STUDIES ON THE MUTATION ABNORMAL OOCYTE AND ITS INTERACTION WITH THE RIBOSOMAL DNA OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

SANDLER (1970) suggested that mutation, abnormal oocyte (*abo*:2-38), may influence the function of the ribosomal RNA cistrons. We have examined the *abo* mutation and its interaction with the ribosomal DNA of *Drosophila melanogaster*. We observed that the expression of the *abo* phenotype is unstable under the appropriate conditions, a behavior which paralleled changes in the phenotypic expression of bobbed mutations during the magnification of the ribosomal DNA. The change in the expression of the *abo* phenotype is correlated with an increase in the redundancy of the ribosomal cistrons, further suggesting a functional interaction of the *abo* and bobbed regions.

THE DNA from which ribosomal RNA is transcribed has been shown to be redundant and clustered in the nucleolus organizer regions of the *Drosophila melanogaster X* and *Y* chromosomes (RITOSSA and SPIEGELMAN 1965). Studies of the stability of the ribosomal RNA cistrons have led to the conclusion that mechanisms are present which function to maintain the redundancy of the ribosomal DNA's. For instance, compensation for the absence of a nucleolus organizer occurs such that flies bearing a single organizer region contain as much ribosomal DNA as flies having two such regions (TARTOF 1971). In larval tissue the compensation events are limited to polytene cells (SPEAR and GALL 1973) and probably reflect regulatory mechanisms influencing the replication of the ribosomal DNA during the growth of the polytene chromosome. Transcriptional controls have also been indicated from biochemical, autoradiographic, and cytological observations in *D. melanogaster* (MOHAN and RITOSSA 1970; KRIDER and PLAUT 1972a,b). SANDLER (1970) has suggested that the chromosomal region characterized by the mutation, abnormal oocyte, may influence the expression of the ribosomal RNA cistrons. We have initiated a study of the interaction of the mutation and the nucleolus organizer region as a means to identify elements in the regulation of transcription and replication of the ribosomal DNA.

The recessive mutation, abnormal oocyte (*abo*), is located on the left arm of the second chromosome of *Drosophila melanogaster*. The phenotype of *abo* is a pronounced excess of female progeny when *abo* homozygous females are mated

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to attached-*XY* males. SANDLER (1970) observed that duplication of the proximal part of the *X* chromosome in either the maternal parent or the male progeny decreased the severity of the sex ratio phenotype of *abo*. Deletions had the opposite effect. The only DNA's of known function in the proximal region of the *X* chromosome are the ribosomal RNA cistrons, suggesting the interaction of the nucleolus organizer region and the mutant *abo* allele. While alternative interpretations were not excluded by the observations, we felt the interaction of the *abo* region with the proximal part of *X* warranted a closer examination.

Our observations show that the expression of the *abo* phenotype is unstable (under the appropriate conditions). We have observed both the loss of the capacity to express the abnormal sex ratio effect in *abo* homozygotes, and the progressive return of the phenotype in populations maintained in the heterozygous condition. These observations had a number of formal similarities to the change in expression associated with deletions of the ribosomal DNA (RITOSSA 1968). Therefore, we examined the influence of *abo* on the redundancy of the ribosomal DNA of adults. The loss of the capacity to generate an abnormal sex ratio in *abo* homozygotes is correlated with a significant increase in the redundancy of the ribsomal RNA cistrons, further suggesting a functional interaction of the *abo* region and the ribosomal DNA's.

MATERIALS AND METHODS

Stocks and culture

All stocks were grown on standard agar, Brewers yeast, and sucrose medium at 22 ± 1°.

The abnormal oocyte (*abo*) stock was kindly provided by DR. LARRY SANDLER (University of Washington, Seattle). The *abo* allele (*abo*:2–38) is maintained in the heterozygous condition against *In(2LR)Cy*. This inversion-bearing chromosome is wild-type for the *abo* region.

The "snoc" stock used in the experiments to demonstrate the *abo* phenotype was obtained from DR. SEYMOUR ABRAHAMSON (University of Wisconsin, Madison) and is of the following genotype: $Y^s \cdot X$ *In(EN) ptg oc* $sn^5 \cdot Y^L$ and *C(1)RM, sc* ct^n *ptg car* $\cdot$ sn^{x^2}, *In(1)d149, Y/O*. The pertinent feature of the stock is the absence of any free *X* or *Y* chromosomes. Thus, when a snoc male is mated to a karyotypically normal female, or vice versa, all the progeny males are *XO*.

The phenotype associated with the *abo* mutation is an excess of females in the progeny of a cross between an *abo/abo* female and an attached-*XY* (no free *Y*) male (SANDLER 1970). To test for the capacity of a stock to generate the abnormal sex ratio, appropriate virgin females were isolated and singly mated to snoc males in vials. After three days the flies were transferred to fresh vials for a second round of egg laying and progeny collection. After removal of the parental flies, all vials were cleared every other day, and the progeny were counted until the vials were exhausted. Vials which produced a total of less than ten progeny were not included in the data. For each mating, some male and female progeny were transferred to fresh media and left for a week, after which the vial was cleared and inspected for a second generation. Since the males of the first generation should be *XO* and sterile, progeny in this case indicated either nondisjunction in the parental *abo* stock, or a free *Y* chromosome in the snoc stock. Test matings where this occurred were discarded and do not appear in the data. A particular *abo/abo* female was arbitrarily considered to have generated an abnormal sex ratio if her progeny deviated from a 1:1 expected ratio by a chi square value of 6.8 or greater. A minimum of thirty females were tested in each of the generations represented in Figures 1 and 2.

Isolation of DNA

The DNA isolation procedure was developed by WEBER (1974) and modified slightly for use with Drosophila. DNA was isolated from adults which had been selected for the appropriate sex

and genotype, and stored at —20° until needed. Since some genotypes were difficult to collect in large quantities, some of the material was stored for a longer period than others. While the yield of DNA decreases on storage, we observe no preferential loss of the rDNA's as compared to DNA extracted from freshly harvested flies. Flies were frozen on dry ice and ground to a fine powder with a mortar and pestle, hydrated for several minutes in a buffer containing 0.1 M NaCl, 0.01 M EDTA, 0.03 M Tris pH 8, and homogenized by several strokes of both the loosely and tightly fitting balls of a dounce homogenizer (Vitro). Approximately ten milliliters of the buffer were used for each gram of starting material and all solutions were maintained at 0–4°. The homogenate was then centrifuged in a Sorvall RC-2B with an SS-34 head at five thousand rpms for five minutes. The pellet was resuspended in a buffer containing 0.1 M EDTA, 0.1 M Tris pH 8.4 using 4 ml of the buffer for each gm of starting material. SDS was added dropwise from a 10% solution to a final concentration of .1%, pre-incubated pronase (Calbiochem, 10 mg/ml 37° for 1 hr) was added to a final concentration of 200 μg/ml, and the material was incubated for 1–2 hrs at 37°. Following the incubation, 6 M sodium perchlorate was added to the material to a final concentration of 1 M and the lysis mixture was extracted by shaking for ten minutes with an equal volume of a mixture of 2 parts phenol to one part Sevag (chloroform-isoamyl alcohol 24:1). The emulsion was broken by centrifugation for ten minutes at approximately 12,100 × gravity and the aqueous upper layer was removed with a large bore pipette. This was reextracted with equal volumes of Sevag until material was no longer visible at the interface following centrifugation.

The DNA was precipitated with one and a half volumes of cold 95% ethanol, redissolved in 0.1 × SSC (SSC is 0.15 M NaCl, 0.015 M Na Citrate) and centrifuged for thirty minutes at 43,000 rpm in the Ti 50 rotor of a Beckman Model L. Ribonuclease A (50 μg/ml, Sigma), ribonuclease T1 (200 units, Sigma) and Tris buffer pH 7.4 (final concentration 50 mM) were added and the supernatant fraction was incubated for two hours at 37°. SDS was added to 0.5%, preincubated pronase was added to 100 μg/ml, and the incubation was continued for 2 hrs at 37°. The salt concentration was then raised to 0.25 M using 5 M sodium chloride, the preparation was extracted with Sevag as described above, the DNA was precipitated with 95% ethanol and rinsed in 75% ethanol. The precipitate was dissolved in 0.1 × SSC, cesium chloride was added to a density of 1.4 gm/cc (density determined with a Zeiss refractometer), and the DNA was pelleted by centrifugation overnight at 42,000 rpm in the Ti-50 head of the model L. The pellet was either frozen for storage at —20°, or redissolved in 0.1 × SSC for loading on filters. DNA prepared in this manner had O.D 260/230 ratios of about 2, and hyperchromicities of 27%–35% when denatured with sodium hydroxide.

Extraction of RNA

Ribosomal RNA was extracted from third instar larvae by the method of BROWN and LITTNA (1964). Larvae of the appropriate age were harvested by flotation away from the medium in 20% sucrose, washed several times in distilled water, and frozen on dry ice. The frozen larvae were ground to a fine powder with a mortar and pestle and stirred for one minute in a buffer containing 0.1 M sodium acetate, pH 5.0, 0.5% SDS, and 40 μg/ml of polyvinyl sulfite. Approximately 10 ml of the buffer were used for each gm of starting material. An equal volume of water-saturated phenol was added and the mixture was stirred for twenty minutes at 0–4°. The emulsion was separated by centrifugation at 10,000 rpm for ten minutes in the SS-34 head of the Sorvall RC-2B, and the phenol extraction of the aqueous phase was repeated until no material was present at the interphase. The salt concentration was raised to 0.25 M with sodium chloride and the RNA was precipitated by two volumes of 95% ethanol and storage at —20° overnight.

To purify the ribosomal RNA, the precipitate was redissolved in a buffer containing 0.01 M sodium acetate pH 5, .005 M magnesium acetate and 1 μg/ml of polyvinyl sulfite to which 25 μg/ml of electrophoretically pure DNAse (Worthington) was added. After incubation at room temperature for 25 minutes, the solution was diluted tenfold with 0.1 M Tris buffered saline (0.1 M sodium chloride, .05 M Tris-HCl pH 7.5) and the RNA was loaded on a methylated albumin Kieselgur column prepared as described by MANDELL and HERSHEY (1960). The column was washed with two volumes of 0.1 M TBS, and tRNA, 5S RNA, and any remaining DNA was eluted with two column volumes of 0.6 M TBS. Ribosomal RNA was eluted from the column

with 1.2 M TBS. The eluent fractions containing the predominant portion of the RNA were collected using an ISCO single beam UV monitor. The RNA was precipitated with two volumes of ethanol and stored until further use at —20°.

Iodination of the RNA

The procedure described here was graciously provided by MAURICE COHEN at Oak Ridge.

^{125}I was obtained at maximum specific activity and concentration from New England Nuclear (NEZ · 33H) and made 0.01 M in sodium sulfite immediately upon receipt. Reactions were carried out in a 2-ml stoppered vessel in a shielded hood facility and all steps were monitored with a hand-held Geiger counter. Ten lambda of the reducel Na ^{12}-I were added to 10 lambda of 2×10^{-4} M potassium iodine in distilled water and the solution was left at room temperature for 30 minutes. Twenty to eighty μg of RNA dissolved in 30 lambda of distilled water, and 50 lambda of a thallic chloride solution (1.2×10^{-2} M $TlCl_3$ in 0.5 M sodium acetate, pH 4) were added and the reaction vessel was placed in a 70° water bath for twenty minutes. After cooling the reactants over ice, 20 lambda of 0.1 M sodium sulfite and 1 ml of a solution containing 1 M ammonium acetate, 1 M ammonium hydroxide pH 8 were included and the mixture was heated at 90° for 15 minutes. The solution was cooled over ice, diluted approximately tenfold with 0.1 M potassium phosphate and applied to a hydroxyl appatite column. The column was washed with 0.12 M phosphate until there was virtually no detectable radioactivity in the wash as monitored with a hand-held Geiger counter. The iodinated product was eluted from the column with 0.5 M phosphate and collected in drop fractions. Those fractions having the major radioactivity (as judged by the hand-held Geiger counter) were pooled and used in the hybridization reactions after determining the specific activity of the RNA. Specific activities of about 10^7 dpm/μg are normally obtained with this procedure.

Preparation of filters and loading of the DNA

Filter disks 25 cm in diameter were dye-cut from nitrocellulose sheets (SCHLEICHER and SCHUELL) to insure uniformity. The filters were treated with 10% TCA at 60° for at least two hours, rinsed several times in cold 10% TCA, washed in several changes of distilled water for thirty minutes each, and left to stand in 6 × SSC overnight. This procedure removes materials which contribute to the background of the fluorescent measurements made on the filters after the completion of the hybridization. Filters treated in this way do not differ in their capacity to retain DNA during the DNA loading, hybridization, or washing steps when compared to untreated filters. DNA dissolved in 0.1 × SSC was denatured with base for ten minutes by the addition of concentrated sodium hydroxide to 0.2 N. The DNA solution was neutralized with HCl, and made 0.05 M Tris, pH 7.4, and 6 × SSC. Approximately 25 μg of DNA was loaded on each filter at a flow rate of 1 ml/minute. The fraction of the DNA bound to the filters was estimated by measuring the optical density of the solution prior to and after filtering; binding efficiency always exceeded 97%. After loading, the filters were air-dried for two hours and then dried in a vacuum oven at 80° for two hours.

Hybridization

For the purpose of DNA-RNA hybridization the ^{125}I ribosomal RNA was normally cut in its specific activity to about 300,000 dpm/μg with cold ribosomal RNA. Filters containing DNA were placed in the hybridization mixture containing 4 × SSC, 0.1% SDS, and 3.6 μg/ml of RNA for 18 hrs at 67°. The reaction was stopped with 2 × SSC and the filters were washed four times for ten minutes each in a swirling beaker with 2 × SSC. RNA which was not in hybrid was removed by the addition of fresh 2 × SSC containing 20 μg/ml of ribonuclease B (Worthington) which had been placed in a boiling water bath for twenty minutes to remove any DNase activity. The washing procedure was then repeated, the filters were dried, and counted in Liquiflor (New England Nuclear). Under our conditions saturation is achieved at an RNA concentration of 2.0 μg/ml. Background and saturation levels of freshly prepared ^{125}I RNA are comparable to ^3H-RNA preparations.

Measurement of DNA on filters

The DNA content of each filter was measured by a modified Di-amino-benzoic acid (DABA) procedure described by KISSANE and ROBBINS (1958). After counting, each filter was washed exhaustively with toluene to remove the scintillants, rinsed in ether, and dried. The filters were minced with a razor blade and incubated at 60° for 60 minutes in 0.1 ml of 25% TCA. To each tube 0.1 ml of freshly prepared 2 M DABA was added and the reaction was continued for another 30 minutes. The reaction was stopped by the addition of 2.5 ml of 0.6N perchloric acid and the samples were read at exciter and analyzer wave lengths of 408 nm and 520 nm, respectively, on a Perkin-Elmer fluorometer equipped with a Xenon light source. Standards were prepared from calf thymus DNA and background was estimated from filters without DNA which had been processed in the same manner as the experimental filters. We found that the measured fluorescence was linearly related to the amount of DNA on the filters over a range of 0.2 μg–75 μg, after subtraction of the background.

RESULTS

Table 1 presents the results of matings between flies bearing the mutant allele, abnormal oocyte (*abo*), and males and females of the snoc stock which have either attached-*X* or attached-*XY* chromosomes. As the data of this table illustrate, *abo* is a recessive allele which is expressed only when homozygous in the maternal parent (e.g., *abo/abo* males and *abo/Cy* females do not produce an abnormal sex ratio). These results are substantially in agreement with those of SANDLER (1970) and serve only to show that our stocks behave in the test situation in a manner similar to that described for the original *abo* isolates. Since the *abo* allele produces no obvious phenotype other than the sex ratio effect, the cross of an *abo* homozygous female to the snoc male was used repeatedly as a means of identifying chromosomes carrying the mutation.

The major new observation is presented in the last cross of Table 1. Males and virgin females lacking the curly (*Cy*) marker (and therefore homozygous for *abo*) were selected from the *abo/Cy* stock and mated to produce a homozygous line of *abo* flies. After several generations in culture, virgin females from this stock were isolated and mated to snoc males. As the table indicate, these females did not generate an abnormal sex ratio in the F_1 progeny. Since the design of the crosses includes a test of the fertility of the male offspring (see MATERIALS AND

TABLE 1

The expression of the abnormal oocyte (abo) *phenotype*

Parental	Progeny	
	Females	Males
abo/abo ♀ × snoc ♂	5,266	817
abo/Cy ♀ × snoc ♂	2,000	2,020
abo/abo ♂ × snoc ♀	2,542	2,470
abo/abo ♀ × snoc ♂	15,604	16,126

The pooled results of single pair matings demonstrate the maternal effect in the expression of the recessive abo phenotype. *Abo* homozygotes and heterozygotes (*abo/Cy*) were mated to snoc flies of the appropriate sex to produce *XO* progeny males. The females of the last mating of the table were taken from a stock in which *abo* had been maintained for several generations in a homozygous form.

METHODS), the accumulation of free *Y* chromosomes in either the parental homozygous *abo* or snoc stocks could be excluded as a cause of the observation (SANDLER 1970).

The failure of the *abo* females from the homozygous line to generate an abnormal sex ratio suggested that either reversion of the *abo* allele or contamination by wild-type chromosomes had occurred in the initial *abo/Cy* stock. It was argued that abo^+ chromosomes might compete with the *abo* allele, thus explaining the loss of the phenotype in the homozygous stock. Therefore, *abo* chromosomes were reisolated from the *abo/Cy* stock (no evidence of contamination was found) and a number of *abo* homozygous lines were initiated. In every case, the loss of the *abo* phenotype was observed. Figure 1A presents the results of following two such homozygous lines over a number of generations with regard of the total females tested that produced an abnormal sex ratio, while the abscissa represents the number of generations the *abo* homozygous line had been cultured. Figures 1B and C present the data from which the above figure was drawn. As in the previous figure, the abscissa represents the number of generations in which the line had been homozygosed, while the ordinate in this case displays the ratio of the male to female progeny of each of the *abo/abo* females tested. Those ratios, which are considered significantly different from the 1/1 expectation, are indicated by the triangles, while those that did not deviate significantly are marked by the circles. As can be seen from these scatter diagrams and Figure 1A, one *abo/abo* homozygous line showed a significant change in the expression of the

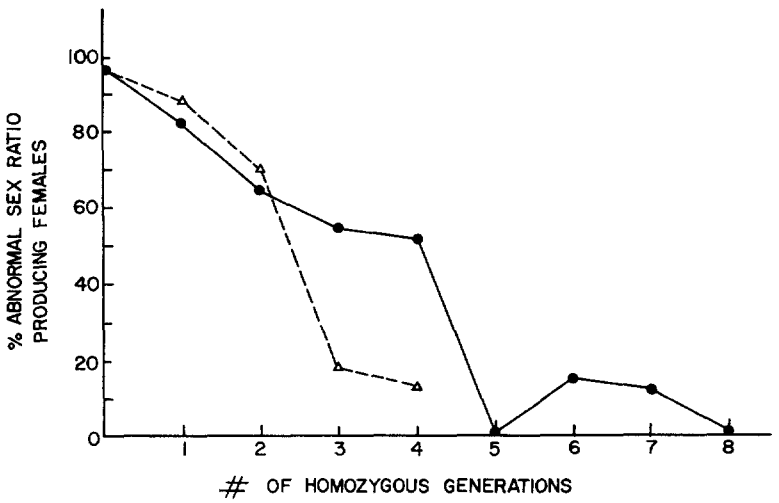

FIGURE 1A.—The loss of the *abo* phenotype in homozygous mutant lines. The results of following two homozygous lines over several generations with regard to the expression of the *abo* phenotype. The ordinate presents the fraction of the total tested females that produced a sex ratio abnormality, while the abscissa expresses the number of generations that each line had been cultured.

.... plotted from data of Figure 1B
——— ——— ——— plotted from data of Figure 1C

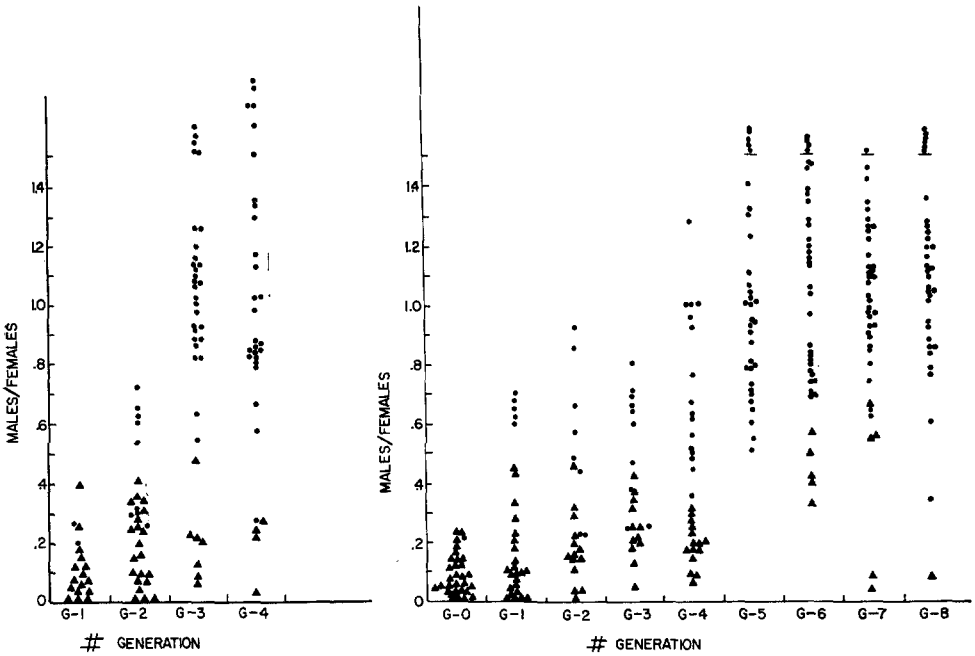

FIGURE 1B and 1C.—Scatter diagrams demonstrating the expression of the *abo* phenotype in homozygous mutant lines. The ordinate presents the ratios of the male to female progeny produced by each of the *abo/abo* females tested in a generation. The abscissa expresses the number of generations that each line had been maintained in a homozygous configuration. Ratios which deviated significantly from the 1/1 expectation are indicated by pyramids; those not significant are represented by solid dots.

abnormal sex ratio expression between the second and third generations, while the other did not show a major change until the fifth generation. Other lines have remained stable for as long as twelve generations.

These observations implied that the loss of the capacity to express the *abo* phenotype was not a consequence of the contamination of the *abo/Cy* stock by wild-type alleles of *abo*. Rather, the change in the expression of the mutant appeared to be associated with its maintenance in a homozygous configuration. This was tested by mating adults from an *abo* homozygous strain, which no longer expressed the abnormal sex ratio, with *Cy* flies. The *Cy* progeny from this cross were isolated and mated to give an *abo*(?)/*Cy* line. In each successive generation *Cy* females were mated to *Cy* males of the same generation, while the *abo/abo* female sibs were mated to snoc males to test for the capacity of the stock to generate a sex ratio. Figure 2A summarizes the results obtained over nine generations using this protocol. Figure 2B presents the data as a scatter diagram as described above. It is apparent from the figures that the fraction of the *abo/abo* females which produced an abnormal sex ratio increased in each generation that the *abo* allele was held in a heterozygous condition. During the second generation of the experiments several *abo* chromosomes were isolated

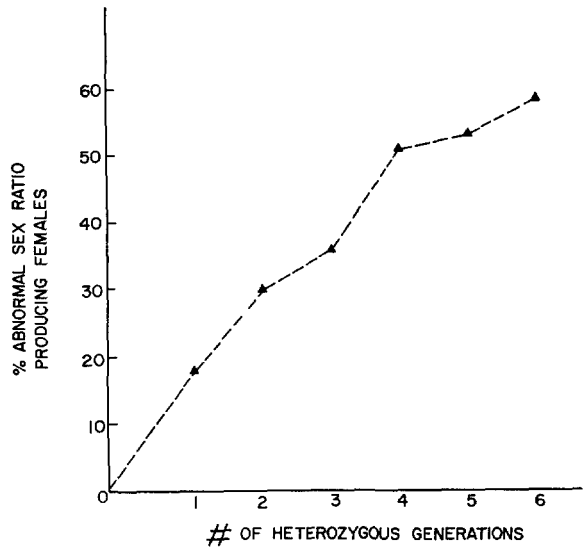

FIGURE 2A.—The return of the *abo* phenotype in a heterozygous line. An *abo* chromosome from a homozygous line that no longer expressed the mutant phenotype was maintained in a heterozygous configuration (*abo/Cy*). In each generation, *abo/abo* virgins were tested for their capacity to produce an abnormal sex ratio. The ordinate expresses the fraction of the total tested females that produced a sex ratio abnormality, while the abscissa presents the number of generations the line was maintained. This figure was drawn from the data of Figure 2B.

from progeny females which had *abo/abo* mothers producing an abnormal sex ratio. When these *abo*-bearing chromosomes were maintained against a *Cy* chromosome, the *abo* alleles were stable. When homozygosed, the *abo* chromosomes showed a decay in the capacity to generate an abnormal sex ratio, as previously observed.

DNA-RNA hybridization

The results of experiments in which ^{125}I-RNA was hybridized to DNA of adult flies are presented in Table 2. Each measurement represents at least two repeat hybridizations with six filters. Only newly iodinated RNA was used in the reactions, all RNA preparations were of comparable specific activity, and all DNA types were included in each of the reaction vessels. However, we were able to perform only one DNA extraction on several of the genotypes because *abo/abo* G-0 flies are difficult to obtain in large quantities. Thus, the variations which may occur between preparations of a single stock are not represented in the data. It should also be mentioned that we have repeated most of the measurements (excepting the *abo/abo* G-0 males) using ^{3}H-RNA and the same DNA preparations used here. These measurements fell within the range of variation of the measurements using the iodinated material. We have chosen to present the ^{125}I-RNA data because it is more complete, and contains a larger number of trials.

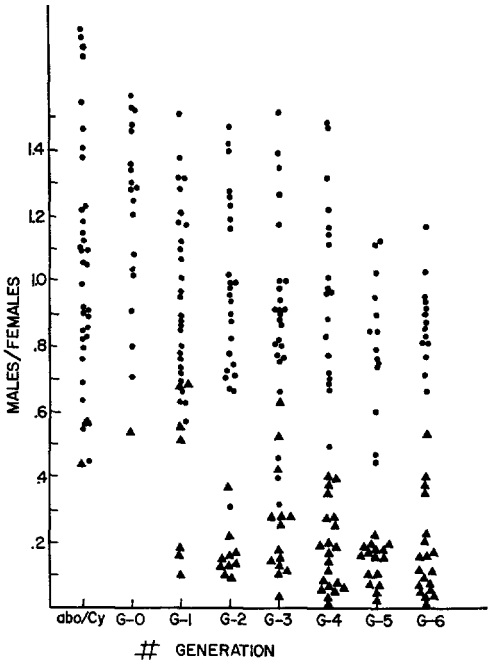

FIGURE 2B.—Scatter diagrams of the change in the expression of the *abo* mutation in a heterozygous line. The ordinate represents the ratios of the male to female progeny produced by *abo/abo* females of the heterozygous line described above. The abscissa presents the number of generations the line had been maintained in a heterozygous configuration. The *abo/Cy* diagram was generated using heterozygous females from a stock in which the homozygous mutant females produced a sex ratio abnormality. The G-0 diagram presents the results of testing the *abo* homozygous females from which the heterozygous line was generated. The pyramids indicate the progeny ratios which deviate significantly from the 1/1 expectation.

TABLE 2

The hybridization of ^{125}I-ribosomal RNA to DNA of abo *and wild-type adults*

Genotype	Phenotype	Percent DNA in hybrid
abo/+ ♀	+	0.245 ± 0.020
abo/abo ♂ G-0	+	0.338 ± 0.029
abo/abo ♀ G-0	mutant	0.417 ± 0.023
abo/abo ♂ G-12	+	0.866 ± 0.031
abo/abo ♀ G-12	+	1.484 ± 0.094

^{125}I-ribosomal RNA was hybridized to DNA from adult flies which were either heterozygous or homozygous for the *abo* mutation. Males and females designated G-0 were newly homozygous, while those labeled G-12 had been maintained in a homozygous stock for twelve to fifteen generations. The phenotype expressed by each class of flies when mated with the appropriate snoc adults is also indicated. Hybridization data are expressed as the fraction of the total DNA which formed hybrids with the rRNA (with standard errors).

DNA was collected from adults of three genotypes. The first, *abo/Cy*, females, were normally used to maintain the *abo* allele in culture. As Table 2 indicates, females of this genotype are phenotypically normal. Since DNA from these females was independently shown to form hybrids to the same degree as DNA from a Canton-S stock (from which the background of the *abo* stock is derived), the *abo/Cy* DNA was considered as appropriate control material. DNA was also obtained from the *abo/abo* siblings of the previous group (G-0). Females of this genotype produced a pronounced sex ratio abnormality, while the males did not. Finally, DNA was extracted from males and females of an *abo/abo* stock which had been homozygous for twelve to fifteen generations (G-12,15). As Table 2 indicates, neither the males nor the females expressed the *abo* phenotype.

DNA extracted from the *abo/abo* females of the G-0 class contained a twofold higher percentage of the genome that would form hybrids with ribosomal RNA than the control material. DNA from *abo/abo* G-0 males also formed more hybrids, but the difference between the experimental material and the control was not as striking as that of the females. The most extreme deviation from the control material was observed in hybridizations in which DNA from the G-12,15 flies was used. The male-derived material had on the order of four times the rDNA redundancy, while the DNA from the females formed nearly seven times the amount of hybrid as the control.

DISCUSSION

We suggest that the expression of the abnormal oocyte (*abo*) region is unstable under the appropriate conditions. When chromosomes bearing an *abo* mutation were maintained in a homozygous configuration for a number of generations, the characteristic sex ratio effect of the mutant was no longer observed. Contamination of the parental *abo/Cy* stock by wild-type second chromosomes, or revertants to wild type, was excluded by several observations. Tests failed to show any evidence of contamination, and when *abo*-expressing chromosomes were reisolated and homozygosed the intial observation was consistently repeated (Table 1 and Figure 1 of the RESULTS). Furthermore, when *abo* chromosomes which no longer produced a sex ratio abnormality were returned to the heterozygous configuration, a return of the capacity of the region to express the phenotype was observed (Figure 2 of the RESULTS).

It would appear that phenotype stability of the *abo* allele is favored when it is maintained against a wild-type allele. Loss of the capacity to express the phenotype was restricted to the homozygous lines in these experiments. As stated in the results, there were substantial variations in the number of generations over which homozygous lines ceased to express the *abo* phenotype. Some homozygous lines have remained stable in their expression for as many as twelve generations. SANDLER has confirmed our initial observation of the loss of the *abo* phenotype in homozygous lines. However, when a marked *X* chromosome was substituted in the *abo/Cy* stock, no loss of the phenotype was observed in the *abo* homozygotes derived from this stock (L. SANDLER, personal communication). Further-

more, PARRY and SANDLER (1974) report that the expression of the *abo* phenotype is dependent, in part, on the origin of the *X* and *Y* chromosomes used in the test. These observations may indicate that there are a number of alternative alleles of *abo* and/or sex chromosome regions with which *abo* interacts. Certainly, we cannot argue that the genetic conditions leading to either stability or change of the *abo* expression are well defined.

The instability of the mutant *abo* expression and the magnification of ribosomal DNA deletions at the bobbed locus (RITOSSA 1968) have a number of formal similarities. For instance, changes in the phenotypic expression of both regions can occur over a number of generations, and the phenotypic change may go in either the mutant to wild type direction or *vice versa* (TARTOF 1974).

SANDLER (1970) has shown that the heterochromatin of the *X* and *Y* chromosomes (in which the ribosomal RNA cistrons are located) can influence the expression of the *abo* region. Because of these considerations we argued that the *abo* mutation may interact with the heterochromatic portions of the *X* and *Y* chromosomes to influence the redundancy of the ribosomal DNA's. Assuming this hypothesis, one might predict that changes in the expression of the *abo* region should be accompanied by alterations in the redundancy of the ribosomal DNA's. The results of hybridization of ^{125}I-ribosomal RNA to adult DNA flies (Table 2) are consistent with this prediction. That is, the change in the expression of the *abo* mutation in homozygous adults was correlated with a significant increase in the fraction of the genome that formed hybrids with ^{125}I-ribosomal RNA.

While these experiments do not establish a causal relationship between the modification of the *abo* expression and the change in ribosomal DNA redundancy, a number of observations provide added support for considering an interaction between the *abo* and bobbed regions.

TARTOF (1971) has demonstrated that the redundancy of the ribosomal RNA cistrons in DNA from XO adults (which have one nucleolus organizer) is approximately equivalent to the redundancy of normal *XY* adults (which have two nucleolus organizers). Similar observations were obtained when DNA from females with an *X* chromosome bearing a deletion of the nucleolus organizer ($In\ sc^4sc^8/X^+$) was compared to DNA from normal females. The observation implied that compensation for the absence of a ribosomal DNA complement had occurred. SPEAR and GALL (1973) have shown that in larval tissues the compensation is limited to polytene cells. They consider that the compensation reflects regulatory events which lead to differential replication of some DNA sequences during the growth of polytene chromosomes. The *abo* mutation most severely influences the viability of those progeny that either lack a sex chromosome (*XO* males) or bear a large deletion of the proximal heterochromatin of the *X* ($In\ sc^4sc^8/X^+$) (SANDLER 1970). Thus, the *abo* phenotypic effect is most extreme in progeny types in which amplification has been demonstrated. Such considerations circumstantially implicate the *abo* product in interactions with mechanisms that establish and maintain the redundancy of the ribosomal RNA cistrons.

PARRY and SANDLER (1975) have concluded that the *abo* allele does not interact directly with the ribosomal RNA cistrons. They utilized free fragments which

bore various portions of the proximal region of the *X* chromosome. By introducing the fragments into *abo/abo* females, they could localize that region which would, when duplicated, eliminate the appearance of the *abo* abnormal sex ratio. Their results indicate that the region of the *X* chromosome that interacts with the *abo* mutation is immediately distal to the nucleolus organizer.

While apparently contradictory, the results of PARRY and SANDLER and those reported here could be resolved in a number of ways. Perhaps the *abo* mutation influences the activity of an array of functions associated with the heterochromatic portion of the *X* chromosome. In such a case, the interactions of the *abo* allele and the ribosomal RNA cistrons may be separable from those events which lead to the aberrant sex ratio. Or, perhaps all of the sequences of this chromosomal region are influenced in their replication, and the site adjacent to the nucleolus organizer includes an initiation or regulatory function. There are numerous other alternatives. In order to distinguish between some of them, we have initiated studies on the kinetics of the loss of the *abo* phenotype and the change of the ribosomal DNA redundancy, the effect of the *abo* allele on the ribosomal DNA's in the progeny of crosses which give abnormal sex ratios, and the effect of *abo* on the redundancy of sequences localized to the regions surrounding the ribosomal RNA cistrons.

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