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Antisense ribosomal protein gene expression specifically disrupts oogenesis in *Drosophila melanogaster*

(antisense gene)

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ABSTRACT **To assess the functional importance of ribosomal protein rpA1 gene expression during development of *Drosophila melanogaster*, we have transformed into the fly's genome an antisense rpA1 gene driven by a heat shock promoter. Antisense rpA1 expression severely disrupted oogenesis and produced a "small egg" female-sterile phenotype. The severities of these defects were proportional to the level of antisense rpA1 expression. Anti-rpA1 expression did not affect larval or pupal development. Quantitative RNA analysis suggested that high anti-rpA1 expression results in a general decrease of mRNA in the ovary.**

The rate of ribosome synthesis during *Drosophila melanogaster* oogenesis is high. On the order of 5×10^{10} ribosomes are stored in the *Drosophila* egg (1), and most of them are synthesized in a matter of hours between stages 8 and 10 of oogenesis (2). By comparison, it takes weeks to synthesize the 9×10^7 ribosomes of the mouse egg or months to synthesize the 7×10^8 ribosomes of the sea urchin egg (3). The demand for new ribosomes during *Drosophila* development varies a great deal according to time or cell type. For instance, the phenotype of mutants in which the synthesis of ribosomes is affected [e.g., bobbed (*bb*), Minute (*M*), and mini (*min*)] suggests that a large output of ribosomes is required in the cells that during pupation give rise to bristles and the abdominal covering (tergites) of the adult (4). *bb* mutants carry deletions of ribosomal RNA genes and are characterized by delayed development, shorter bristles, and lower fertility. *M*s are a class of 50 or so recessive lethal mutations whose dominant phenotype is similar to that of *bb*. *M*s are haploinsufficient; that is, deletion of one allele will produce the dominant phenotype (5). The similarity between the *bb* and the *M* phenotype suggests that these mutations affect common cellular processes: the synthesis of ribosomes. Several recently isolated ribosomal protein (r-protein) genes have been localized near *M* loci (6–8), pointing to the possible connection between r-protein genes and *M* mutations. By suppressing the phenotype of an *M* fly with an exogenously introduced r-protein gene, Kongsuwan *et al.* (9) have shown that a previously undescribed *M* results from the deletion of the gene encoding r-protein rp49. This result supports the hypothesis that *M* mutations correspond to r-protein genes. But there is *a priori* no reason to believe that mutations in all r-protein genes lead to a *M* phenotype; the generalization of this correlation needs more evidence.

We have recently isolated the gene encoding r-protein rpA1 by using the unique translational pattern of its mRNA during early embryogenesis (8, 10). The rpA1 gene was localized to the polytene chromosomal band 53CD, which is in the same region where the *M* mutation *M(2)S7* has been genetically mapped (5, 11). We have examined the relationship between rpA1 and *M(2)S7* by introducing a wild-type rpA1 gene into *M(2)S7* flies. This extra copy of the wild-type rpA1 gene did not suppress the *M(2)S7* phenotype. Moreover, rpA1 mRNA is of normal abundance and size in *M(2)S7* flies (S.Q. and M.J.-L., unpublished data), suggesting that rpA1 and *M(2)S7* are unrelated. The *M(2)S7* locus probably corresponds to a different gene, and a mutation in the rpA1 gene remains to be identified.

rpA1 has strong amino acid sequence homology with the eukaryotic A type acidic r-proteins, which appear to play an important role in the initiation and elongation steps of protein synthesis (8, 12). It is therefore reasonable to expect that rpA1 has a vital function. The decrease or loss of rpA1 function could lead to abnormal development or disruption of certain aspects of the fruit fly life cycle. Because the rpA1 gene is located in a relatively unexplored area of the second chromosome, there are no readily available genetic approaches for isolating rpA1 mutations. The haploid insufficiency of the rp49 *M* suggested that the cellular function of r-proteins is dosage dependent, making the rpA1 gene a suitable target for antisense inhibition. That antisense inhibition of r-protein synthesis is possible has been shown in *Xenopus* by microinjection of antisense rpL1 RNA into oocytes (13). We adopted a different antisense inactivation approach by integrating an antisense rpA1 gene into the fly's genome and measuring the effects that antisense rpA1 expression has on developmental processes.

MATERIALS AND METHODS

Transposon Construction. A 1.05-kilobase (kb) *Aha* III–*Xba* I fragment from the rpA1 genomic clone p5D (8) containing the entire rpA1 transcribed sequence was inserted into *Xba* I–*Bam*HI and *Hin*cII–*Xba* I sites of the pUC18 polylinker producing constructs A and B, respectively. Relative to the *Hin*dIII site of the pUC18 polylinker, the rpA1 sequence is in antisense orientation in construct A and in sense orientation in construct B. A 400-base-pair (bp) DNA from the 3′ end of the fushi tarazu (*ftz*) gene (*Pvu* II–*Sac* I fragment, ref. 14) was inserted at the *Sma* I–*Sac* I site of the polylinker of construct A to ensure the proper transcription termination and polyadenylylation. The inserts of the plasmids A and B were released from pUC18 by *Hin*dIII–*Eco*RI double digestion.

The promoter of the gene for the 70-kDa heat shock protein, hsp70, was derived from the plasmid pHAP (15). The 600-bp hsp70 promoter was excised from pHAP by *Sal* I–*Hin*dIII digestion and inserted into the *Sal* I–*Hin*dIII site of pUC18. The insert was then released from pUC18 by *Bam*HI–*Hin*dIII double digestion. The hsp70 promoter was ligated with the released rpA1 inserts of plasmids A and B at

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Abbreviation: r-protein, ribosomal protein.

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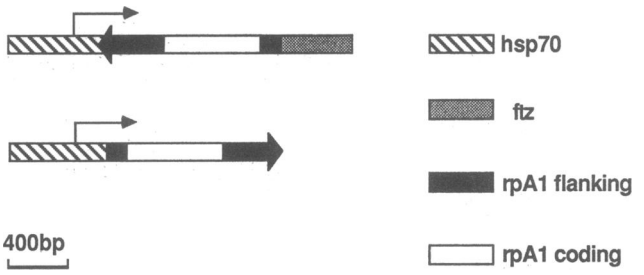

FIG. 1. rpA1 antisense and sense constructs. A 1.05-kb DNA fragment containing the entire rpA1 coding region was ligated in antisense and sense orientation (thick arrows) to a *Drosophila* heat shock protein (hsp70) promoter. A 400-bp *ftz* polyadenylylation signal sequence was added to the 3′ end of the antisense construct. These two constructs were inserted into a *P*-element vector and introduced into the germ line by transformation. The thin arrows above the constructs indicate the start point and direction of transcription from the hsp70 promoter.

the *Hin*dIII site and inserted into a *Bam*HI–*Eco*RI-digested shuttle vector pHSS7 (constructed by F. Heffron and G. M. Rubin, University of California, Berkeley) in a three-fragment ligation. The resulting two plasmids have the hsp70 promoter–rpA1–*ftz* (antisense) and the hsp70 promoter–rpA1 (sense) configurations (Fig. 1).

The antisense and the sense constructs were excised from the pHSS7 shuttle vector by *Not* I digestion and inserted into the *Not* I site of a newly modified Carnegie 20 *P*-vector (16), giving rise to the antisense and sense transformation constructs.

Germ Line Transformation. ry506 embryos were injected with the transformation constructs and transformant flies were selected according to the methods of Rubin and Spradling (17). The chromosomal sites of transposon integration were determined by *in situ* hybridization as described (8).

Induction of the Transformed Genes. To induce expression of the transformed genes, adult flies were transferred to glass vials and heat shocked by submerging the vials into a 37°C water bath for 1 hr. The larvae and pupae were transferred to Microfuge tubes containing paper towels moistened with Ringer's solution and heat shocked in a water bath as for the adult flies.

RNA Blot Analysis. Total RNA was extracted from flies, larvae, or pupae as described (18). RNA was fractionated by electrophoresis in 1.5% (vol/vol) formaldehyde agarose gels and blotted onto GeneScreen membranes (New England Nuclear). The filters were hybridized either with a double-stranded rpA1 probe labeled by nick-translation or with single-stranded rpA1 RNA probes labeled by bacteriophage T3 or T7 RNA polymerase transcription. The single-stranded RNA probes were derived from the 1.05-kb *Xba* I–*Aha* III rpA1 fragment inserted into the Bluscribe vector (Stratagene, La Jolla, CA) and the conditions for RNA·RNA hybridization were according to the Stratagene protocol. DNA·RNA hybridizations were done at 42°C in a buffer consisting of 50% (vol/vol) formamide, 0.75 M NaCl, 0.5% sodium dodecyl sulfate, 0.01% Pipes at pH 6.8, denatured salmon sperm DNA at 100 μg/ml, and poly(A) (Boehringer Mannheim) at 100 μg/ml.

RESULTS

Expression of the Antisense rpA1 Gene in Transformed Flies. So far, to our knowledge, the only reported antisense inactivation experiments in *Drosophila* made use of *in vitro* synthesized antisense RNA injected into early embryos (19, 20). However, our objectives required a generalized inactivation of rpA1 expression at different stages of development. To be able to inactivate rpA1 expression at critical developmental stages, we sought to construct flies that could express endogenous antisense rpA1 RNA in a controlled manner. The strong and inducible expression of antisense rpA1 RNA was obtained by placing the rpA1 coding region in reverse orientation under the control of the hsp70 promoter. The conditional promoter avoids the possible lethal effect of high constitutive expression of antisense rpA1 RNA. As a control, the rpA1 coding sequence was placed in sense orientation under the control of the same promoter (Fig. 1). The sense and antisense constructs were introduced into the genome of the fly by *P*-element transformation. Three antisense and four sense transformant lines were obtained. The integration sites of all three antisense and of one sense transformant lines were determined by *in situ* hybridization and are listed in Table 1. All transformant lines were made homozygous with respect to the integrated sequence except for line B, which is homozygous lethal.

Table 1. Effect of temperature on oogenesis of flies transformed with the antisense rpA1 gene

Transformant line	Integration site: Chromosome	Integration site: Band	Small eggs laid, % 18°C	22°C	29°C	37°C
rpA1(−)						
A1	X	12A	8–10	20–30	40	60–80
B	3L	73A	1–2	3–10	35–40	40–50
D4	2L	27EF	1–2	10–30	70	60–80
rpA1(+)						
H	3R	82CD	1–2	0	≤10	1

Orientations of the integrated rpA1 genes are antisense (−) or sense (+). Flies were either maintained at 18°C, 22°C, or 29°C or subjected to a 1-hr treatment at 37°C (heat shock).

The expression of the transformed genes in the different lines of transgenic flies was assessed by RNA blot analysis. The blots were hybridized with a double-stranded probe, thus allowing the simultaneous detection of transcripts from the resident and from the transformed genes (the transcripts have different sizes). The transcript from the resident gene served as a reference for quantitation of the transcripts from the transformed genes. Fig 2 shows that upon heat treatment, transcripts from the transformed genes quickly accumulated to high levels in all three antisense transformant lines and in

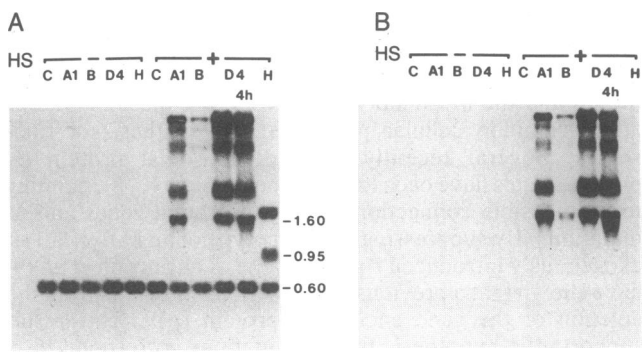

FIG. 2. Heat induction of the integrated antisense and sense rpA1 genes in adult females. (*A*) RNA was extracted from the antisense (A1, B, D4) and sense (H) transformants and the ry506 parental (C) female flies with (+) or without (−) being subjected to a prior 1-hr heat treatment (HS). The RNAs were analyzed on a Northern blot using a double-stranded rpA1 probe labeled with ^{32}P by nick-translation. Sizes are in kb. The 0.6-kb band corresponds to the endogenous rpA1 transcript; 0.95 kb is the predicted transcript size for the sense construct, and 1.60 kb is the predicted transcript size for the antisense construct. The larger bands in each lane correspond to readthrough transcripts. The D4 lane marked with "4h" shows the RNA extracted 4 hr after the heat pulse. (*B*) The filter shown in *A* was dehybridized and rehybridized with a strand-specific RNA probe that detects only the antisense rpA1 transcripts.

the control line H. A major antisense RNA band has the predicted size of 1.6 kb. In addition, a number of larger readthrough transcripts containing vector sequences were produced, suggesting that the transcription termination signals from the *ftz* gene did not function effectively or that the pre-mRNAs were not processed properly after heat shock (21). Similarly, besides the predicted 0.95-kb band, a larger readthrough sense transcript was also produced in the control line H. The amount of the antisense RNA accumulated during an 1-hr heat pulse can be a fewfold higher than the endogenous sense transcript. In the experiment shown in Fig. 2, which was done with newly transformed flies, the antisense RNA seems to be stable for at least 4 hr after heat treatment. It should be noted, however, that the antisense RNA was less stable after the fly stocks had been maintained for several months. The amounts of antisense RNA expression in larvae and pupae were similar to those in whole flies or dissected ovaries (data not shown).

Induction of Antisense rpA1 Gene Expression Severely Disrupts Oogenesis. Oogenesis, the third larval instar, and pupation are rapid cell growth phases during *Drosophila* development and may be particularly sensitive to a decrease in the rate of protein synthesis. The effect of antisense rpA1 induction was first examined during larval and pupal development. Transgenic larvae and pupae from synchronized embryo collections were allowed to develop at 25°C and were subjected to regular heat pulses (see *Materials and Methods*) to induce antisense gene expression. Surprisingly, heat treatment of even once every 6 hr throughout postembryonic development produced no apparent defects specific to the antisense transformants. Although frequent heat shock caused mild lethality in both control and experimental flies, no developmental delay or abnormal bristles typical of *M* mutants were detected in treated antisense-transformed flies. However, antisense rpA1 severely disrupted ovarian development.

Oogenesis in antisense-transformed flies is extremely sensitive to induction of the antisense rpA1 gene. When anti-rpA1 expression was induced in newly eclosed females by a 1-hr heat pulse at 37°C, there was a significant delay of ovarian maturation and an approximate 70% decrease in the rate of egg laying. Moreover, a high percentage (up to 80%; Table 1) of the eggs laid by these flies were much smaller than normal (Fig. 3 *Upper*). Most of these small eggs were not fertilized, and the few that were did not hatch. In comparison, none of the four control lines transformed with the sense DNA exhibited these traits after identical treatment. Moreover, in a parallel experiment, we constructed eight lines of flies transformed with an antisense D-*raf* oncogene (22), and none of those flies showed any oogenesis defects after anti-(D-*raf*) induction (unpublished observation). The appearance of the small-egg phenotype after a brief heat pulse is time dependent; after a lag of about 1 day, the proportion of small eggs increases, peaks about 2 days later, and then decreases again (Fig. 3 *Lower*). The transcriptional inactivity (and presumed lack of antisense RNA expression) of germ cells of late-stage egg chambers probably explains the initial lag. The relatively sharp peak observed in Fig. 3 *Lower* indicates that specific stages of oogenesis (possibly vitellogenic stages 8 to 10) are more affected than others.

The defect in oogenesis exhibits a clear temperature dependence: at 22°C, all three antisense transformant lines laid a significant proportion of abnormal eggs. When the antisense flies were reared at 18°C, the defects were greatly reduced; in contrast, at 29°C, more than half of the eggs were abnormal (Table 1). The defect displayed at 22°C and 29°C is likely due to the leaky expression of the hsp70 promoter, although we did not detect antisense RNA on Northern blots when flies were reared at these temperatures (Fig. 2).

The severity of the phenotype was also dependent on gene dosage and on the level of antisense expression (Table 1 and

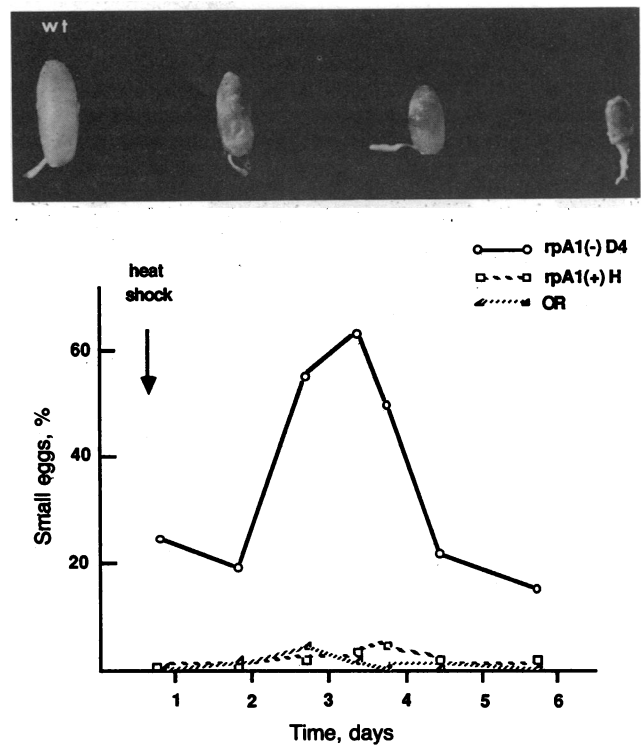

FIG. 3. Abnormal eggs are produced by transformed flies in response to antisense rpA1 gene induction. (*Upper*) Heat treatment of antisense transformants induces the formation of a high percentage of abnormal eggs which are significantly smaller than wild-type (wt) eggs. The small eggs were mostly unfertilized, and the few that were fertilized failed to hatch. The small eggs vary in size as shown here. (×30.) (*Lower*) Time course of appearance of the small egg phenotype after a heat pulse. Equal numbers of flies of the antisense line D4, sense line H, and wild-type Oregon R (OR) were kept at 22°C before the heat pulse. Flies were heat-treated by submerging the rearing vials into a 37°C water bath for 1 hr. The laid eggs were examined at the times indicated and the percentages of small eggs (see *Upper*) were recorded.

Fig. 2). Line B is less affected than lines A1 and D4 and also produces substantially less antisense RNA than the other two lines (line B is heterozygous with respect to the inserted sequence). When the antisense genes of the A1 and D4 lines were genetically combined into a single fly, the resulting A1/D4 females had only rudimentary ovaries (Fig. 4*A*) and were almost completely sterile when reared at 25°C. By contrast, the progenitor A1 and D4 females yielded large number of progeny at 25°C. The A1/D4 males were fertile. The sterile phenotype of A1/D4 females raised at 25°C could not be rescued by shifting them to lower temperatures, while flies of the same genotype raised continuously at 18°C did produce progeny. These observations suggest that female germ cells are uniquely sensitive and may die due to leaky anti-rpA1 expression during larval or pupal development at 25°C.

Possible Mechanisms of Anti-rpA1 Action. In an attempt to determine how the antisense rpA1 RNA exerts its effects, we assayed RNA duplex formation by using an RNase protection assay. Female flies were subjected to a 1-hr heat pulse at 37°C and allowed to recover for 3 hr at 22°C. Either flies or dissected ovaries were homogenized, and either the homogenate or the purified RNA was digested with various amounts of RNase A or T1. No protected RNA fragments were detected when the digestion products were analyzed on RNA blots. Control double-stranded rpA1 RNA synthesized *in vitro* yielded a protected fragment even at the highest concentration of RNase used (data not shown). These results suggest that substantial amounts of double-stranded RNA do

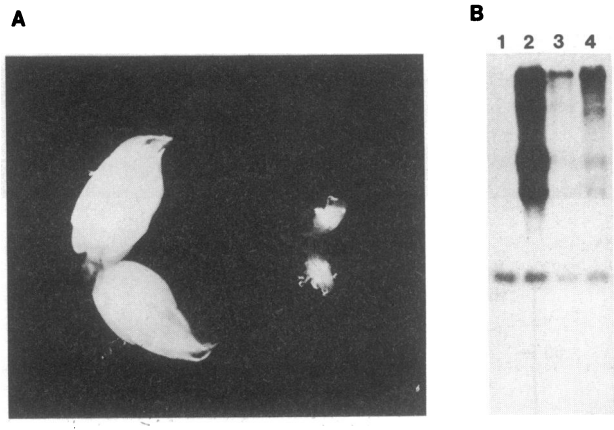

FIG. 4. Dependence of ovarian development on antisense gene dosage. (*A*) The anti-rpA1 gene dosage was increased by genetically combining the antisense genes from the A1 and D4 lines into a single fly. The resulting "doubly transformed" A1/D4 females carry 4 copies of anti-rpA1 per diploid genome. The A1/D4 females were sterile when reared at 25°C. Most of their ovaries (right) lack late-stage egg chambers, in contrast to ovaries from control H females (left), which appear normal. The ovaries were from 5-day-old flies. (×20.) (*B*) Northern analysis of anti-rpA1 expression in A1/D4 and D4 females. Lane 1, A1/D4 before heat shock; lane 2, A1/D4 immediately after heat shock; lane 3, A1/D4, 4 hr after heat shock; lane 4, D4 immediately after heat shock. The filter was hybridized with a double-stranded nick-translated rpA1 probe. The dramatically enhanced sterility of the doubly transformed flies is correlated with a large increase in antisense expression.

not form *in vivo*, that such RNA is unstable, or that the double-stranded region is too small to be detected by the techniques employed.

The doubly transformed A1/D4 flies expressed antisense rpA1 RNA at much higher level than either the A1 or D4 parental flies (Fig. 4*B*). Further analysis indicated that such high antisense expression resulted in a substantial decrease of a number of different mRNAs. Relative to ribosomal RNA, the mRNA abundance of not only rpA1, but also of actin, tubulin, and rp49 decreased significantly in these doubly transformed flies 4 hr after anti-rpA1 induction (Fig. 5). Apparently, such a decrease in cellular mRNA was responsible for the disruption of oogenesis.

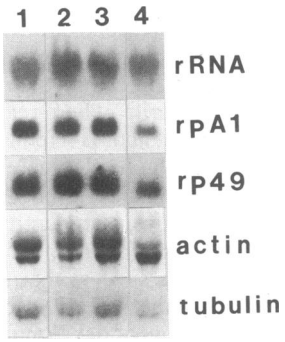

FIG. 5. High induction of the antisense rpA1 gene in doubly transformed A1/D4 flies results in a decrease in the rpA1, rp49, actin, and tubulin mRNAs. Four hours after heat treatment, RNAs were extracted from control H (lane 1), antisense A1 (lane 2), antisense D4 (lane 3), and doubly transformed A1/D4 (lane 4) females and analyzed on a Northern blot. The same blot was sequentially hybridized with rpA1, rp49, actin, tubulin, and ribosomal RNA (rRNA) probes. The actin probe hybridizes to two size classes of actin mRNAs. The rRNA provided a reference for the amount of RNA analyzed in each lane (10 μg per lane). Note that in the doubly transformed flies (lane 4) the levels of rpA1 mRNA and of other mRNAs are substantially reduced relative to rRNA. Also note that A1/D4 flies produce considerably more antisense rpA1 RNA than other transformed flies upon heat induction (Fig. 4*B*).

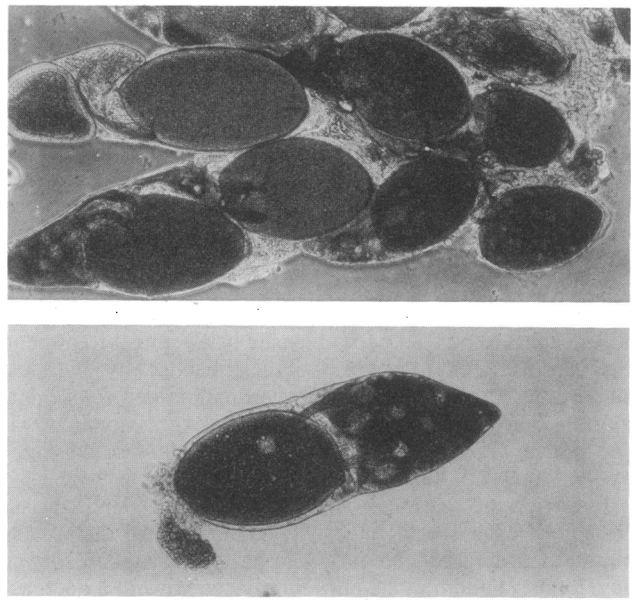

FIG. 6. Defective ovary and egg chamber phenotype of antisense transformant flies. (×90.) (*Upper*) Cluster of small, round mature oocytes from the ovary of a D4 fly that was subjected to a 1-hr heat pulse. Small stage-14 oocytes associated with clusters of nurse cells are visible in the lower portion of the field. (*Lower*) Detail of a mature, small stage-14 oocyte capped by a nurse-cell cluster.

One possible interpretation of the small-egg phenotype is that the egg chamber fails to synthesize its constituents in sufficient amount. However, microscopic examination of dissected ovaries reveals that the defect may be of a different nature. In flies that express the antisense gene, it is common to observe mature oocytes, complete with dorsal appendages, that have a "cap" of nurse cells over them (Fig. 6). A likely interpretation of this observation is that the small size of the egg results from the failure of the nurse cells to transfer their contents to the oocyte at the end of oogenesis.

DISCUSSION

Transformation of an antisense r-protein gene into the *Drosophila* genome resulted in a conditional pseudo-female-sterile phenotype. Sterility in these flies can be induced either by growth at elevated temperatures as in conventional temperature-sensitive mutants or by a brief treatment at 37°C. Several lines of evidence indicate that the observed phenotype is due specifically to the expression of the antisense rpA1 RNA. The possibility that the phenotype is due to insertional mutagenesis is ruled out by the fact that three independent antisense lines with the transposon integrated at different chromosomal locations (Table 1) exhibit the same defects. Also arguing in favor of a specific role for the antisense rpA1 sequences is the observation that the severity of the defects correlates with the degree of antisense expression (Table 1, Fig. 2, and Fig. 4). Finally, the fact that expression of an unrelated antisense RNA (D-*raf*) to high levels had no effect on oogenesis (unpublished observation) also supports the specificity of the phenotype observed in response to anti-rpA1 expression.

When the expression of the antisense rpA1 gene was enhanced in the doubly transformed A1/D4 flies, we detected the reduction of the endogenous rpA1 mRNA. The decrease in abundance of several mRNAs in addition to rpA1 mRNA (Fig. 5) was unexpected. Differing from this result, previous antisense experiments have shown that the expression of an antisense discoidin gene in *Dictyostelium* and an antisense c-*fos* gene in 3T3 mouse cells led to a specific degradation of

the endogenous target mRNAs without affecting other transcripts (23, 24). The actual mechanism of anti-rpA1 action remains unclear, and several possibilities can be envisioned. The decrease of cellular mRNAs that we observed may be related to the function of rpA1. Since rpA1 may play a crucial role in translation initiation and elongation (8), a partial repression of rpA1 synthesis would result in the synthesis of defective ribosomes lacking this essential r-protein. The defective ribosomes would be inefficient in mRNA translation and consequently lead to an increased mRNA turnover. Levels of ribosomal RNAs would not be affected because they are protected within the ribosomes. Another possibility is that the accumulation of high levels of anti-rpA1 transcripts stimulated an RNase activity and triggered a general degradation of cellular mRNAs. However, this effect must be specific to anti-rpA1 expression, since high anti-(D-*raf*) expression had no effect on oogenesis. Therefore, the decrease in cellular mRNA and the disruption of oogenesis could not result merely from the expression of a foreign RNA.

M loci have been correlated with r-protein genes (see introduction). Interestingly, several (but not all) of the phenotypes that we observed are also those of *M* mutants. For instance, reduced female fertility or even sterility is often associated with the *M* phenotype. Li (25) noted that there is a maternal effect of *M(2)l* on both the viability of the progeny and the shape of the eggs. Li described the eggs laid by *M(2)l* females as "smaller than normal in size and more oval in shape." These observations were confirmed 30 years later by Farnsworth (26). Despite the similarity of the phenotype concerning oogenesis, anti-rpA1 flies exhibited none of the characteristic postembryonic *M* phenotypes, even though the antisense transcripts appear to be as abundant and as stable in postembryonic stages as in oogenesis (data not shown). It is possible that mutations in the rpA1 gene will have a phenotype different from that of *M*s. In *Xenopus*, a duplex-RNA-unwinding activity was detected in eggs but not in oocytes (27, 28). The presence of such an activity in *Drosophila* somatic tissues but not in germ cells could provide an explanation for our results. However, we were unable to detect any double-stranded RNA in the ovary.

Previous antisense experiments in *Drosophila* relied on the injection of very large excess of antisense over sense RNA (19, 20). That synthesis of excess antisense RNA is not an absolute prerequisite for gene inactivation is indicated by the significant number of abnormal eggs laid by the antisense flies that were kept at room temperature (Table 1). Under these conditions the levels of antisense RNA should be low. The exquisite sensitivity of oogenesis to antisense RNA may be related to the extremely high rates of ribosome synthesis (see introduction). During late vitellogenic stages, surplus r-protein mRNA and ribosomes may be very low or may not exist at all (29, 30), explaining why even a modest reduction in the rate of ribosome synthesis has a profound effect on oocyte development.

The abnormal egg chambers observed in ovaries of antisense transformant flies (Fig. 5) suggest that rather than failure to accumulate sufficient amounts of egg constituents, the small-egg phenotype is the result of incomplete transfer of the nurse cell contents to the oocyte. Possibly, expression of the antisense gene causes an uncoupling of the nurse cell and follicle cell developmental programs. If follicle cells are less affected by the antisense RNA, they would synthesize the protective coverings of the egg ahead of the nurse cell's schedule, thus blocking the transfer of their contents to the oocyte. A number of "dumpless" mutants with similar phenotype have been recently isolated by T. Schupbach (personal communication). Our observations point to the possibility that the functions affected in at least some of these mutants may not be required specifically for oogenesis but rather for more general cellular processes. The analysis of null "dumpless" mutations will be essential to clarify this possibility.

Antisense inhibition at the organism level has been achieved in *Dictyostelium* (23, 31), in plants (32), in mice (33), and in *Drosophila* (this report). These results promise future applications of antisense mutagenesis in the functional analysis of cloned genes. Nevertheless, a few points suggested by the present study should be considered when applying this approach at the whole organism level. Although the results from the control sense rpA1 and antisense D-*raf* transformed flies support the specific role of the antisense rpA1 RNA, it remains to be verified that the inhibition of rpA1 synthesis is directly responsible for the decrease of cellular mRNA. Moreover, antisense gene expression might in many cases not be effective in bringing substantial reduction of the target gene activity. Suitable targets for antisense mutagenesis would be those genes that are expressed with little or no surplus relative to cellular needs. Antisense inhibition may be more effective in some cell types than in others. In the present experiments, the female germ cells appeared to be particularly sensitive. Finally, this approach should not be attempted for obtaining null mutations.

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