Rescue from the *abnormal oocyte* Maternal-Effect Lethality by *ABO* Heterochromatin in *Drosophila melanogaster*

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ABSTRACT

The euchromatic maternal-effect mutation *abnormal oocyte* (*abo*), of *Drosophila melanogaster* interacts with regions of heterochromatin known as *ABO*, which reside on the *X*, *Y* and second chromosomes. Here, we show that survival of progeny from *abo* females depends in part upon the maternal dosage of *ABO* heterochromatin. A comparison was made of the recovery of genotypically identical progeny from *abo* mothers bearing sex chromosomes of various *ABO* contents. The results show that the recovery of daughters was decreased if mothers were ABO^-/ABO^-. However, no decrease was observed if mothers were ABO^+/ABO^-. In addition, the survival of daughters was greater when they received an ABO^- *X* chromosome from an ABO^-/ABO^+ mother rather than the father. We suggest that these results reflect a complementation or interaction between the *ABO*-deficient *X* and the *ABO* heterochromatin in the maternal genome. This proposed interaction could occur early in oogenesis in the mother or prior to completion of meiosis I in the fertilized egg. To determine if zygotic dosage of *ABO* heterochromatin might also be important at very early stages of embryogenesis, we examined the timing of zygotic rescue by paternally donated *ABO* heterochromatin using a second mutation, *paternal loss* (*pal*). Homozygous *pal* males produce progeny which lose paternally derived chromosomes during the early zygotic divisions. Zygotes that have lost a paternal sex chromosome in a fraction of their nuclei will be mosaic for the amount of *ABO* heterochromatin. By monitoring the recovery of *pal*-induced mosaics from *abo* and abo^+ females, we could determine the temporal and spatial requirements for *ABO* function. Results show that the survival of progeny from the *abo* maternal-effect lethality was increased if *ABO* heterochromatin was present prior to the *pal*-induced loss event. Analysis of mosaic patterns did not reveal a specific lethal focus. We conclude from these results that *ABO* heterochromatin serves its vital function prior to completion of the early cleavage divisions in progeny of *abo* mothers.

THE *abnormal oocyte* mutation, *abo*, is a recessive maternal-effect mutation which maps to position 38 on the left arm of chromosome *2* (salivary bands 32 A–F), of *Drosophila melanogaster* (SANDLER 1970; MANGE and SANDLER 1973; TOMKIEL 1990). The viability of progeny from *abo* mothers is reduced compared to progeny of abo^+ mothers, but this maternal-effect lethality can be partially rescued by heterochromatin in the zygote (SANDLER 1970; PARRY and SANDLER 1974; PIMPINELLI *et al.* 1985). Regions of heterochromatin shown to be effective in rescuing zygotes from the *abo* maternal-effect lethality have been termed *ABO*. The *X*, both the short and long arms of the *Y*, and the right arm of the second chromosome have been demonstrated to contain *ABO* heterochromatin (PARRY and SANDLER 1974; B. S. GANETZSKY and J. S. HAEMER, cited in SANDLER 1977; YEDBOVNICK, KRIDER, and LEVINE 1980; PIMPINELLI *et al.* 1985).

Two questions regarding the interaction between the *abo* maternal effect and *ABO* heterochromatin are addressed here. First, we asked if there are maternal effects of *ABO* heterochromatin, in other words, if the maternal dosage of *ABO* could influence the survival of zygotes from the *abo* maternal-effect lethality. Second, using mosaics created by zygotic loss of sex chromosomes, we asked if the presence of *ABO* heterochromatin in *abo*-derived zygotes during the initial cleavage divisions was sufficient to affect viability.

Evidence for a maternal effect of *ABO* heterochromatin has been previously obtained by SANDLER (1970) and MALVA *et al.* (1985). Sandler found that progeny of *abo* mothers survived better if the maternally contributed heterochromatin came from a female with an additional heterochromatic *Y* chromosome. He compared the survival of progeny of *X-Y*/O fathers (where *X-Y* denotes the attachment of the *X* and *Y* chromosomes to the same centromere, and O denotes the absence of a free sex chromosome homolog) and either *X/X;abo* or *X/X-Y;abo* mothers were compared. Relative to the same *X-Y/X* sisters, The *X*/O sons of *X/X* mothers are recovered at a much lower frequency (0.09) than *X*/O sons of *X/X-Y* mothers (0.37). MALVA *et al.* found that survival of progeny

[1] Deceased.

from *In(1)sc⁴;abo* mothers was decreased relative to the survival of progeny from *In(1)sc⁴/+;abo* mothers. *In(1)sc⁴* is an *X* chromosome inversion which translocates heterochromatin to distal euchromatin. It was proposed that this reduction in progeny survival resulted from a maternal effect caused by a partial disruption of *ABO* function by this rearrangement.

Neither of these previous studies, however, demonstrated that the effects observed mapped to the regions containing *ABO* heterochromatin. We tested the effects of maternal *ABO* constitution more directly by using cytologically defined heterochromatic deletions which had previously been demonstrated to remove *ABO* function in assays of zygotic rescue. We monitored the effect of maternal *ABO* dosage on survival of *abo*-derived progeny, and also measured the effects on zygotic viability when an *ABO*-deficient chromosome was contributed paternally *vs.* maternally. Results suggest that maternal as well as zygotic dosage of *ABO* heterochromatin is important in determining the viability of progeny of *abo* mothers.

The second question we asked was when in development the interaction between *ABO* heterochromatin and the *abo* maternal defect was critical. A proportion of embryos from *abo* mothers die before hatching (SULLIVAN 1985; TOMKIEL 1990). SANDLER (1970) showed that the *abo* maternal-effect lethality which could be rescued by heterochromatin was temperature sensitive, and that the temperature-sensitive period was prior to completion of embryogenesis. To determine more precisely when the presence of *ABO* heterochromatin was important, we used the paternal-effect mutation *paternal loss* (*pal*) (BAKER 1975) to create mosaics in which *ABO* heterochromatin had been eliminated from a fraction of nuclei early in development. We then monitored the recovery of these mosaics from *abo vs. abo⁺* mothers to determine the developmental stage during which the presence of *ABO* was necessary for rescue. The distribution of tissues with decreased amounts of heterochromatin in these mosaic progeny was also analyzed to determine tissue or region-specific requirements for *ABO* function. An increase in the recovery of *abo*-derived progeny was correlated with the presence of *ABO* heterochromatin prior to *pal*-induced chromosome loss. This result suggests that *ABO* heterochromatin rescued prior to *pal*-induced loss events. We conclude that the critical period for the presence of *ABO* heterochromatin is either in the father during spermatogenesis, or during the initial cleavage divisions in the zygote. No tissue-specific requirements for *ABO* heterochromatin were found.

MATERIALS AND METHODS

Drosophila crosses: 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 GRELL (1968) or LINDSLEY and ZIMM (1987). For progeny per mother counts, two males and one female were mated in vials on day 0, removed on day 6, and progeny were counted until day 18. All other crosses were single pair matings, but were otherwise treated in the same manner. For egg to adult counts, two males and one female were mated in vials on day 0, and transferred to vials containing fresh media every twelve hours until day 6. Eggs were counted after each collection, and daughters were counted which eclosed within 18 days after the collection was made.

For a comparison of recovery values, the Mantel-Haenzel estimate of odds ratio was used to generate expected matrix values (BISHOP, FIENBERG, and HOLLAND 1975). These were then used to calculate chi-square values. All other statistical comparisons were made using a standard $2 \times N$ contingency test.

Correction for a haplo-insufficient locus: An adjustment was necessary to estimate progeny per mother yields in crosses involving *In(1)sc^{4L}sc^{8R}/Df(1)C3* mothers. *X* chromosome recombination in *In(1)sc^{4L}sc^{8R}/Df(1)C3* mothers produces chromosomes duplicated or deleted for the region between the *sc^{4L}* breakpoint of the *In(1)sc^{4L}sc^{8R}* chromosome and the *w^{m5l6}* inversion breakpoint of the *Df(1)C3* chromosome. Using an *In(1)sc^{4L}sc^{8R}* chromosome marked with *y* and *w* we found that this region contained a haplo-insufficient locus. When *In (1)sc^{4L}sc^{8R}, y w/Df(1)C3* females were mated to *y w* males, no *y w⁺* progeny were recovered. This finding, in combination with results of STEWART and MERRIAM (1973), places a haplo-insufficient locus between salivary bands 2A and 3C1-2. Because levels of *X* chromosome recombination were expected to be higher in control *abo/Cy* mothers due to the interchromosomal effect (LUCCHESI 1976) and the meiotic defect associated with *abo* (CARPENTER and SANDLER 1974), we adjusted progeny per mother values to compensate for the absence of the haplo-insufficient class. To do this, we estimated the frequency of recombination between *In(1)sc^{4L}sc^{8R}, y w* and *Df(1)C3* in both *abo* and *abo/Cy* females by counting the percentage of duplication-bearing (*y⁺ w*) daughters produced (10.8% from *abo* and 25.3% from *abo/Cy* mothers; data not shown). In crosses involving *In(1)sc^{4L}sc^{8R}/Df(1)C3* mothers, we increased the daughter per mother values by these amounts.

Analysis of gynandromorphs and mosaics: Gynandromorph progeny (denoted *X/O//X/X*) from the mating of *y w sn³/y w sn³; abo/abo* or *abo/Cy* females to *+/y⁺Y; pal* males were compared as in BAKER (1975). Thirty-eight adult cuticle structures per side were scored for loss of the paternal *X*, indicated by the phenotype of the recessive maternal *X*-linked mutations *y,w* and *sn³*. To determine the proportion of *X/O* tissue in a gynandromorph , a structure was given a score of zero if wild type, one if *y w sn³*, or 0.5 if mosaic. To compare the distribution of patch sizes between progeny of *abo/abo vs. abo/Cy* mothers, gynandromorphs (n = 138 and 227, respectively) were classified into ten groups of equal size by percent *X/O* tissue per fly, and the two distributions were compared using a 2×10 contingency test. Similar comparisons were made considering only tissue in the head, or only in the thorax, or only in the abdomen. The frequency of chromosomal loss events occurring in each cuticle structure was calculated as the average score for that particular structure.

Progeny of *abo/abo* and *abo/Cy* females which were mosaic for *Y* or fourth chromosome loss were obtained by mating *y w sn³* females to either *+/y⁺Y;pal/pal* males to recover *X/O//X/Y* mosaics, or to *y/Y;pal/pal;y⁺·spa^{pol}/y⁺·spa^{pol}* males to recover *4/O//4/4* mosaics. In both of these cases, paternal chromosome loss was indicated by phenotypically yellow

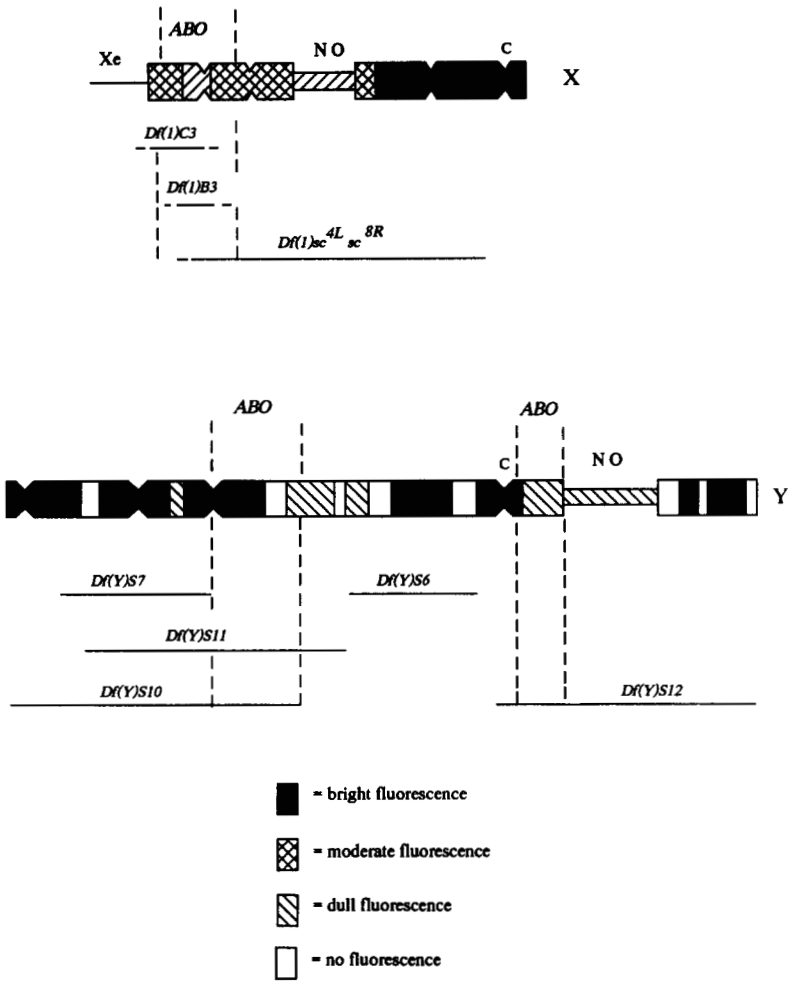

FIGURE 1.—Diagram of the heterochromatin of the *X* and *Y* chromosome, illustrating the cytologically distinguishable subunits. The extent of the chromosome deficiencies used in this study are indicated by solid lines below each chromosome. "C" indicates the position of the centromere, "NO" indicates the nucleolus organizer. The diagram and the cytological characterization of the deficiencies are from GATTI and PIMPINELLI (1976) and S. BONACCORSI, S. PIMPINELLI and M. GATTI, in PIMPINELLI *et al.* (1985).

tissue. These mosaics were compared in the same manner as the gynandromorphs described above.

RESULTS

***ABO* heterochromatin has a maternal effect:** To test for an influence of maternal dosage of *ABO* heterochromatin on the severity of the *abo* maternal defect, we counted the numbers of female progeny produced by *abo* mothers of various sex chromosome constitutions. Male progeny were not compared in these crosses because of viability differences owing to the various *X* chromosome deficiencies used. Mothers bearing combinations of the following *X* chromosomes were used: + [a wild-type *Canton-S X* with intact heterochromatin (ABO^+)], *In(1)dl-49* (a balancer *X*, ABO^+), and *Df(1)C3* and $In(1)sc^{4L}sc^{8R}$, chromosomes with heterochromatic deficiencies that remove *ABO* heterochromatin (ABO^-) (Figure 1). These chromosomes have previously been characterized cytologically, and assessment of their *ABO* content has been made on the basis of the ability to rescue zygotes from the *abo* maternal-effect lethality (PIMPINELLI *et al.* 1985).

The results in Table 1 show that a deletion of *ABO* heterochromatin on one *X* of an *abo* mother had no measurable effect on the survival of ABO^+/ABO^+ daughters. Recoveries of *In(1)dl-49*/+ or +/+ daughters were the same from *In(1)dl-49*/+, *In(1)dl-49*/*Df(1)C3* and $+/In(1)sc^{4L}sc^{8R}$ mothers. However, daughters which received an *ABO*-deficient *X* from their mothers survived only 75% as well as their sisters which received an intact maternal *X*. This result reflects the ability of *ABO* heterochromatin to increase survival when present in the zygote (SANDLER 1970; PARRY and SANDLER, 1974; PIMPINELLI *et al.* 1985). When both *X* chromosomes of an *abo* mother were deficient for *ABO* heterochromatin, as in $In(1)sc^{4L}sc^{8R}$/*Df(1)C3;abo* females, we observed an enhancement of the *abo* maternal effect. The recovery of $+/In(1)sc^{4L}sc^{8R}$ daughters from these ABO^-/ABO^- mothers was significantly lower than the recovery of the same class of progeny from ABO^+/ABO^- mothers ($P < 0.01$). Similarly, the recovery of +/*Df(1)C3* daughters from ABO^-/ABO^- mothers was significantly lower than their recovery from ABO^+/ABO^- mothers ($P < 0.01$).

To determine if the reduced recovery of daughters from ABO^-/ABO^-;*abo/abo* mothers resulted from a decrease in zygotic viability or in fecundity, we counted the number of eggs produced from the same crosses as in Table 1, and the percent of those eggs

TABLE 1

The effect of maternal dosage of *ABO* heterochromatin on the *abo*-induced maternal effect

X Chromosomes of mothers	Cross	No. of mothers	Female progeny				Daughters per mother ± SE		Relative survival	
			+; Cy	y; Cy	+; +	y; +	+	y	+	y
In(1)dl-49 y Hw m² g⁴ / +	C	72	912	828	852	782	24.5 ± 0.79	22.4 ± 0.67	0.47	0.50
	E	132			1515	1467	11.6 ± 0.79	11.2 ± 0.80	0.43 ≤ × ≤ 0.52	0.45 ≤ × ≤ 0.56
In(1)dl-49 y Hw m² g⁴ / *Df(1)C3*	C	118	1257	1256	1380	1279	22.3 ± 0.81	21.5 ± 0.78	0.36	0.47
	E	230			1847	2353	8.0 ± 0.52	10.2 ± 0.61	0.32 ≤ × ≤ 0.40	0.43 ≤ × ≤ 0.49
In(1)sc⁴ᴸsc⁸ᴿy cv v B / +	C	76	828	799	817	810	21.6 ± 0.85	21.2 ± 0.87	0.51	0.37
	E	148			1635	1141	11.1 ± 0.75	7.8 ± 0.60	0.46 ≤ × ≤ 0.57	0.33 ≤ × ≤ 0.42
In(1)sc⁴ᴸsc⁸ᴿy cv v B / *Df(1)C3*	C	115	1421	948	1422	1007	24.7 ± 0.93 (27.6)	17.0 ± 0.67	0.17	0.22
	E	233			1002	874	4.3 ± 0.34 (4.6)	3.8 ± 0.28	0.15 ≤ × ≤ 0.20 (0.17)	0.20 ≤ × ≤ 0.25

The female offspring from crosses of sister *abo/In(2LR)Cy* (C) and *abo/abo* (E) females carrying the indicated sex chromosomes by *abo*⁺ males carrying a normal *X* chromosome marked with *y* and a normal *Y* chromosome. The numbers in parentheses are estimated to compensate for haplo-insufficient recombinants as explained in MATERIALS AND METHODS.

which survived to adults. Again, only daughters were considered because of the differences in the sons' viablities which were unrelated to *abo*. Table 2 shows that egg production by *abo/abo* mothers relative to control *abo/Cy* sisters was unaffected by their *X* chromosome constitution. Thus, the differences in recovery of daughters observed in Table 1 can be attributed to differences in progeny viabilities. On the basis of these data, it was assumed that the recovery of daughters in the remainder of the crosses also reflected viability rather than fecundity differences.

Enhancement of the *abo* maternal effect requires deficiencies of *ABO* on both maternal *X* chromosomes: A possible reason why we observed no maternal effect of *ABO* dosage when mothers were deficient on only one *X* chromosome is that the decrease in maternal heterochromatin in ABO^-/ABO^+ mothers may not have been sufficient to affect the viability of the ABO^+/ABO^+ progeny class we monitored. To determine if an effect could be revealed in ABO^+/ABO^- zygotes, we monitored the recovery of progeny from ABO^+/ABO^+ *vs.* ABO^+/ABO^- mothers crossed to fathers which carried the *ABO*-deficient *X*, *Df(1)C3*. We also monitored the recovery of progeny from ABO^-/ABO^- mothers and *Df(1)C3* fathers males to determine if the maternal effect of *ABO* dosage was more severe when zygotes received two deficient *X* chromosomes (ABO^-/ABO^-).

The data in Table 3 show that a deficiency for *ABO* heterochromatin on only one maternal *X* was not sufficient to enhance the *abo* maternal effect, even when the zygotic *ABO* content was also reduced. We again observed an enhancement of the *abo* maternal effect when both maternal *X* chromosomes lacked *ABO* heterochromatin, but saw no effect when only one *X* was deficient. The recovery of ABO^+/ABO^- zygotes was the same whether mothers were ABO^+/ABO^+ or ABO^+/ABO^-. Furthermore, a decrease in maternal *ABO* content had the same effect regardless of zygotic *ABO* content. The twofold decrease in recovery of ABO^-/ABO^- zygotes from ABO^-/ABO^- *vs.* ABO^+/ABO^- mothers (0.09 *vs.* 0.17) was similar to the decrease in recovery of ABO^+/ABO^- zygotes from the same mothers (0.17 *vs.* 0.36). These results demonstrate a maternal effect of *ABO* dosage which is independent of the overall zygotic *ABO* content.

Parental source effects on rescue from the *abo* maternal-effect lethality: The recovery of ABO^+/ABO^- daughters was greater if the source of the ABO^- chromosome was maternal. A comparison of recoveries of progeny when specific chromosomes were either paternally or maternally contributed is summarized in Table 4. *In(1)sc⁴ᴸsc⁸ᴿ*/+ and *Df(1)C3*/+ progeny which received the ABO^- chromosome from their mother were recovered better than +/*Df(1)C3* daughters which received the ABO^- chromosome from their father. (In crosses involving *Df(1)C3*, it should be noted that *Df(1)C3* is lethal in the homozygous condition because of a euchromatic deficiency of proximal material, therefore all the *Df(1)C3*-bearing daughters from *Df(1)C3* fathers and *Df(1)C3*-bearing mothers will have received this chromosome from their fathers.) This result is unlikely to reflect a paternal effect owing to differences between the total heterochromatic content of *Df(1)C3* and + fathers, since they have approximately the same amount of *ABO* heterochromatin. *Df(1)C3* fathers carried *Ymal*⁺, a *Y* chromosome with a duplication of proximal *X* euchromatin which is deleted from *Df(1)C3*. Based on genetic tests, *Ymal*⁺ appears to also be duplicated for *ABO*

TABLE 2

The relative effect of *abo* on egg production and egg hatch in relation to *ABO* dosage

X chromosomes of mothers	Cross	No. of Mothers	No. of Eggs	No. of Daughters	Per mother Eggs	Per mother Daughters	E ÷ C Eggs	E ÷ C Daughters
In(1)dl-49 y Hw m² g⁴ / +	C	45	3247	1193	72.2	26.5		
	E	45	2338	323	52.0	7.2	0.72	0.38
In(1)dl-49 y Hw m² g⁴ / *Df(1)C3*	C	10	948	169	94.8	16.9		
	E	16	1076	94	67.5	5.9	0.71	0.49
In(1)sc⁴ᴸsc⁸ᴿ y cv v B / +	C	45	3159	1079	70.2	24.0		
	E	45	2223	222	49.4	4.9	0.70	0.29
In(1)sc⁴ᴸsc⁸ᴿ y cv v B / *Df(1)C3*	C	10	649	103	64.9	10.3		
	E	16	756	10	47.3	0.6	0.73	0.08

The results of crosses of sister *abo/In(2LR)Cy* (C) and *abo/abo* (E) females carrying the indicated sex chromosomes by *abo*$^+$ males carrying a normal *X* chromosome marked by *y* and a normal *Y* chromosome. The experimental details are given in the text.

TABLE 3

The effect of maternal dosage of *ABO* heterochromatin on the *abo*-induced maternal effect is independent of the zygotic dosage of *ABO*

X chromosomes of mothers	Cross	No. of mothers	Female progeny	Daughters per mother +	Daughters per mother B	Relative survival +	Relative survival B
In(1)dl-49 y Hw m² g⁴ / +	C	48	2524	52.6 ± 1.59		0.32	
	E	103	1769	16.9 ± 1.41		0.29 ≤ × ≤ 0.36	
In(1)dl-49 y Hw m² g⁴ / *Df(1)C3*	C	54	1252	23.2 ± 1.03		0.27	
	E	109	682	6.3 ± 0.51		0.24 ≤ × ≤ 0.31	
In(1)sc⁴ᴸsc⁸ᴿ y cv v B / +	C	47	2485	27.0 ± 0.80	25.8 ± 0.85	0.30	0.17
	E	92	1156	8.1 ± 0.63	4.5 ± 0.44	0.27 ≤ × ≤ 0.33	0.15 ≤ × ≤ 0.20
In(1)sc⁴ᴸsc⁸ᴿ y cv v B / *Df(1)C3*	C	114	1907		16.7 ± 0.83 (21.0)		0.10
	E	215	362		1.7 ± 0.15 (1.8)		0.09 ≤ × ≤ 0.12 (0.09)

The female offspring from crosses of sister *abo/In(2LR)Cy* (C) and *abo/abo* (E) females carrying the indicated sex chromosomes by *abo*$^+$ males carrying *Df(1)C3/Ymal*$^+$. The numbers in parentheses are estimated to compensate for haplo-insufficient recombinants as explained in MATERIALS AND METHODS.

heterochromatin (PIMPINELLI *et al.* 1985). Thus, any differences in paternal *ABO* dosage would have been restricted to postmeiotic stages, during spermatid maturation.

Using *pal* to test the timing of *ABO* zygotic rescue: The zygotic function of *ABO* heterochromatin during embryogenesis was investigated using the paternal-effect mutation, *paternal loss* (*pal*) (BAKER 1975). Paternally derived chromosomes are lost in progeny of males homozygous for *pal*. Such loss events occur at a low frequency and can involve any chromosome. When sex chromosome loss occurs, *X*/O//*X*/*X* gynandromorphs or *X*/O//*X*/*Y* mosaics are produced. The average size of the *X*/O patches in such progeny is large, suggesting that loss occurs mainly during the initial cleavage divisions (BAKER 1975). Because *ABO* heterochromatin resides on both the *X* and *Y* chromosomes, *pal*-induced loss of either sex chromosome creates individuals mosaic for differing amounts of *ABO* (Figure 2). The recovery of such mosaics among progeny of *abo* females was monitored to identify spatial and temporal requirements for paternally derived *ABO* heterochromatin. The following questions were asked: First, does the presence of *ABO* prior to *pal*-induced loss result in significant rescue? If so, is this rescue dependent on when the chromosome loss occurred? In other words, must *ABO* be present at a specific time during embryogenesis to rescue? Finally, does the pattern of chromosome loss in adult cuticle structures reflect region-specific requirements for *ABO* in embryogenesis?

In addition to mosaics, *X*/O males are also produced

TABLE 4

A summary of the relative recoveries of daughters of *abo* mothers according to heterochromatic dosage

X chromosomes of mothers	X chromosomes of daughters[a]			
	+/+	+/*Df(1)C3*	+/*In(1)sc^{4L}sc^{8R}*	*Df(1)C3*/*In(1)sc^{4R}sc^{8L}*
In(1)dl-49 y Hw m^2 g^4 / +	0.47, 0.50	0.32		
In(1)dl-49 y Hw m^2 g^4 / *Df(1)C3*	0.47	0.27/0.36[b]		
In(1)sc^{4L}sc^{8R} y cv v B / +	0.51	0.30	0.37	0.17
In(1)sc^{4L}sc^{8R} y cv v B / *Df(1)C3*		0.17	0.17[c]	0.09[c]

The entries are the relative survivals from the data in Tables 1 and 3.

[a] "+" here represents *ABO*$^+$ and may be *In(1)dl-49* or *Canton-S*.

[b] The first number represents the recovery when *Df(1)C3* is paternally derived, the second number is the recovery when the same chromosome is maternally derived.

[c] These numbers have been corrected for the haplo-insufficient recombinant class as explained in MATERIALS AND METHODS.

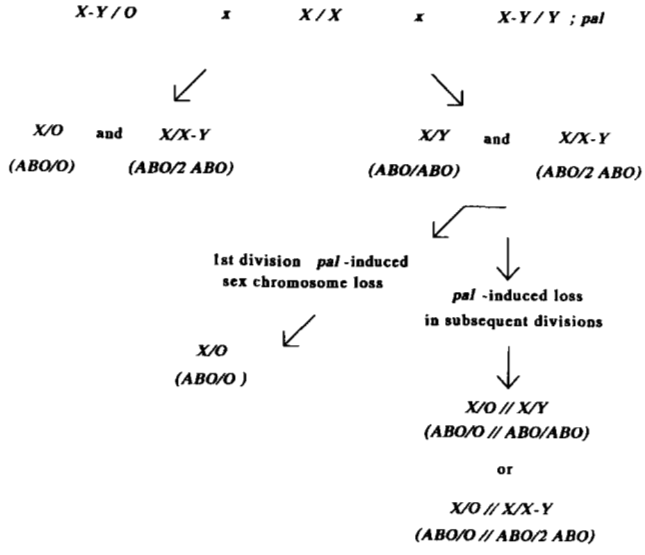

FIGURE 2.—Schematic of alternative modes of production of X/O males. Nullo-*X*, nullo-*Y* gametes are produced by the *X-Y*/O males shown on the left as a consequence of normal meiosis. Fusion with an *X*-bearing maternal gamete produces X/O sons, which do not receive a paternal sex chromosome *ABO* locus. *X-Y/Y;pal* males shown on the right produce either *X-Y* or *Y* bearing gametes. All regular progeny receive a paternal *ABO* locus. X/O sons result from *pal*-induced loss of the paternal sex chromosome prior to completion of the first zygotic division. Sex chromosome mosaics arise from loss of the paternal *X-Y* or *Y* chromosome in subsequent divisions. The sex chromosome *ABO* constitution is indicated in parentheses below each genotype.

from *pal* fathers. Although it is impossible to know if such individuals result from meiotic or zygotic chromosome loss events, a number of lines of evidence suggest that a large proportion of the X/O individuals result from zygotic chromosomal loss. First, *pal* does not increase nondisjunction (BAKER 1975). The frequency of paternal diplo-exceptional progeny in crosses presented here is comparable to background levels of nondisjunction and does not differ among progeny of *pal/pal* and *pal/Cy* fathers (see Table 6). Assuming an equal frequency of paternal nullo-exceptional and diplo-exceptional progeny, we expect less than 10% of the X/O progeny in these crosses to have arisen as a result of nondisjunction. Second, the maternal genotype can affect the frequency of X/O males produced by *pal* fathers (BAKER 1975). This implies that at least some of *pal*-induced X/O progeny arise from postfertilization events rather than meiotic loss. Finally, a cytological examination of embryos from *pal* fathers indicates that a high frequency of zygotic chromosomal loss occurs before completion of the first division (TOMKIEL 1990). Cytologically, chromosomal loss events in subsequent divisions are observed at low frequencies, and therefore only a small fraction of nullo-exceptions are expected to result from coincident loss of a given chromosome at later divisions. Thus, when X/O sons are produced by *X* (*ABO*$^+$)/*Y* (*ABO*$^+$);*pal* fathers, most will have had paternal sex chromosome *ABO* heterochromatin present between fertilization and the first division loss event. Recovery of such X/O progeny from *pal* fathers and *abo* females should reflect any rescue by the paternal sex chromosome *ABO* prior to its loss, *i.e.*, prior to completion of the first division.

The *pal* and *abo* defects do not interact: The above rationale is based on the assumption that the *pal* and *abo* mutations act independently. That is, that the severity of the *abo* maternal effect is not altered by *pal*, and that the cytoplasm of eggs from mutant *abo* females does not affect frequencies of *pal*-induced chromosomal loss. To test these assumptions, the

TABLE 5

The effect of the *abo* maternal-effect on *pal*-induced fourth chromosome loss

Genotype of mother	Constitution of male gametes producing recovered non-mosaic progeny				Mosaic progeny			Frequency of *pal* loss events (per 1000 progeny)		
	Y, 4	*X*, 4	*Y*, O	*X*, O	*X*/*X*//*X*/O	*X*/*X*//*X*/O, 4/4//4/O	4/4//4/O	Nullo-4	4/4//4/O Mosaics[a]	*X*/*X*//*X*/O Mosaics[a]
abo/abo	1521	1891	8	11	6	1	60	5.43	17.43	2.86
abo/SM1, Cy	2275	2737	14	20	11	7	119	6.56	24.31	3.47

Progeny from crosses of sister females bearing *y w sn*^{3} *X* chromosomes and the indicated second chromosomes by *y*/*Y*; *pal*; *y*$^+$·*pol* males. The experimental details are given in the text.

[a] These numbers include progeny which are mosaic for both the *X* and fourth chromosome.

numbers of *pal/abo* progeny per cross were determined from the matings of *Y*^{S}*X*·*Y*^{L},*In(1)EN/y*$^+$*Y;pal/pal* or *pal/Cy* males to *y w sn*^{3}*;abo/abo* or *abo/Cy* females. We observed that the reduction in progeny per mother owing to the *abo* maternal-effect lethality is the same whether fathers are *pal/pal* or *pal/Cy*. From crosses of *pal/pal* fathers and *abo/abo* or *abo/Cy* mothers, numbers of *pal/abo* progeny per mother were 27.05 ± 0.37 (n = 824) and 27.65 ± 0.53 (n = 397), respectively. From crosses of *pal/Cy* fathers and *abo/abo* or *abo/Cy* mothers, numbers of *pal/abo* progeny per mother were 18.67 ± 1.07 (n = 96) and 19.00 ± 0.81 (n = 89), respectively. Note that the *pal/abo* progeny class represents a different proportion of the total progeny for each cross. In crosses with either *pal/pal* or *pal/Cy* fathers, the survival of progeny owing to the *abo* maternal effect is approximately 50%. In addition, recovery of *pal/abo* progeny did not differ significantly from recovery of *pal/Cy* or *abo/Cy* progeny in these same crosses (data not shown). We conclude from these results that the paternal effects owing to the *pal* mutation do not influence the survival of progeny from the *abo* maternal-effect lethality.

To test if the *abo* maternal cytotype altered levels of *pal*-induced chromosome loss, we compared the ratio of progeny per *pal/pal* father to progeny per *pal/Cy* father from the same crosses to *abo/abo* or *abo/Cy* females. A reduction in progeny per father is observed from crosses of *pal/pal* fathers compared to their *pal/Cy* brothers, presumably owing to production of nonviable aneuploids via *pal*-induced autosome loss. The ratio of progeny per *pal/pal* father to progeny per *pal/Cy* father is the same whether mothers are *abo/abo* or *abo/Cy*, suggesting that autosomal loss is not significantly affected by the *abo* cytotype.

This conclusion was assessed more directly by comparing the frequencies of *pal*-induced fourth chromosome loss from *abo/abo vs.* sister *abo/Cy* females. These results also indicate that the *abo* and *pal* mutations do not act synergystically to increase zygotic chromosome loss (Table 5). The fourth chromosome does not contain *ABO* heterochromatin (L. SANDLER, unpublished results), and therefore the recovery of mosaics from *abo/abo* females should directly reflect levels of fourth chromosome loss. No significant differences in fourth chromosome loss were observed from *abo/abo vs. abo/Cy* females. Furthermore, distributions and patch sizes of haplo-4 tissue in adult cuticle did not differ between mosaics from *abo/abo vs. abo/Cy* females, indicating that the haplo-four tissue was not preferentially recovered from *abo/Cy* females (data not shown).

***ABO* rescues the early embryonic *abo* maternal-effect lethality prior to first division *pal*-induced loss events:** Genotypically identical progeny were produced by two means 1) from nullo-*X*, nullo-*Y* sperm produced as a consequence of normal meiosis in males in which the *X* and *Y* chromosomes are attached (males bearing *Y*^{S}*X*·*Y*^{L},*In(1)EN*, denoted as *X-Y*/O), and 2) as the result of a *pal*-induced sex chromosome loss event prior to completion of the first zygotic mitosis. These two classes of *X*/O males differ with respect to *ABO*. Those produced by the latter means will have had additional *ABO* heterochromatin present during the time period between fertilization and the subsequent loss of a sex chromosome. To assess the rescue by *ABO* prior to the first zygotic division, a comparison of the survival of these two classes of *X*/O progeny from *abo* mothers was made.

Table 6 shows the results of crosses between males homozygous or heterozygous for *pal* and females homozygous or heterozygous for *abo*. The recovery of sons relative to their *X-Y/X* sisters from *abo/abo* mothers in the experimental (E) crosses was monitored and compared to the recovery of the same classes from control (C) crosses with *abo/Cy* mothers. The data are expressed as the ratio of these two values and reported in Table 5 as "male recovery." This adjustment is necessary to account for sex ratio differences generated for reasons unrelated to *abo*, such as the presence of recessive *X*-linked deleterious polymorphisms segregating in the *abo* stock, and the meiotic loss observed in *X-Y*/O males (SANDLER and BRAVER 1954).

When *abo/abo* females were crossed to *X-Y*/O males, *X*/O sons were recovered only 29% as frequently as their *X-Y/X* sisters (Table 6). The decreased recovery

TABLE 6

The effect of *ABO-Y* on the recovery of progeny from *abo* mothers and *pal* fathers

Y and second chromosomes of male	Cross	Phenotype of Progeny						Male recovery[a]	
		w sn	y B/+	y w sn	w sn//y w sn mosaics	y B/+//y w sn gynandromorphs	B/+	X/Y	X/O
O; *pal/pal*	E		319	125		1			0.29
	C		745	1,007		0			
O; *pal/SM1, Cy*	E		3,408	1,297		0			0.32
	C		2,736	3,231		0			
y^+ *Y; pal/pal*	E	9,983	12,307	35	4	5	2	0.85	0.73
	C	11,806	12,312	48	4	5	2		
y^+ *Y; pal/SM1, Cy*	E	1,443	1,925	1	0	0	0	0.82	
	C	2,586	2,829	3[b]	0	0	1[b]		
Df(YL)S10, y^+*; pal/pal*	E	5,071	8,154	16	15	6	4	0.67	0.42
	C	6,325	6,811	32	20	2	2		
Df(YL)S10, y^+*; pal/pal*	E	433	690	0	0	1	0	0.65	
	C	423	435	0	0	0	0		
Df(YL)S11, y^+*; pal/pal*	E	1,824	2,235	10	4	1	0	0.65	0.38
	C	6,352	5,095	60	16	3	0		
Df(YL)S11, y^+*; pal/SM1, Cy*	E	386	506	1	0	0	0	0.70	
	C	577	527	0	0	0	0		
Df(YL)S6, y^+*; pal/pal*	E	4,665	6,822	12	3	4	1	0.75	0.45
	C	6,384	6,967	27	6	4	4		
Df(YL)S6, y^+*; pal/SM1, Cy*	E	747	1,154	0	0	1	1	0.78	
	C	529	635	0	0	0	0		
Df(YL)S7, y^+*; pal/pal*	E	1,251	1,601	7	1	1	1	0.79	0.55
	C	2,473	2,509	20	9	2	0		
Df(YL)S7, y^+*; pal/SM1, Cy*	E	430	550	2	0	1	0	0.78	
	C	919	911	0	0	0	1		
Df(YS)S12, y^+*; pal/pal*	E	4,376	6,619	29	16	5	1	0.73	0.51
	C	5,937	6,580	57	28	10	3		
Df(YS)S12, y^+*; pal/SM1, Cy*	E	906	1,273	1	0	0	0	0.76	
	C	1,010	1,075	1	0	0	0		

The progeny of sister *abo/abo* (E) females and *abo/Cy* (C) females bearing *X* chromosomes marked with *y w* sn^3 by males bearing the attached-*XY* chromosome *YSX.YL, In(1)EN y B* and the indicated *Y* homolog and second chromosomes.

[a] Calculated as (E males ÷ E females)*(C females ÷ C males).

[b] Owing to nondisjunction in one vial.

of sons compared to their sisters is a consequence of the difference in zygotic dose of *ABO* heterochromatin on the sex chromosomes. It is important to note that the majority of these *X*/O progeny were produced as a consequence of normal meiotic segregation rather than *pal*-induced loss. Both the low frequency of gynandromorphs from *X-Y*/O;*pal/pal* males and the low frequency of *X*/O progeny from *X-Y/Y;pal/pal* males in subsequent crosses suggest that the *X-Y* chromosome is relatively insensitive to the *pal* defect. Thus, although the *X-Y*/O fathers are homozygous for the *pal* mutation, we believe that the fraction of *X*/O progeny produced by *pal*-induced loss of the *X-Y* chromosome in these first two crosses is insignificant. The recovery of these *X*/O progeny served as the control value for comparison to the recovery of *X*/O males produced as a result of *pal*-induced loss.

The recovery of *X*/O progeny from *abo/abo* mothers is greater when those progeny were produced via a *pal*-induced loss event rather than as a result of normal meiotic segregation. That is, *pal*-induced *X*/O progeny from *X-Y/Y* fathers have a significantly higher recovery value than do *X*/O progeny from *X-Y*/O fathers (Table 6). Furthermore, the increase in *X*/O recovery is proportional to the amount of *ABO* heterochromatin on the paternal *Y* chromosome. This is evidenced by a comparison of the *X*/O recoveries from crosses in Table 6 which involve males bearing one of a series of deficiency *Y* chromosomes. Each deficiency *Y* chromosome is originally derived from the intact y^+Y chromosome (M. SCHWARTZ, unpublished results, cited in GATTI and PIMPINELLI 1983); the cytological extent of each deficiency is shown in Figure 1 (data of GATTI and PIMPINELLI 1983). The *ABO* rescue by each *Y* chromosome is reflected in the *X/Y* male recovery values. The amount of rescue prior

to the first division *pal*-induced loss event can be correlated with the amount of the *ABO* heterochromatin on the chromosome which was lost.

Note that in these experiments the *Df(Y)S6* and *Df(Y)S7* chromosomes behave as if they are partially deficient for *ABO* function. PIMPINELLI *et al.* (1985) found no measurable effect of either of these deficiencies on *ABO* rescue; both *Df(Y)S6* and *Df(Y)S7* rescued *abo*-derived progeny as well as an intact *Y*. We confirmed their results in a recent test performed with *abo* females of the same genetic background which they used (data not shown). Thus, the decrease in zygotic rescue by *Df(Y)S6* and *Df(Y)S7* observed here cannot simply be accounted for by changes in the *Y* chromosomes. More likely, the differences between the two studies are owing to differences in the maternal genetic backgrounds of they *w sn*^{3}*;abo* stock used here and the *abo* stock used in the previous study. This may mean that some *ABO* sequences are contained within the regions deleted by *Df(Y)S6* and *Df(Y)S7*, and the viability of progeny of *y w sn*^{3}*;abo* mothers is sensitive to smaller changes in *ABO* content. This issue is more fully addressed in TOMKIEL (1990).

The *ABO* loci on the *X* and *Y* act similarly: The recoveries of two classes of *X*/O progeny from *abo* mothers were compared. The first class was produced via *pal*-induced loss of the *X* chromosome. In these progeny, paternal *X* chromosome *ABO* heterochromatin was delivered to the zygote and subsequently lost. The second class did not have paternal *X* chromosome *ABO* heterochromatin present at any time.

To produce *X*/O progeny via *pal*-induced loss of the paternal *X*, *abo/abo* and *abo/Cy* females were crossed to *pal/pal* males bearing either a wild-type *X* (*Canton S*, *ABO*$^+$) or *Df(1)B3*, an *X* chromosome bearing a partial deficiency of *ABO* heterochromatin (Figure 1). The low frequency of y^+Y loss in previous crosses, and the high ratio of *X*/O//*X*/*X* gynandromorphs to *X*/O//*X*/*Y* mosaics produced in these crosses provides evidence that the majority of the *pal*-induced *X*/O sons from these crosses resulted from *X* rather than *Y* loss.

For comparison, *X*/O sons were produced which lack paternal *X* chromosome *ABO* heterochromatin throughout development by crossing *Df(1)sc*^{4L}*sc*^{8R}*;pal/pal* fathers to the same classes of females. The *Df(1)sc*^{4L}*sc*^{8R} chromosome is deficient for almost all of the *X* heterochromatin, including the *ABO* heterochromatin (Figure 1). *X*/O sons from these fathers can be produced either by *pal*-induced *X* chromosome loss prior to the first zygotic division, or by meiotic loss (GERSHENSON 1933; SANDLER and BRAVER 1954). For the purpose of this experiment, *X*/O sons produced by either means can be considered identical with respect to *ABO* content. If the *X ABO* heterochromatin functions similarly to the *Y ABO* heterochromatin, then the survival of these males is expected to be lower than the survival of *X*/O sons produced by *pal*-induced loss of the wild-type *X* or the partially deficient *Df(1)B3*.

In Table 7 the recoveries of both *X*/*X* and *X*/O progeny are given relative to *X*/*Y* brothers, which comprise a genetically identical control class for all three sets of crosses. Recovery of *X*/O sons of the *X*-*Y*/y^+Y males from the previous crosses was also compared to the same control class of *X*/*Y* brothers. *X*/O progeny resulting from *pal*-induced *X* loss were recovered at a significantly higher frequency than *X*/O progeny which did not receive paternal *X ABO* heterochromatin.

In addition, recovery of *pal*-induced *X*/O sons was proportional to the amount of *ABO* heterochromatin on the paternal *X* chromosome. The relative recovery rates for *X*/O progeny of *X*-*Y*/*X*, *X*/*Y*, *Df(1)B3*/*Y* and *Df(1)sc*^{4L}*sc*^{8R}/*Y* fathers, respectively, are consistent with the amount of *ABO* heterochromatin on each *X* chromosome. These results suggest that the *ABO* heterochromatin on the *X* chromosome rescues zygotes from the *abo* maternal effect prior to the completion of the first zygotic division.

***ABO* rescues the *abo* maternal-effect lethality prior to *pal*-induced chromosome loss in mosaics and gynandromorphs:** The analysis of gynandromorphs and mosaics is consistent with the idea that rescue from the early *abo* maternal-effect lethality by *ABO* heterochromatin occurs very early in development. The survival of *X*-*Y*/*X*//*X*/O gynandromorphs relative to their *X*-*Y*/*X* sisters was determined for the crosses shown in Table 6. Since the frequency of *pal*-induced loss of the *X*-*Y* chromosome is very low, all of the *X*-*Y*/*X* females and *X*-*Y*/*X*//*X*/O gynandromorphs produced from *pal/pal* males in Table 6 were summed for the purpose of this comparison. Gynandromorphs were recovered as frequently from *abo/abo* mothers as from their *abo/Cy* sisters (23/38,563 *vs.* 26/41,454, respectively). This suggests that the *ABO* heterochromatin on the *X*-*Y* chromosome is not important for rescue from the *abo* maternal effect after the time of the chromosomal loss events in these flies. The average amount of adult cuticle showing a chromosome loss event in these gynandromorphs was 35%. Cytological examination of *pal*-induced chromosome loss events suggests that chromosomes are lost prior to replication, such that when a loss event occurs in a dividing nucleus, both daughter nuclei fail to receive the lost chromosome (TOMKIEL 1990). Thus, these results suggests that *ABO* function is complete prior to the third zygotic division.

A similar comparison was made between the recoveries of *X*/*Y*//*X*/O mosaics and their *X*/*Y* brothers from crosses in Tables 3 and 4. In this case, the progeny of the various crosses are of differing genotypes and therefore must be considered independently. In all crosses, the recovery of *X*/*Y*//*X*/O mosaics

TABLE 7

The effect of *ABO-X* on the recovery of progeny from *abo* mothers and *pal* fathers

X and second chromosomes of male	Cross	Phenotype of progeny					Paternal diploexceptions	F_1 recovery[a]	
		w sn	w^+ sn^+	y w sn	+w sn//y w sn mosaics	+//y w sn gynandromorphs		X/Y	X/O
+; *pal/pal*	E	10,026	12,136	59	7	67		1.01	0.60
	C	9,821	11,772	95	9	158			
+; *pal/SM1, Cy*	E	806	957	2	0	0		0.98	
	C	375	453	1	0	0			
Df(1)b-3, y^2; pal/pal	E	2,809	3,902	19	2	23	1	0.93	0.52
	C	2,908	4,360	38	7	64	2		
Df(1)b-3, y^2; pal/ SM1, Cy	E	595	662	0	0	0	1	0.90	
	C	438	542	0	0	0	1		
Df(1)$sc^{4L}sc^{8R}$, yB; pal/ pal	E	338	628	57	0	1	9	0.62	0.29
	C	383	1,156	225	0	0	12		
Df(1)$sc^{4L}sc^{8R}$, yB; pal/ SM1, Cy	E	383	711	33	1	1	5	0.59	0.32
	C	333	1,049	89	0	0	14		
XSX.YL yB; pal/pal	E	9,983	12,307	35	4	5	1	1.18	0.72
	C	11,806	12,312	48	4	5	2		

The progeny of sister *abo/abo* (E) females and *abo/Cy* (C) females bearing *X* chromosomes marked with *y w sn^3* by males bearing a *y^+Y* and the indicated *X* homolog and second chromosomes.

[a] Calculated as (E (females) + E (males))•(C (males) + C (females)) for female recovery and (E (X/O) + E (X/Y))•(C (X/Y) + C (X/O)) for X/O male recovery.

did not significantly differ from the recovery of their *X/Y* brothers, yet these results must be interpreted with caution since the numbers of mosaics compared in each case is very small. The overall average amount of adult cuticle showing chromosome loss in these mosaics was 46%, and therefore these results are consistent with a function for *ABO* heterochromatin which is completed prior to the end of the second zygotic mitosis.

Finally, the recoveries of *X/X//X/O* gynandromorphs and their *X/X* sisters from crosses in Table 7 were compared. Gynandromorphs from both *+/Y* and *Df(1)B3/Y;pal/pal* fathers survive only 40% as well as their *X/X* sisters. This result can only be reconciled with the above data by proposing this reduction in recovery is unrelated to *ABO* function. A further analysis of these gynandromorphs (below) suggests that this is a reasonable proposition.

Analysis of timing and distribution of *pal*-induced loss events: The patterns of chromosome loss in *X/O//X/X* gynandromorphs from *abo/abo* and *abo/Cy* mothers were analyzed to determine if there were specific temporal and/or spatial requirements for the presence of *ABO* heterochromatin.

No significant differences were found when frequencies of *X* chromosome loss within any given structure were compared in gynandromorphs from *abo vs. abo/Cy* mothers. Nor were any significant differences found in the frequency of loss when the head, thorax and abdomen were considered separately. This suggests either that *ABO* function is nonautonomous or is complete before *pal*-induced loss events in these mosaics. These findings are consistent with results of a similar study which monitored the loss of a ring-*X* chromosome in progeny of *abo* mothers (HAEMER 1977).

The sizes of tissue with chromosome loss in these same gynandromorphs were compared to determine if a requirement for *ABO* function exists after the first division. If *ABO* factors rescue later than the first division, a preferential survival of gynandromorphs showing later loss would be expected from *abo/abo* mothers. Patch size ranged from 3 to 97% in both sets, and the average patch size showing chromosome loss did not differ significantly between progeny of *abo/abo vs. abo/Cy* mothers (42.4% and 42.8%, respectively). Furthermore, when individuals within each set were grouped by patch size, the two distributions obtained appeared identical (data not shown). These observations are consistent with a function of *ABO* prior to completion of the first embryonic mitosis.

***abo* females produce an increased number of polyspermic progeny:** Mosaic adults were recovered from the crosses described in Tables 3 and 4 which were likely to have been products of polyspermy. These flies exhibited the genetic markers of both the paternal *X* and *Y* chromosomes, but in non-overlapping tissues. That is, from *X-Y y B/y^+Y* males mated to *y w sn^3* females, flies were produced which bore *y^+ w sn^3* male tissue and *y B or B/+* female tissue. From *+/y^+Y* males mated to the same females, *y^+ w sn^3* male//+ female gynandromorphs were produced. These individuals were observed at a significantly higher fre-

quency among progeny of *abo/abo vs. abo/Cy* females (38/109,304 *vs.* 5/125,597), and the frequency of such progeny was independent of *pal*. One of these flies, from a *pal/Cy* father, had one straight wing and one Cy wing. All others were Cy^+. In five of these mosaics, the proportion of differing tissue types were approximately equal, and all tissues appeared to be diploid. However, the remainder had on average only 12.9% w^+ sn^+ tissue. This tissue was usually anteriorly located, was always female when it encompassed sexually dimorphic structures, and appeared to be haploid, based on cell size and bristle density.

Polyspermy can account for both of these mosaic types, either by fusion of a secondary sperm with a meiotic product other than the egg pronucleus, or by haploid division of a secondary sperm. There are numerous reports describing mosaicism resulting from fusion of an additional sperm with a polar body in *D. melanogaster* [see STERN (1968) for review]. This event would result in the first type of mosaic described above. Cytological observations suggests that the other type of mosaics may have resulted from continued division of an *X*-bearing accessory sperm to form a haploid patch of tissue (J. TOMKIEL, unpublished observations). Haploid/diploid mosaics have also previously been described, although the proposed mechanism by which they arose was different [see HALL, GELBART and KANKEL (1976) for review).

A cytological analysis of early embryogenesis indicates that the frequency of multiple fertilization events is more than twofold higher in eggs from *abo/abo* females than from their *abo/Cy* sisters (56/882 *vs.* 44/1424, respectively), (TOMKIEL 1990). However, the survival of such progeny from an *abo/abo* female must also be increased to fully account for the increased recovery. We suggest that the increased recovery of these individuals from *abo* females may reflect the rescue by additional paternal *ABO* heterochromatin during early embryogenesis.

DISCUSSION

The results of the above experiments are consistent with a function of *ABO* heterochromatin during gametogenesis and/or prior to the first mitotic division in zygotes from *abo* mothers. The survival of *abo*-derived progeny was decreased when mothers bore two *X* chromosomes which were deficient for *ABO* heterochromatin. This suggests that the presence of *ABO* maternally can influence the *abo* maternal defect. However, to observe this maternal effect of *ABO* dosage, heterochromatin had to be removed from both *X* chromosomes in the mother. No enhancement of the *abo* maternal-effect lethality was observed when mothers were deficient for *ABO* on only one *X*, even when the zygotic dose of heterochromatin was lowered to increase the sensitivity of the assay. In addition, we observed that survival of zygotes which received an ABO^- *X* from an ABO^+/ABO^- mother was increased relative to the survival of the genotypically identical progeny class which received the ABO^- *X* from their father. We suggest that maternal *ABO* heterochromatin on one *X* chromosome may interact with or complement a deficiency for *ABO* on its homolog, and thus increase the ability of the ABO^- homologue to rescue *abo*-derived progeny. We imagine that this complementation might occur either in oogenesis, or early in embryogenesis prior to completion of meiosis I in the fertilized egg.

Results of experiments monitoring the survival of *pal*-induced *X*/O progeny from *abo* mothers suggest a function of *ABO* heterochromatin before the completion of the first division in the zygote. The presence of *ABO* heterochromatin on the paternal sex chromosomes prior to first division *pal*-induced chromosomal loss events increased the recovery of zygotes from *abo* mothers. Analysis of mosaics produced from later chromosomal loss events was consistent with this finding. These results suggest either that paternally derived *ABO* functions postmeiotically in the sperm, or that it acts in the egg prior to completion of the first division. The increased recovery of polyspermic progeny from *abo* mothers provides evidence in support of the latter. Increased survival of such individuals is consistent with rescue by additional paternally derived *ABO* heterochromatin during the early cleavage divisions in the zygote.

A particularly attractive model that can account for all these results is that the critical period for the function of both maternal and zygotic *ABO* heterochromatin is after fertilization but prior to the first division in the egg. It seems economical to suggest that both the effect of maternal dosage of *ABO* heterochromatin and the rescue by paternally derived heterochromatin prior to *pal*-induced loss reflect a function of *ABO* during this single time period.

In Drosophila, the mature oocyte is arrested in metaphase of meiosis I prior to fertilization. At or around fertilization, when the meiotic chromosomes are presumably in an equivalent state, the *ABO* heterochromatin of the entire maternal genome may function within the egg. Thus a deficiency of *ABO* heterochromatin in the presumptive egg pronucleus may be partially complemented by *ABO* heterochromatin in the chromosomes of the remaining meiotic products. This would explain why a reduction in progeny survival was observed only when both maternal *X* chromosomes were deficient for heterochromatin.

The differences in survival depending on the parental source of *ABO* can also be accounted for by this hypothesis. In zygotes which receive an ABO^- chromosome from an ABO^-/ABO^+ mother, the presence of the maternal ABO^+ chromosome in the oocyte could contribute to rescue from the *abo* maternal-effect

lethality. An analogous complementation of a paternally contributed *ABO*$^-$ *X* chromosome by its *ABO*$^+$ homologue would not be expected, since male meiosis is completed prior to fertilization, and the paternal *ABO*$^+$ homologue is at no time present in the egg. This would account for the enhanced survival of *ABO*$^-$/*ABO*$^+$ zygotes when an *ABO*$^-$ *X* was contributed maternally from an *ABO*$^+$/*ABO*$^-$ mother rather than paternally.

In summary, our results suggest a critical function of heterochromatin very early in embryogenesis. Although the requirement for *ABO* is not evident in embryos derived from *abo*$^+$ mothers, we propose a role of heterochromatin in normal embryogenesis as well. We suggest that the maternal *abo* defect may be required merely to enhance the sensitivity of the system and allow us to detect this function. There are several means by which this might occur. The function of the *abo* gene product may be analogous to that carried out by the heterochromatin, such that *abo* and *ABO* are functionally redundant, as proposed by PIMPINELLI *et al.* (1985). Alternatively, the maternal *abo*$^+$ product may positively regulate an early heterochromatic function, either directly or indirectly. Thus, lack of *abo*$^+$ product might be compensated by an increase in heterochromatin. Finally, the *abo*$^+$ product and *ABO* heterochromatin may act in the same or a related biochemical pathway, such that the effects of removing both are more severe than either one alone.

If heterochromatin does indeed play an important function in early development of wild-type embryos, it seems unlikely that such a function would be limited to an interaction with the *abo* gene or gene product. Broader testing of other maternal-effect genes may uncover similar interactions with heterochromatin. While such tests to date have been limited to a small number of mutations, it is already clear that *abo* is not unique in its interaction with heterochromatin (SANDLER, 1977). Thus, we suggest that the *abo* system may provide a useful model for further characterizing a general phenomenon of an involvement of heterochromatin in early development.

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