatched box below the map shows the location of the 9-kb *Bam*HI DNA fragment inserted in the CaSpeR vector and used for germline transformations. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I; X, *Xho*I.

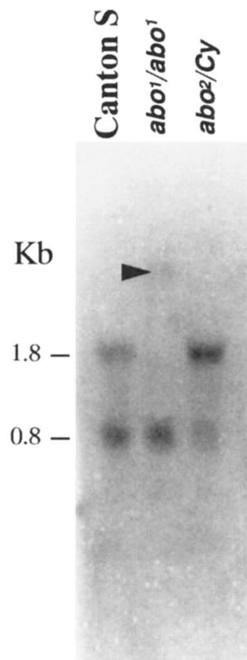

FIGURE 6.—Autoradiograph of a Northern blot of total RNA (10 μg RNA per lane) from adult females probed with the 2.3-kb *Xho*I-*Eco*RI fragment of the wild-type genomic phage clone. Two fragments of 1.8 and 0.8 kb are present in both the wild-type and *abo²/Cy* lanes. However in *abo¹* females, the 1.8-kb band is replaced by larger transcript (arrowhead).

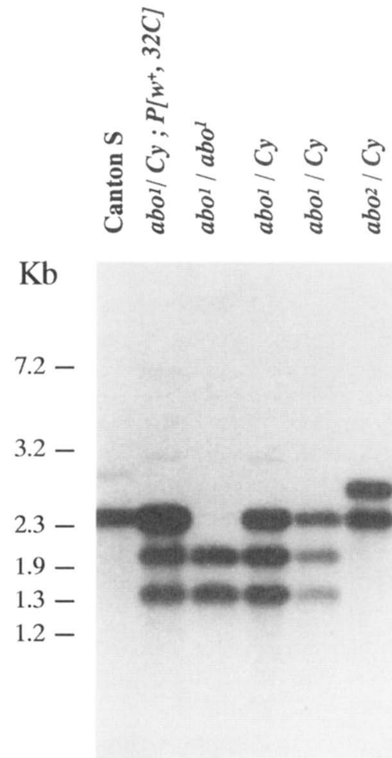

FIGURE 7.—Autoradiograph of a Southern blot of genomic DNAs from adult flies of the indicated genotypes, digested with *Xho*I and *Eco*RI and probed with the 2.3-kb *Xho*I-*Eco*RI fragment of the genomic phage clone. Note that the wild-type 2.3-kb fragment is replaced by a 3-kb fragment on the *abo²* chromosome and by 1.9 and 1.4 kb fragments on the *abo¹* chromosome.

DISCUSSION

This report describes the initial genetic and molecular characterization of *abo²*, a *P*-element-induced allele of *abnormal oocyte*. The results have allowed us to clarify certain features of the *abo* mutant phenotype and reexamine the nature of the interaction of *abo* with the heterochromatic *ABO* elements.

Whereas previous reports suggested that *ABO* heterochromatin rescues a preblastoderm embryonic lethality (PIMPINELLI *et al.* 1985; SULLIVAN 1985), we failed to find evidence of an early lethality among the progeny of homozygous *abo¹* mothers or heterozygous *abo¹/abo²* mothers. We did, however, find that the *abo¹* chromosome causes a recessive fertilization defect that shows considerable variability. The phenotype of unfertilized eggs is consistent with the phenotype of the *abo¹* "early lethality" described by SULLIVAN (1985). The use of an antibody to the Drosophila sperm tail in the present studies has provided the means to accurately distinguish between a fertilization defect and a failure in early embryogenesis.

We have demonstrated that the progeny of *abo* mutant mothers die predominately late in embryogenesis with some lethality occuring during the larval stages. The lethal embryos show a range of cuticular defects that indicate that they fail to complete the movements of gastrulation, such as normal germband formation, head involution and dorsal closure. Our data show that the *ABO* elements rescue the late embryonic lethality. We have also confirmed earlier studies that show that the paternally derived *abo⁺* allele rescues this same stage of lethality, as well as the larval lethality. These findings allow for models in which the *ABO* heterochromatin may exert its effects on the expression of zygotically active genes. It is important to note, however, that the studies by TOMKIEL *et al.* (1991) show that the presence of *ABO* heterochromatin in oogenesis or in the earliest stages of embryogenesis is sufficient for the rescuing effect. That is, *ABO* heterochromatin serves its function before the completion of the early cleavage divisions; induced loss of *ABO* factors subsequent to these divisions has no effect on viability of embryos from *abo* mutant mothers. These results, taken together with those of the present study, suggest that the influence of heterochromatin on embryogenesis occurs well before transcriptional activation of the zygotic genome but that the effects are manifested in later developmental stages.

We may now reconsider the models that have been proposed to explain the nature of the interaction of *abo* and *ABO* elements. One class of models invokes the direct suppression of the *abo* mutation by *ABO* heterochromatin. LAVORGNA *et al.* (1989) have suggested such a model based on the interaction of the gypsy transposable element with the *su(Hw)* gene, a known suppressor

TABLE 7

Rescue of the *abo*[1] maternal effect defect by P[w^+, 32C]

Maternal genotype	Paternal genotype	Males	Females	Male recovery[a]
abo[1]/*abo*[1];+/+	X-Y/O	564	1626	0.37
abo[1]/*abo*[1];P[w^+, 32C]+		1196	1951	0.66
abo[1]/*abo*[1];P[w^+, 32C]/P[w^+, 32C]		230	318	0.78
abo[1]/Cy;+/+		1677	1805	
abo[1]/*abo*[1];+/+	X-Y/Y	443	702	0.66
abo[1]/*abo*[1];P[w^+, 32C]/+		1153	1500	0.81
abo[1]/*abo*[1];P[w^+, 32C]/P[w^+, 32C]		80	192	0.99
abo[1]/Cy		1604	1694	

[a] Calculated as the ratio of sons to daughters among the progeny of *abo* females divided by the same ratio among progeny of *abo*/*Cy* females used as a control.

of the mutagenic effect of gypsy (CORCES and GEYER 1991). These authors mapped a copia-like transposable element, the *blood* element to 32E, the presumed site of the *abo*[1] mutation. They proposed that the insertion of the blood element into or near the *abo* gene caused a mutant phenotype that could be directly suppressed by *ABO* heterochromatin. Our data argue strongly against this model. First, we have localized the *abo* mutation to salivary chromosome band 32C, rather than 32E. Second, we have found no evidence for a *blood* element associated with either the *abo*[1] or *abo*[2] mutation (data not shown). Recent cloning of the *abo*[1] mutation reveals that a doc-transposable element is inserted in an exon of the *abo* gene (M. BERLOCO and S. PIMPINELLI, unpublished data). We conclude that the insertion of a *blood* element is not relevant to the *abo* mutant phenotype.

Although other models that invoke direct heterochromatic suppression of the *abo* mutant phenotype are possible, we view these as unlikely. We have shown in crosses with *abo*[1]/*abo*[2] mothers and fathers with various amounts of heterochromatin that the heterochromatin can rescue progeny bearing either the *abo*[1] allele or the *P*-element-induced *abo*[2] allele with equal efficiency. Thus, the rescue by *ABO* heterochromatin must not depend on some unique characteristic of the *abo*[1] mutation. The *abo*[1] and *abo*[2] mutations are unlikely to share similar molecular lesions and it is difficult to imagine a mechanism by which heterochromatin directly suppresses the expression of both of these euchromatic alleles.

We cannot as yet distinguish between models that propose that *abo* and *ABO* have similar functions and models that suggest that the loci have different but interacting roles in development. Clearly, the most straightforward way of addressing these models will be through knowledge of the structure of the *abo* and *ABO* loci and their gene products. If the model proposed by PIMPINELLI *et al.* (1985) holds true, then the heterochromatic *ABO* is expected to be transcriptionally active in the zygote before blastoderm formation and to produce a product similar or identical to abo^+ gene product.

Models that evoke a regulatory interaction between *abo* and *ABO* make different predictions, depending upon the level of interaction. The suggestion that *ABO* heterochromatin acts as a sink to bind products that interact either directly or indirectly with the abo^+ product (SULLIVAN 1985) is similar to the titration models previously proposed to account for the quantitative and trans-acting effects of heterochromatin on position-effect variegation (SCHULTZ 1936; ZUCKERKANDL 1974). Similarly, one could imagine a number of ways how the titration of products by *ABO* sequences might enhance the viability of *abo*-derived progeny. Competition for heterochromatin-associated proteins by *ABO* sequences may have a general effect on chromatin structure. This in turn may affect levels of zygotic transcription of genes whose products are critical to the survival of *abo*-derived progeny. The resulting increase in zygotic viability may be mediated through changes in chromatin structure as a consequence of decreased availability of heterochromatin-associated proteins.

A variant of this titration model is that *ABO* heterochromatin may reduce the availability of products that are responsible for a function that is normally negatively regulated by abo^+ (SULLIVAN 1985). Thus, the abo^+ gene product might itself have a role in regulating heterochromatin. This idea is consistent with observations that changes in heterochromatic sequences occur in stocks that are maintained homozygous for *abo*[1] (KRIDER and LEVINE 1975; KRIDER *et al.* 1979; YEDBOVNICK *et al.* 1980; GRAZIANI *et al.* 1981; MANZI *et al.* 1986; SULLIVAN and PIMPINELLI 1986).

The cloning of the euchromatic *abo* gene and identification of its mRNA product represent the first steps toward a molecular understanding of *abo* function. The molecular tools will allow us to test various aspects of the models, including examining the effect of adding increasing amounts of *ABO* on the timing and levels of expression of the *abo* mRNA and protein products.

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