Translational regulation of a specific gene during oogenesis and embryogenesis of *Drosophila*

(protein synthesis/development)

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ABSTRACT Polysomal and postpolysomal mRNAs were prepared from *Drosophila* egg chambers or embryos of different developmental stages. Cell-free translation of these mRNAs followed by two-dimensional gel electrophoresis of the products indicated the presence of a specific mRNA that appears to be translated (polysome-associated) during oogenesis. This mRNA, designated T1 mRNA, is selectively excluded from polysomes in 3-hr- and 5-hr-old embryos and is again translated in 18-hr-old embryos. A clone containing DNA complementary to T1 mRNA was selected from a library of recombinant DNA prepared from polyadenylylated ovary RNA. This clone was positively identified by hybrid-selected translation followed by two-dimensional gel electrophoresis and autoradiography. T1 mRNA is polyadenylylated and codes for a small, acidic protein. The cloned probe hybridizes to a unique site (2L-39CD) of the polytene chromosomes, very close to the histone genes. The results suggest that this mRNA is under specific translational regulation in contrast to a background of a large number of other abundant mRNAs that are translated at all developmental stages examined.

As mosaic organisms, insect eggs are believed to store substantial information needed for early development. Examples of findings that support this assertion are: the isolation in *Drosophila* of a large number of maternal-effect mutants having broadly varying phenotypes (1, 2); the identification of germ-cell determinants located in the posterior region of the *Drosophila* egg (3, 4); and the demonstration of determinants of anterior structures located at the anterior tip of *Smittia* eggs (5). Little is known about the maternal molecules in the egg that encode key developmental information. Whatever their nature, they must be synthesized during oogenesis. We have shown that the RNA stored in *Drosophila* eggs has the relatively low complexity of 1.2×10^7 nucleotides (6) and that egg chambers synthesize RNA of approximately equal complexity from the beginning of oogenesis (7). We are interested in the differential utilization of informational molecules along the oogenesis–embryogenesis developmental axis. Whereas in mature *Drosophila* oocytes a few mRNAs are not translated, they become polysome-associated in 3-hr-old embryos (8). It has not been determined whether those mRNAs are ever translated during oogenesis. Here we report on a previously undetected mRNA whose translation appears to be uniquely regulated during early *Drosophila* development. This mRNA is polysome-associated during oogenesis but is excluded from polysomes in early embryos, where it is found as an untranslated postpolysomal messenger ribonucleoprotein (RNP). This mRNA species again becomes polysome-associated in late embryos. We isolated a bacterial clone carrying a recombinant plasmid complementary to this mRNA. Using DNA from this clone as a probe, we de-

termined the location of the corresponding gene on polytene chromosomes by *in situ* hybridization.

MATERIALS AND METHODS

Egg Chambers and Embryos. Wild-type *Drosophila melanogaster* of the Oregon R strain were reared at 25°C in population cages and fed live yeast. Egg chambers were fractionated as described (9) except that stage-10 egg chambers were manually selected to assure uniformity of developmental stage (10). The buffer used for egg-chamber fractionation was supplemented with 50 μg of cycloheximide per ml to prevent polysome run-off. Embryos were collected for 30-min periods and aged at 25°C so that they were at the desired age at the time of homogenization. Synchrony of each embryo collection was assessed by microscopic observation (11) after the chorion was rendered transparent with Halocarbon series 27 oil (Halocarbon Products, Hackensack, NJ). More than 88% of the embryos were always at the desired stage.

Isolation and Fractionation of RNA. Unless otherwise stated, all operations were performed at 4°C. Egg chambers and embryos were homogenized at a concentration of about 4,000 per ml in homogenization buffer: 250 mM NaCl/50 mM MgCl$_2$/50 mM Tris·HCl, pH 7.5/0.2% Triton X-100 containing 10 mg of heparin and 1 μg of cycloheximide per ml. A postmitochondrial supernatant was obtained by centrifuging the homogenate for 10 min at 20,000 × g. A 100-μl aliquot of the supernatant was used to determine the polysome content by centrifugation through an analytical 5-ml linear 15–50% sucrose gradient for 40 min at 265,000 × g maximum with a Beckman SW 50.1 rotor. The sucrose was prepared in buffer A: 250 mM NaCl/25 mM MgCl$_2$/50 mM Tris·HCl, pH 7.5. The remaining postmitochondrial supernatant was centrifuged on a preparative 35-ml linear 15–30% sucrose gradient in buffer A for 21 hr at 65,000 × g maximum with a Beckman SW 27 rotor. Fractions from this gradient were combined into two pools (see Fig. 1): postpolysomal RNPs, containing material sedimenting between about 20 and 40 S, and polysomes, containing material sedimenting at more than 80 S (8). The RNA from the two pools was extracted (6). The poly(A)-containing RNA was obtained by column chromatography on oligo(dT)-cellulose as described (12) except that all buffers contained 0.5% NaDodSO$_4$. The RNA was precipitated with ethanol twice and resuspended in 10 mM Tris·HCl (pH 7.5).

In Vitro Translation and Two-Dimensional Gel Electrophoresis. Poly(A)-containing RNA was translated at a final concentration of 20 μg/ml in a nuclease-treated reticulocyte cell-free system (New England Nuclear). Incubation was for 1 hr at

30°C in the presence of 1 mCi (1 Ci = 3.7×10^{10} Bq) of [^{35}S]-methionine (New England Nuclear) per ml. The *in vitro* translation products were analyzed by two-dimensional gel electrolation products were analyzed by two-dimensional gel electrophoresis (13) except that the samples (containing $0.5-1.0 \times 10^6$ cpm of acid-precipitable radioactivity) were dissolved in isoelectric focusing sample buffer containing 0.2% NaDodSO$_4$. The second dimension was run on a 12.5% polyacrylamide slab gel (14), and fluorography was carried out with sodium salicylate (15). Preflashed Kodak X-Omat R film was exposed to the dried gels usually for 2–3 days at −70°C.

Screening the cDNA Library. The cDNA library was constructed by cloning DNA complementary to poly(A)-containing RNAs from egg chambers stages 11–14 (16). High-density plasmid screening (17) was used for plating, growth, and colony hybridization of the library. Replica filters were screened with a [^{32}P]-labeled cDNA probe (1×10^6 cpm per filter) complementary either to poly(A)-containing postpolysomal RNA from stage-14 unfertilized eggs or to poly(A)-containing polysomal RNA from 3-hr-old embryos. RNAs were reverse-transcribed in 100 μl incubations containing 40 mM KCl; 8 mM MgCl$_2$; 50 mM Tris·HCl (pH 8.3); 500 μM each of dATP, dGTP, and dTTP; 20 μM [^{32}P]dCTP; 4 mM Na$_2$P$_2$O$_7$; 0.4 mM dithiothreitol; and 20 μg of oligo(dT)$_{(12-18)}$, 200 units of avian myeloblastosis virus reverse transcriptase (a gift from J. W. Beard), and 20 μg of poly(A)-containing RNA per ml. Preincubation was for 10 min at 15°C, and incubation was for 1 hr at 37°C. After hybridization and autoradiography, colonies of interest were picked with sterile toothpicks and rescreened as above.

Hybrid-Selected Translation. Cloned plasmid DNA (50–100 μg) was denatured with 0.3 M NaOH for 10 min at 65°C. Samples were cooled in ice; after addition of 1 vol of 2 M NH$_4$OAc (final pH, ≈ 9.5) they were applied by gravity onto 13-mm nitrocellulose filters (Schleicher & Schuell, BA85). All operations were performed at 4°C. Filters were rinsed twice with 0.3 M NaOH/2 M NH$_4$OAc, 1:1 (vol/vol), and twice with 4× standard saline citrate (NaCl/Cit; 1× is 0.15 M NaCl/0.015 M sodium citrate, pH 7). They were then blotted dry and baked under vacuum overnight at 68°C. The filters were prehybridized at 50°C in hybridization buffer: 600 mM NaCl/25 mM Hepes/NaOH, pH 6.7/1 mM EDTA/65% deionized formamide/100 μg of poly(A) per ml. After 1 hr the hybridization buffer was removed and replaced by hybridization buffer containing 250 μg of polyadenylylated RNA from stage-14 egg chambers per ml; 40 μl of this solution was added per filter. The samples were incubated for 12 hr at 50°C. The filters were washed, and the hybridized RNA was eluted (18). The hybrid-selected RNA was translated, and the products were fractionated by two-dimensional gel electrophoresis as described above.

***In Situ* Hybridization to Polytene Chromosomes.** Third-instar larva salivary glands were fixed and squashed (19). The RNA digestion and denaturation of chromosomes was as described (20) except that treatment with HCl was omitted. DNA used for the hybridization was labeled *in vitro* by nick-translation (21). The reaction mixture (final volume, 10 μl) contained 50 mM Tris·HCl (pH 7.5); 5 mM MgOAc$_2$; 5 μg of bovine serum albumin per ml, 20 μM each of dATP, dCTP, and dGTP; 5 μM (0.5 mCi/ml) [^{3}H]TTP; and 12.5 μg of plasmid DNA, 2.5 ng of DNase, and 125 units of DNA polymerase I (New England Nuclear) per ml. Incubation was at 14°C for 2 hr. Nonincorporated nucleotides were removed by filtration through Bio-Gel P60 (Bio-Rad). Hybridization was carried out in 50 μl of hybridization buffer containing 2× NaCl/Cit, 1× Denhardt's solution (22), and 4.5×10^4 cpm of plasmid DNA nick-translated with [^{3}H]TTP to 10^7 cpm/μg. The slides were incubated in a plastic chamber containing hybridization buffer to prevent

evaporation of the reaction mixture. After incubation at 65°C for 16 hr, the probe was removed and the slides were washed three times with 2× NaCl/Cit at 60°C and twice at room temperature, dehydrated in 70% and then in 95% ethanol, and dried in air. Autoradiography was performed by dipping the slides in Kodak NTB-2 liquid emulsion, exposing at 4°C, developing, and staining with Giemsa (23).

RESULTS

Isolation of Polysomal and Postpolysomal RNAs. To test if selective translational regulation occurs in early development of *Drosophila*, mRNAs were prepared from polysomal and postpolysomal compartments and examined by *in vitro* translation followed by two-dimensional gel electrophoresis. Postmitochondrial supernatants from egg chambers or embryos were fractionated on preparative sucrose gradients (Fig. 1B). RNP particles with sedimentation coefficients of up to 80 S were found within the gradient, whereas polysomes were recovered from the pellet. Because the polysomal profile is not displayed in this gradient, it was important to ascertain that the polysomes were undegraded and had not run off. Therefore, for every experiment, an aliquot of the material to be fractionated preparatively was analyzed on small sucrose gradients (Fig. 1A). Only preparations showing intact polysomal profiles were used. Fractions
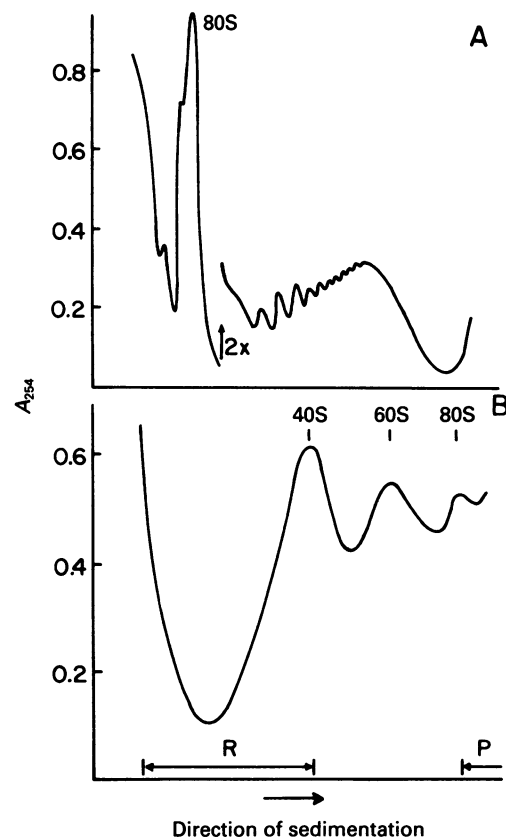

FIG. 1. Sedimentation profiles of postmitochondrial supernatants from stage-14 unfertilized eggs. (A) Analytical gradient (displays polysomal profile): 2 A_{260} units of postmitochondrial supernatant were centrifuged through a 5-ml 15–50% sucrose gradient. At the point indicated, the scale sensitivity was increased 2-fold. (B) Preparative gradient (displays postpolysomal profile): 70 A_{260} units of the same postmitochondrial supernatant were centrifuged through a 35-ml 15–30% sucrose gradient. Monosomes (80 S) and ribosomal subunits are contained in the gradient, polysomes are in the pellet. The horizontal bars indicate the pooled fractions. R, postpolysomal fraction; P, polysomal fraction (includes the pellet).

of preparative sucrose gradients were pooled as in Fig. 1B. In recent experiments, preparative gradients were centrifuged so that polysomes were contained within the gradient (Fig. 1A) rather than in the pellet (Fig. 1B). Equivalent fractions were pooled from these gradients, yielding similar results. RNA was extracted from each pool, and poly(A)-containing RNA was prepared by oligo(dT)-cellulose column chromatography.

Comparison of Translational Products from Polysomal and Postpolysomal mRNAs of Different Developmental Stages. Fig. 2 shows the distribution of translational products from polysomal and postpolysomal mRNAs of 3-hr-old embryos analyzed by two-dimensional gel electrophoresis. Similar patterns were obtained from corresponding fractions of other developmental stages. In general, the pattern obtained from postpolysomal mRNAs (Fig. 2A) was simpler than the one obtained from polysomal mRNAs (Fig. 2B). This result can be attributed to at least two reasons. (*i*) Not all postpolysomal mRNPs were pooled, but only the relatively slow-sedimenting ones were (Fig. 1B). (*ii*) Some mRNAs may be very efficiently translated *in vivo*, and such mRNAs would be expected to be associated almost exclusively with polysomes. Comparison of the translational patterns of postpolysomal and polysomal RNAs from different developmental stages showed significant differences in the acidic, low molecular weight region of the gels (indicated by the rectangle in Fig. 2 A and B). One difference relates to a prominent translational product of $\approx$16,000 daltons, T1 in Fig. 3. Three translational products migrating in the same region of the gel (a, b, and c) were used as references. Fig. 3 demonstrates that association of T1 mRNA with polysomes was specifically regulated during development. A significant proportion of T1 mRNA was associated with polysomes during mid and late oogenesis (Fig. 3 A–D), whereas during early embryogenesis T1 mRNA was hardly detectable among translational products of polysomal mRNAs (Fig. 3 E–H). By 18 hr of embryogenesis, however, T1 mRNA was found again to be associated with polysomes *in vivo* (Fig. 3 I and J).

The results shown in Fig. 3 suggest the following interpretations. First, the observed changes of T1 mRNA association with polysomes is specific because the translation of the majority of other abundant mRNAs is not modulated in the same way (e.g., translational products a, b, and c). Moreover, the independent assortment of a, b, and c mRNAs and T1 mRNA between RNPs and polysomes serves as an internal control for possible artifacts, such as RNA degradation during fractionation or increase
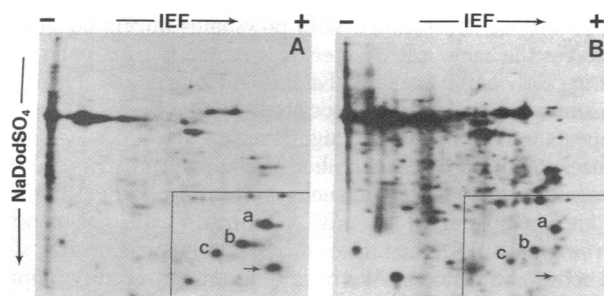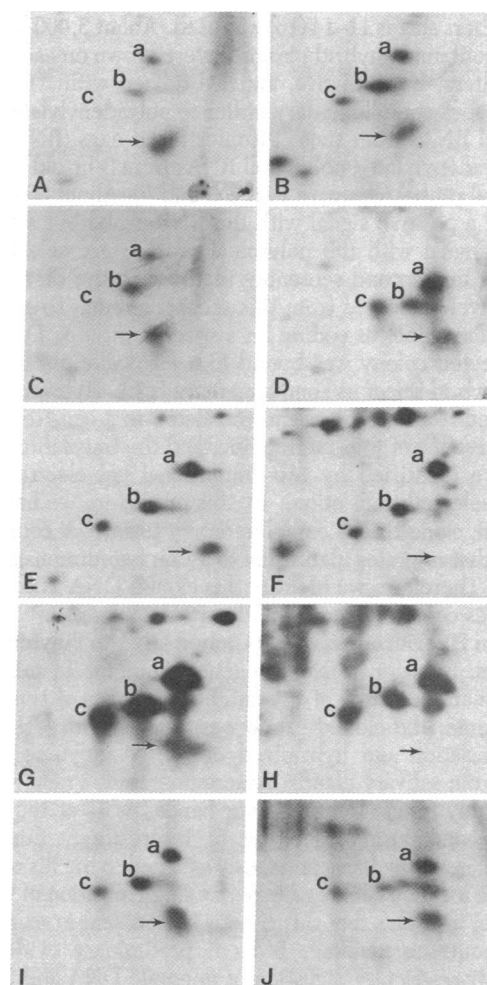

FIG. 3. Changes in *in vitro* [35S]methionine-labeled translated products of polyadenylylated mRNAs prepared from the postpolysomal (*Left*) and polysomal (*Right*) fractions of different developmental stages. The areas of autoradiograms shown correspond to those delimited in Fig. 2. The source of the RNA that was translated is as follows: stage-10 egg chambers (mid-oogenesis) (*A* and *B*); stage-14 oocytes (unfertilized eggs) (*C* and *D*); 3-hr-old blastula embryos (*E* and *F*); 5-hr-old gastrula embryos (*G* and *H*); 18-hr-old embryos (*I* and *J*). Identification of the products as in Fig. 2.

FIG. 2. Two-dimensional gel analysis of *in vitro* translation products of postpolysomal and polysomal mRNAs. Polyadenylylated RNAs obtained from postpolysomal and polysomal fractions (compare with Fig. 1B) of 3-hr-old embryos were translated in a reticulocyte cell-free system in the presence of [35S]methionine, followed by two-dimensional gel electrophoresis of the products and autoradiography. (*A*) Poly(A)-containing RNA from the postpolysomal fraction was translated. (*B*) Poly(A)-containing RNA from the polysomal fraction was translated. The rectangles delimit the area to which the translation product of T1 mRNA migrates (→), and *a*, *b*, and *c* are reference proteins; this area of the gel is shown in each panel of Fig. 3. IEF, isoelectric focusing.

of mRNA levels in the postpolysomal fraction because of polysome run-off. Second, T1 mRNA is not degraded *in vivo* but is present at all developmental stages examined in a form that is translatable in a cell-free system. This reinforces the interpretation that regulation occurs at the translational rather than pretranslational level. Notice that the mRNA coding for protein c (Fig. 3) also appears to be regulated at the translational level. During oogenesis most of this mRNA is polysome-associated (Fig. 3 A–D), whereas during embryogenesis the same mRNA species is substantially represented in both polysomal and postpolysomal fractions (Fig. 3 E–J).

Selection of a Specific Cloned DNA and Its Identification by Hybrid-Selected Translation. The previous section provided evidence for the presence of a relatively abundant RNA species (T1 mRNA) among postpolysomal mRNAs of unfertilized eggs (Fig. 3C) and its scarcity or absence among polysomal mRNAs of 3-hr- and 5-hr-old embryos (Fig. 3 F and H). This is a favorable situation for the selection from a cDNA library of a recombinant DNA clone coding for this mRNA by the differential screening technique (24). A library of recombinant plasmids prepared from cDNA to poly(A)-containing RNAs of

egg chambers stages 11–14 (16) was used. About 3,000 bacterial colonies containing hybrid plasmids were grown on each of two replica filters, lysed *in situ*, and hybridized with ^{32}P-labeled cDNA probes complementary either to polyadenylylated post-polysomal RNAs from stage-14 unfertilized eggs (RNP probe) or to polyadenylylated polysomal RNAs from 3-hr-old embryos (polysomal probe) (compare Fig. 3 *C* and *F*). About 50 colonies that gave a positive signal with the RNP probe but a weak or negative signal with the polysomal probe were selected and subjected to a second screening identical to the first. Eleven colonies were selected from this second screen. To positively identify the clones as coding for a specific mRNA, DNA from each selected colony was bound to a nitrocellulose filter and used to hybrid-select its complementary RNA. Hybridized RNA was eluted from the filter and translated in a cell-free system in the presence of [^{35}S]methionine, and the translational products were identified by two-dimensional gel electrophoresis (hybrid-selected translation). By this procedure we have identified one clone that is complementary to mRNA coding for a protein that migrates identically to T1 on two-dimensional gels (Fig. 4). Therefore, we identify this cloned DNA as containing sequences complementary to T1 mRNA.

In Situ Hybridization of the Cloned DNA to Polytene Chromosomes. In order to map the gene coding for T1 mRNA and to obtain an indication of the number of copies of this gene in the genome, the cloned probe was labeled with [^{3}H]TTP by nick-translation and hybridized *in situ* to polytene chromosomes from salivary glands. Autoradiographic grains were observed only in one location, over bands 39CD of the left arm of the second chromosome (Fig. 5). Interestingly, this location is very close to the locus previously assigned to the histone genes (25). The location of this gene over a single position of the chromosome, indicates that this gene is not present in many copies throughout the genome. Indeed, preliminary evidence obtained by restriction digestion of genomic DNA and Southern

FIG. 5. Localization on the polytene chromosomes of the gene coding for T1 mRNA. DNA from the clone containing T1 mRNA sequences (compare Fig. 4) was labeled with [^{3}H]TTP and hybridized to chromosomes from salivary gland squashes, followed by autoradiography. Each squash was hybridized with 45,000 cpm of DNA and exposed to autoradiographic emulsion for 21 days. (*A*) Grains were detected at a single site on the left arm of the second chromosome at position 39CD (arrow). (*B*) *A* at higher magnification, showing hybridization to the position 39CD.

blotting (data not shown) suggests that this may be a unique gene.

DISCUSSION

At any stage of *Drosophila* oogenesis and early embryogenesis, ≈50% of the ribosomes are found associated with mRNAs in polysomes (10, 26). These polysomal mRNAs are presumably actively translated by the cell, whereas mRNAs associated with postpolysomal RNP particles (mRNPs) are not being actively translated by the cell at the time of homogenization. A species of mRNA that is under negative translational regulation is expected to be enriched in the postpolysomal mRNP fraction and depleted from the polysomal fraction. A substantial proportion of T1 mRNA is associated with polysomes during oogenesis, whereas the same mRNA species is excluded from polysomes during early embryogenesis. Later in embryogenesis, T1 mRNA becomes again polysome-associated. Regulation of T1 mRNA appears to be selective and significant in that it occurs against a background of a large number of abundant mRNAs that apparently are not subject to such a control. In particular, translational regulation of T1 mRNA contrasts with that of other *Drosophila* mRNAs coding for proteins of known function, such as actins and histones. We have determined recently (unpublished data) that a substantial proportion of these mRNAs are associated with polysomes both during oogenesis and early embryogenesis. In addition to the findings of Mermod and Crippa (8) mentioned earlier, an interesting precedent exists for specific translational regulation during *Drosophila* oogenesis: mRNA coding for chorion protein s15-1 can be detected in the cytoplasm of stage-9 egg chambers at the time of chorion gene amplification but is selectively excluded from polysomes at this stage of oogenesis. The s15-1 mRNA appears to be degraded shortly thereafter, then resynthesized, and translated later in oogenesis (27). In other organisms large changes in overall rates
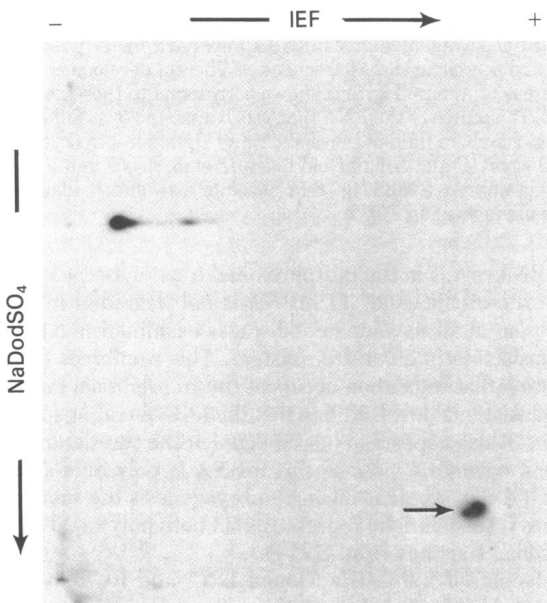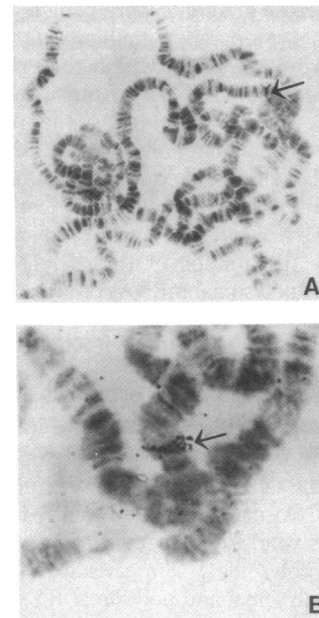

FIG. 4. Identification by hybrid-selected translation of a cDNA clone coding for T1 mRNA. DNA from a selected recombinant cDNA clone (see text) was immobilized on a nitrocellulose filter and used to hybrid-select its complementary RNA. This RNA was translated *in vitro* in the presence of [^{35}S]methionine, and the products were analyzed by two-dimensional gel electrophoresis followed by autoradiography. The arrow points to the translational product from the selected mRNA. The spots on the upper-left of the gel correspond to endogenous products of the cell-free system. IEF, isoelectric focusing.

of protein synthesis at oocyte maturation or at fertilization is a common finding. However, in some cases translation of defined mRNAs does not follow the same pattern as that of the bulk mRNAs. Notably, selective translational regulation occurs in Spisula (28), in sea urchins (H3 and α-type histones; refs. 29 and 30), and in mouse (31). Transcription in *Drosophila* embryos is very low during the first 3 hr of development (32–34). This raises the possibility that the same T1 mRNA molecules that were translated during oogenesis are excluded from polysomes in early embryos. However, formal proof of this fact requires further experimentation. At 18-hr of embryonic development, T1 mRNA abundance is approximately the same as that at 3 hr (our unpublished observations). Therefore, the T1 mRNA that is polysome-associated in 18-hr-old embryos could be either maternal in origin or newly synthesized if significant T1 mRNA turnover occurs.

Although it is not possible to infer from the reported experiments the exact mechanism of selective translational regulation, certain possibilities can be excluded and others considered. Selective degradation or irreversible alteration of T1 mRNA does not seem to occur because this mRNA is found in the embryos associated with the postpolysomal RNP fraction in a form that is translatable in a cell-free protein-synthesizing system. Although only polyadenylylated mRNA was analyzed for the reported experiments, selective deadenylylation of T1 mRNA is not likely to be responsible for our observations. When early embryo RNA that was retained on an oligo(dT)-cellulose column was assayed by RNA blotting, in parallel with a large excess of RNA that was not retained by the column, T1 mRNA was detected only in the retained fraction (data not shown). It is possible that differential translation of the mRNA is due to differences in the protein constituents of the T1 mRNPs at different developmental stages. This protein may directly affect the binding of ribosomes or alter the secondary or tertiary structure of T1 mRNA. Moreover, changes in the 5'-terminus cap structure such as methylation or demethylation could influence the translation of T1 mRNA. These possibilities are now being investigated.

1. Zalokar, M., Audit, C. & Erk, I. (1975) *Dev. Biol.* 47, 419–432.
2. Mohler, J. D. (1977) *Genetics* 85, 259–272.
3. Illmensee, K. & Mahowald, A. P. (1974) *Proc. Natl. Acad. Sci. USA* 71, 1016–1020.
4. Okada, M., Kleinman, I. A. & Schneiderman, H. L. (1974) *Dev. Biol.* 37, 43–54.
5. Kalthoff, K. (1979) in *Determinants of Spatial Organization*, eds. Subtelny, S. & Konisberg, I. R. (Academic, New York), pp. 97–126.
6. Hough-Evans, B., Jacobs-Lorena, M., Cummings, M. R., Britten, R. J. & Davidson, E. H. (1980) *Genetics* 95, 81–94.
7. Jacobs-Lorena, M., Hough-Evans, B. R., Britten, R. J. & Davidson, E. H. (1980) *Dev. Biol.* 76, 509–513.
8. Mermod, J.-J., Schatz, G. & Crippa, M. (1980) *Dev. Biol.* 75, 177–186.
9. Jacobs-Lorena, M. & Crippa, M. (1977) *Dev. Biol.* 57, 382–385.
10. Ruddell, A. & Jacobs-Lorena, M. (1983) *Wilhelm Roux's Arch. Dev. Biol.*, in press.
11. Bownes, M. (1975) *J. Embryol. Exp. Morphol.* 33, 789–801.
12. Faust, C. H., Jr., Diggelmann, H. & Mach, B. (1973) *Biochemistry* 12, 925–931.
13. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* 12, 1133–1142.
14. Laemmli, U. K. (1970) *Nature (London)* 227, 680–685.
15. Chamberlain, J. P. (1979) *Anal. Biochem.* 98, 132–135.
16. Spradling, A. C., Digan, M. E. & Mahowald, A. P. (1980) *Cell* 19, 905–914.
17. Hanahan, D. & Meselson, M. (1980) *Gene* 10, 63–67.
18. Ricciardi, R. P., Miller, J. S. & Roberts, B. E. (1979) *Proc. Natl. Acad. Sci. USA* 76, 4927–4931.
19. Atherton, D. & Gall, J. G. (1972) *Drosoph. Inf. Serv.* 49, 131–133.
20. Gall, J. G. & Pardue, M. L. (1971) *Methods Enzymol.* 21, 470–480.
21. Rigby, P. W. J., Dieckmann, M., Rhoses, C. & Berg, P. (1977) *J. Mol. Biol.* 113, 237–251.
22. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* 23, 641–646.
23. Pardue, M. L. & Gall, J. G. (1975) in *Methods in Cell Biology*, ed. Prescott, D. (Academic, New York), Vol. 10, pp. 1–34.
24. St. John, T. P. & Davis, R. W. (1979) *Cell* 16, 443–452.
25. Pardue, M. L., Kedes, L. H., Weinberg, E. S. & Birnstiel, M. L. (1977) *Chromosoma* 63, 135–151.
26. Mermod, J.-J. & Crippa, M. (1978) *Dev. Biol.* 66, 586–592.
27. Thireos, G., Griffin-Shea, R. & Kafatos, F. C. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5789–5793.
28. Rosenthal, E. T., Hunt, T. & Ruderman, J. V. (1980) *Cell* 20, 487–494.
29. Wells, D. E., Showman, R. M., Klein, W. H. & Raff, R. A. (1981) *Nature (London)* 292, 477–478.
30. Herlands, L., Allfrey, V. G. & Poccia, D. (1982) *J. Cell Biol.* 94, 219–223.
31. Cascio, S. M. & Wassarman, P. M. (1982) *Dev. Biol.* 89, 397–408.
32. McKnight, S. L. & Miller, O. L., Jr. (1976) *Cell* 8, 305–319.
33. Zalokar, M. (1976) *Dev. Biol.* 49, 425–437.
34. Anderson, K. V. & Lengyel, J. A. (1979) *Dev. Biol.* 70, 217–231.