Translational Regulation of mRNAs for Ribosomal Proteins during Early *Drosophila* Development†

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ABSTRACT: In *Drosophila*, the vast majority of mRNAs that are polysome associated during oogenesis are also polysome associated during early embryogenesis. We have previously identified an exceptional mRNA that appears to be depleted from early-embryo polysomes [Fruscoloni, P., Al-Atia, G. R., & Jacobs-Lorena, M. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3359-3363]. This mRNA has been subsequently identified as coding for a ribosomal protein (r-protein) [Kay, M., & Jacobs-Lorena, M. (1985) *Mol. Cell. Biol.* (in press)]. Changes in association with polysomes of two r-protein mRNAs during early *Drosophila* development were investigated for this report. Hybridization of cloned DNA probes to blots of RNA obtained from sucrose gradient fractions reveals that r-protein mRNAs are substantially associated with polysomes during oogenesis, depleted from polysomes during early embryogenesis, and again polysome associated during late embryogenesis. Thus, translation of r-protein mRNAs parallels transcription of ribosomal RNA (rRNA) during this time of development. By contrast, no such differences were observed when actin and histone probes were used as controls and hybridized to the same blots. The abundance of mRNAs for r-proteins as a function of development was also measured. Abundance was relatively high and constant during oogenesis and embryogenesis (when translational regulation is apparent), somewhat decreased in larval and pupal stages, and low in adult nonovarian tissues. Coordination between r-protein and rRNA synthesis appears to be achieved by regulating translation of r-protein mRNAs in early embryos and by decreasing their abundance in adult tissues.

The rate of ribosome synthesis varies considerably during early development of *Drosophila*. Ribosomes are synthesized at high rates during oogenesis (Mermod et al., 1977), but synthesis drops to undetectable levels during the first 3 h of embryogenesis (McKnight & Miller, 1976) after which it resumes and gradually increases during the remainder of embryonic development (Anderson & Lengyel, 1979). In contrast to RNA, most proteins are actively synthesized during early embryogenesis (Zalokar, 1976; Santon & Pellegrini, 1981). Since abundant and moderately abundant proteins are synthesized at times of undetectable ribosomal RNA (rRNA)[1] synthesis, the question arises of how r-protein synthesis is regulated during this period of time. In general, the same abundant and moderately abundant proteins are synthesized at all developmental times (Mermod et al., 1980; Loyd et al., 1981; Sakoyama & Okubo, 1981; Savoini et al., 1981). However, we have recently identified an exceptional mRNA (termed T1 mRNA) that is excluded from polysomes in early embryos but is polysome associated at other times (Fruscoloni et al., 1983). Further investigations in our laboratory revealed that this mRNA codes for an acidic r-protein (and is henceforth refered to as rpA1 mRNA) and that most r-protein mRNAs appear to be similarly regulated (Kay and Jacobs-Lorena, 1985).

The above-mentioned arguments for translational regulation can only be considered suggestive, for a quantitative assessment is not possible from the cell-free translation and two-dimensional gel analyses that were used in those experiments. Here we present evidence obtained with the use of recombinant DNA probes suggesting that translational regulation plays a major role in controlling r-protein gene expression during early *Drosophila* development. A different (pretranslational) regulatory mechanism appears to operate in the adult, where r-protein mRNA abundance is significantly depressed relative to other developmental stages.

Materials and Methods

Live Materials. Wild-type *Drosophila melanogaster* (Oregon R, P-2 strain) were reared at 25 °C in population cages and fed live yeast. Egg chambers were fractionated as previously described (Ruddell & Jacobs-Lorena, 1983) except that the buffers contained 50 μg/mL cycloheximide to prevent polysome runoff. Briefly, flies were homogenized in a Sorvall Omnimixer blender, and the homogenate was fractionated on a stack of metal nets of different mesh size that were immersed in *Drosophila* Ringer (DR) (80 mM NaCl, 64 mM KCl, 4 mM $MgSO_4$, 1 mM calcium acetate, 2.9 mM Na_2HPO_4, and 2.1 mM NaH_2PO_4, pH 6.8). To assure high developmental synchrony, egg chambers of the desired stage were individually picked from the material recovered from the appropriate net. Stage 10 egg chambers are at the completion of vitellogenesis, and stage 14 is the final stage of oogenesis. Embryos were collected for 30-min periods and aged to the desired stage (better than 85% synchrony) at 25 °C. A description of Drosophila oogenesis and embryogenesis can be found in King (1970), Fullilove & Jacobson (1978), and Mahowald & Kambysellis (1980).

Polysome Fractionation and Preparation of RNA. Experiments were performed at 4 °C. Egg chambers or embryos were homogenized with a Dounce homogenizer at a concen-

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[1] Abbreviations: r-protein, ribosomal protein; rRNA, ribosomal RNA; Tris, tris(hydroxymethyl)aminomethane; MOPS, 4-morpholinepropanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; RNP, ribonucleoprotein particle; kb, kilobase.

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tration of about 2000 per milliliter of homogenization buffer: 500 mM NaCl, 25 mM magnesium acetate, 50 mM Tris-HCl, pH 7.5, 0.2% Triton X-100, 10 mg/mL heparin, and 1 μg/mL cycloheximide. The homogenate was centrifuged for 10 min at 20000g. Up to 13 A_{260} units or 90 A_{260} units of this supernatant were applied respectively onto 5- or 35-mL linear 15–50% sucrose gradients. The sucrose was prepared in buffer containing 250 mM NaCl, 25 mM magnesium acetate, and 50 mM Tris-HCl, pH 7.5. The 5-mL gradients were centrifuged for 40 min at 265000g_{max} (47 000 rpm) in a Beckman SW50.1 rotor; the 35-mL gradients were centrifuged for 3.5 h at 126000g_{max} (26 500 rpm) in a Beckman SW27 rotor. Gradients were analyzed with an ISCO UV-5 monitor with continuous recording at 254 nm. The profiles obtained from either type of gradient were identical. Fractions were collected, and RNA was extracted from individual or pooled fractions. Release of mRNA from polysomes by puromycin was essentially as indicated (Blobel & Sabatini, 1971; Ruddell & Jacobs-Lorena, 1984). Preparation of RNA from whole organisms or tissues (Hough-Evans et al., 1980) and preparation of polyadenylated RNA (Faust et al., 1973) were as described.

RNA Blot Analysis. RNA was dissolved in MOPS buffer (20 mM MOPS, 5 mM sodium acetate, and 1 mM EDTA, pH 7.5) containing 3% formaldehyde, 0.1% $NaDodSO_4$, 10% sucrose, and 0.06% Bromophenol Blue. After being heated for 2 min in a boiling water bath, the RNA was subjected to electrophoresis at 30 V for 16 h in 1.5% agarose gels containing MOPS buffer and 6% formaldehyde. The running buffer was 3% formaldehyde in MOPS buffer. The RNA was transferred from the gels to nitrocellulose as described (Thomas, 1980), except that the transfer buffer contained 2% formaldehyde (this modification improves the efficiency of transfer). The filters were baked, prehybridized (for 3 h at 42 °C), and hybridized (Thomas, 1980) with nick-translated (Rigby et al., 1977) ^{32}P-labeled probes. The following recombinant DNA probes were used: cloned rpA1 cDNA coding for ribosomal protein A1 (Fruscoloni et al., 1983; unpublished experiments); rp49, a pBR322 subclone (HR 0.6) of phage C25 coding for ribosomal protein 49 (Vaslet et al., 1980); actin, the 3.4-kb *Hind*III fragment from phage DmA2, cloned in pBR322 (this probe contains most of the actin coding sequence and cross-hybridizes with mRNAs from all six actin genes) (Fyrberg et al., 1980); histone, the cDm500 plasmid coding for all five *Drosophila* histones (Lifton et al., 1977); ribosomal, the plasmid pKB7 containing the *Drosophila* 18S and 28S rRNA sequences (Wensink et al., 1979). The autoradiographic signals were quantitated by scanning densitometry of films exposed to within the linear response range (Ruddel & Jacobs-Lorena, 1984).

Results

Association of Ribosomal Protein mRNAs with Polysomes Is Uniquely Regulated during Development. To measure association of different classes of mRNAs with polysomes as a function of development, postmitochondrial supernatants from egg chambers or embryos of different ages were fractionated on sucrose gradients. It is assumed that mRNAs sedimenting slower than monoribosomes are not being translated and that mRNAs cosedimenting with polyribosomes *are* being translated at the time of homogenization. The latter assumption was further corroborated by experiments that disaggregated polysomes with puromycin (see below). Figure 1 displays a representative absorbance profile of a postmitochrondrial supernatant from stage 10 egg chambers analyzed on a sucrose gradient. This profile is representative of all developmental stages in this study [see also Mermod & Crippa

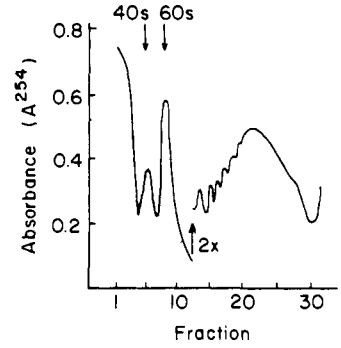

FIGURE 1: Absorbance profile of polysomes and postpolysomal particles fractionated on a sucrose gradient. A postmitochondrial supernatant from about 6000 stage 10 egg chambers was centrifuged on a 35-mL 15–50% sucrose gradient. The absorbance was monitored continuously, and fractions were collected. At the point indicated by the upward arrow, the sensitivity was increased 2-fold. Monosomes are dissociated into subunits (40 S and 60 S) under the conditions employed. The identity of the 40S and 60S peaks was verified by electrophoresis of the extracted RNAs on nondenaturing gels.

(1978) and Ruddell & Jacobs-Lorena (1983)]. Gradient fractions were pooled in groups of two, and the nucleic acids were extracted and subjected to electrophoresis in denaturing agarose gels. Blots were prepared from these gels and sequentially hybridized with cloned probes coding for either r-proteins (rpAl and rp49) or nonribosomal proteins (actins and histones) as controls. The actin probe was chosen for control experiments because the corresponding mRNAs were known not to be translationally regulated during early development (Ruddel & Jacobs-Lorena, 1984). Histones were chosen because they represent a class of mRNAs that are translated on polysomes of similar size as are r-protein mRNAs. Messenger RNA distribution from the following developmental periods was analyzed in this manner: stage 10 egg chambers (mid to late oogenesis), stage 14 egg chambers (mature oocytes), 3-h-old embryos (late blastula), 5-h-old embryos (gastrula), and 18-h-old embryos (late organogenesis). Examples of autoradiograms obtained after hybridization of the RNA blots to the various probes are shown in Figure 2. The position of sedimentation in the sucrose gradient (cf. Figure 1) of the ribosomal subunits and of polyribosomes is indicated in the figure. In some cases (e.g., rpAl mRNA in stage 14 egg chambers), there was a biomodal distribution of the mRNA in the polysome region. A similar observation has been made by Ballinger & Pardue (1983). This result was not reproducible, and its significance is unknown. The distribution of a particular mRNA between postpolysomal and polysomal fractions was quantitated by densitometric scanning of the autoradiograms, using exposures that were within the linear response range. The resulting profiles are illustrated in Figure 3. The calculated percentage of each mRNA that is associated with polyribosomes at the different developmental stages is presented in Table I. In general, all mRNAs that were measured appear to be very efficiently translated at mid-oogenesis (stage 10). Thereafter, association of r-protein mRNAs with polysomes declines considerably to reach a minimum at 5 h of embryogenesis. In late embryos, association of r-protein mRNAs with polysomes increases to intermediate levels between that of early embryos and oogenesis. Note that the change in association with polysomes as a function of developmental stage is particularly pronounced for rp49 mRNA (Figure 3 and Table I). By contrast, the pattern of actin and histone mRNA association with polyribosomes is very different: despite a tendency toward reduced association with polysomes during embryogenesis, this decrease is of lesser

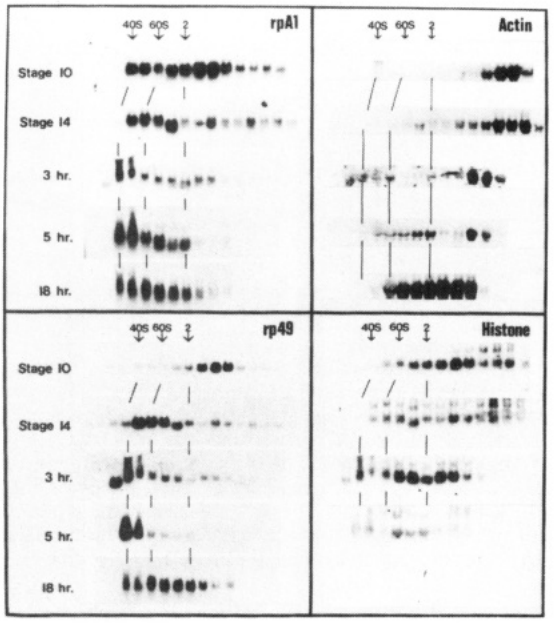

FIGURE 2: Analysis of the distribution of ribosomal and nonribosomal mRNAs between polysomes and postpolysomal supernatant by RNA blotting. Postmitochondrial supernatants from egg chambers or embryos were fractionated on sucrose gradients (cf. Figure 1 for representative profile). RNA was extracted from fractions pooled in groups of two and subjected to electrophoresis in denaturing agarose–formaldehyde gels. Blots prepared from these RNA samples were hybridized with the ^{32}P-labeled cloned DNA probe indicated at the top of each panel. The corresponding autoradiograms are shown. The actin and histone probes hybridize to mRNAs of two size classes. Downward arrows indicate the position of migration on the sucrose gradients of ribosomal markers (cf. Figure 1): "40 S" and "60 S", ribosomal subunits; "2", polysomes containing two ribosomes. The stage of oogenesis or age of the embryos that served as a source for the postmitochondrial supernatant is indicated at the left of each blot.

Table I: Association of mRNAs with Polysomes as a Function of Developmental Stage[a]

	percentage of mRNA associated with polysomes			
developmental stage	rpA1	rp49	actins	histones
oogenesis stage 10	81	96	86	71
oogenesis stage 14	54	25	86	66
3-h-old embryos	37	16	64	59
5-h-old embryos	23	3	75	51
18-h-old embryos	36	49	79	

[a] RNA extracted from fractions of sucrose gradients was subjected to electrophoresis, blotted, and hybridized with the indicated probes, followed by densitometry of the autoradiographs. Further details are outlined in the legend to Figure 3 and under Materials and Methods. The listed values for actin and histones include results from a separate set of analogous experiments (Ruddell & Jacobs-Lorena, 1984, 1985). Histone mRNA abundance in late embryos was too low to measure.

magnitude and does not show (at least for actins) an increase during late embryogenesis. Histone mRNA abundance in late embryos was too low to measure. It should be emphasized that for each experiment the same blot was hybridized with the different cloned DNAs. In this way, the nonribosomal probes act as an "internal control", precluding the possibility that the observed differences were due to variations in sample preparation or to differences in starting materials.

To ascertain that the association of mRNAs with polysomes was functionally significant and not due to spurious interactions, the following control experiment was performed. Polysomes from stage 14 oocytes were prepared on sucrose gradients and divided into two aliquots. One control aliquot was kept on ice while the other aliquot was incubated with puromycin. Both aliquots were then centrifuged on sucrose gradients, and the nucleic acids in each fraction were analyzed by gel electrophoresis and RNA blotting. As illustrated in Figure 4A, puromycin caused complete dissociation of the polysomes. RNA blot analysis of fractions of these gradients with the rpAl probe (Figure 4B) followed by densitometric quantitation of the autoradiograms (Figure 4C) indicates that most if not all of the rpAl mRNA that originally cosedimented with polysomes sediments with postribosomal particles after puromycin treatment. Since puromycin requires an enzymatic translocation step in order to disrupt polysomes, these results suggest that association of rpAl mRNA with polysomes is a functional one.

Abundance of mRNA for Ribosomal Proteins Is High throughout Early Development and Low in Adult Tissues. To measure the abundance of r-protein mRNAs, polyadenylated RNAs were prepared from representative stages of *Drosophila* development and analyzed by RNA blot analysis with r-protein probes (Figure 5). Since an equal mass of RNA from each stage was applied to the gels, differences in intensities of the autoradiograms can be directly correlated at each stage, with the abundance of the tested mRNA among other polyadenylated RNAs. Figure 5 indicates that little change in r-protein mRNA abundance occurs during embryogenesis, while a modest decrease (about 2-fold) occurs during larval development. Adult nonovarian tissues, however, are depleted of r-protein mRNAs by about 6–8-fold relative to embryonic levels. Actin mRNAs follow a similar profile (Fyrberg et al., 1983; our unpublished experiments).

A large excess (500 μg per sample) of poly(A)-deficient RNA that failed to bind to oligo(dT) was analyzed in the same manner. No hybridization to any of the probes was detected (results not shown). This suggests that no significant amount of these r-protein mRNAs is poly(A) deficient at the developmental stages examined.

Discussion

Ribosomal RNA synthesis in *Drosophila* varies from very high levels during oogenesis (Mermod et al., 1977) to undetectable levels during early embryogenesis (McKnight & Miller, 1976) and then gradually increases to reach moderate levels in late embryogenesis (Anderson & Lengyel, 1979). However, r-protein mRNAs are abundant at all these developmental stages (Figure 5). We find that translation of r-protein mRNAs closely parallels ribosomal RNA synthesis (Figure 3 and Table I): association of at least two r-protein mRNAs with polysomes is maximal during oogenesis (stage 10), minimal during early embryogenesis (5 h), and intermediate during late embryogenesis (18 h). In our earlier experiments that used the less sensitive cell-free translation analysis (Fruscoloni et al., 1983), rpAl mRNA was hardly detectable among polysomal mRNAs of early embryos. The results of the experiments presented here indicate that the exclusion of rpAl and rp49 from early embryos is not complete. Recent experiments in our laboratory show that most r-protein mRNAs are regulated to varying degrees at the translational level in a manner similar to that of rpAl and rp49 (Kay & Jacobs-Lorena, 1985).

The observed translational regulation of r-protein mRNAs is specific because translation of nonribosomal mRNAs follows a different pattern (Figure 3 and Table I). The latter results completely agree with previous findings showing that the spectrum of proteins synthesized at all developmental stages is extremely similar (Loyd et al., 1981; Sakoyama & Okubo, 1981; Savoini et al., 1981). Some of our results are not consistent with those of Santon & Pellegrini (1980, 1981), who

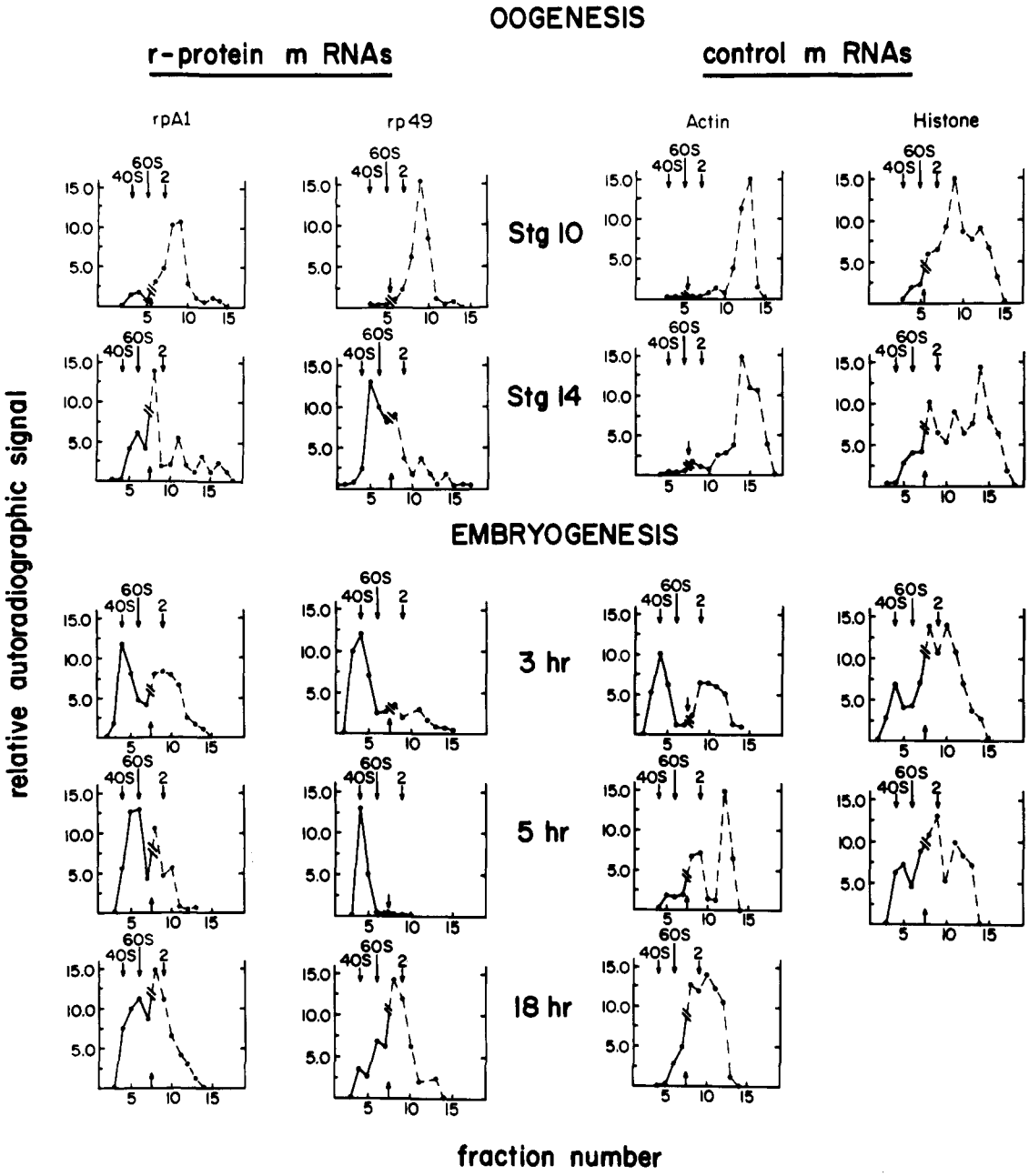

FIGURE 3: Densitometric quantitation of mRNA distribution between polysomes and postpolysomal supernatant. Postmitochondrial supernatants were fractionated on sucrose gradients, and the RNA from each fraction was assayed by RNA blot analysis as indicated in Figure 2. The resulting autoradiograms were analyzed by scanning densitometry. Different exposures were used to assure that signals were within the linear response range. The relative signal intensity (in arbitrary units) measured for each lane of the autoradiogram is indicated on the ordinates. The numbers on the abscissa correspond to the sucrose gradient fractions (cf. Figure 1) that were pooled in groups of two. The upward arrows and the double slashes on the profiles indicate the transition point between polysomal and postpolysomal compartments that were used for computing the values in Table I. Points to the right of the double slashes are plotted on a 2-fold expanded scale. Developmental stages analyzed (middle column of values) and hybridization probes (top of each column of panels) are as indicated in the legend of Figure 2. "40 S", "60 S", and "2" indicate the position of sedimentation of small and large ribosomal subunits and of polysomes containing two ribosomes, respectively.

reported high rates of r-protein synthesis in early embryos. It is possible that this apparent discrepancy is only quantitative since we find a small proportion of r-protein mRNA associated with polysomes even during early embryogenesis. Corroborating the evidence presented in this paper, M. Kay and M. Jacobs-Lorena (1985) observed that r-proteins are hardly detectable among proteins synthesized in vivo by early embryos while r-proteins are easily detectable among proteins synthesized in late embryos.

It appears as if the coregulation of rRNA and r-protein gene expression bears a casual relationship. In procaryotes, r-protein translation is feedback inhibited by excess r-protein (Lindhal & Zengel, 1982), and, on the basis of our observations, it is possible to suggest a similar model for *Drosophila*. Accordingly, after cessation of rRNA synthesis, r-proteins would accumulate and preferentially inhibit the translation of r-protein mRNAs. There is a lag between the decrease of rRNA synthesis and the shift of r-protein mRNAs from polysomes to postpolysomal RNPs during *Drosophila* development. Ribosomal protein mRNAs are still substantially associated with polysomes in stage 14 oocytes when little rRNA synthesis is detected, while this association reaches a minimum at 5 h of embryogenesis when rRNA synthesis has already resumed. This lag could be accounted for by the large volume of the egg and the need to accumulate enough r-proteins (or another regulatory substance) to inhibit translation.

Translational regulation of r-protein synthesis has been demonstrated in a number of organisms (Warner et al., 1980).

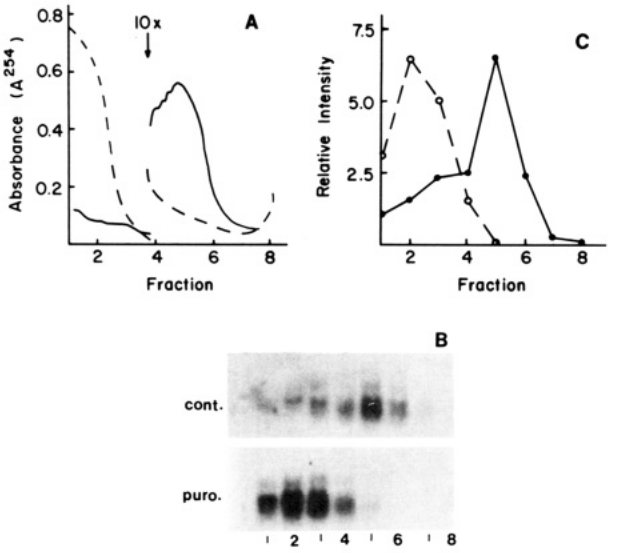

FIGURE 4: Release of mRNAs from polysomes by incubation with puromycin. Polysomes were prepared from stage 14 oocytes by centrifugation of a postmitochondrial supernatant (13 A_{260} units per gradient) on two 5-mL sucrose gradients. The pooled polysomal fractions were divided into two aliquots. One aliquot (control) was kept at 4 °C; the other aliquot (puromycin) was incubated with 0.4 mM puromycin for 10 min at 37 °C. These aliquots were analyzed on 25–60% linear sucrose gradients as indicated below. (A) Absorbance profiles. (B) Fractions from the gradients were analyzed by RNA blot hybridization with the rpA1 probe. Autoradiograms are shown. The numbered lanes correspond to gradient fractions in (A). (C) Autoradiograms in (B) were quantitated by scanning densitometry. Under the centrifugation conditions employed, the center of the monoribosome peak is at fraction 4. [(—) and "cont."] Control sample; [(---) and "puro."] puromycin-treated sample.

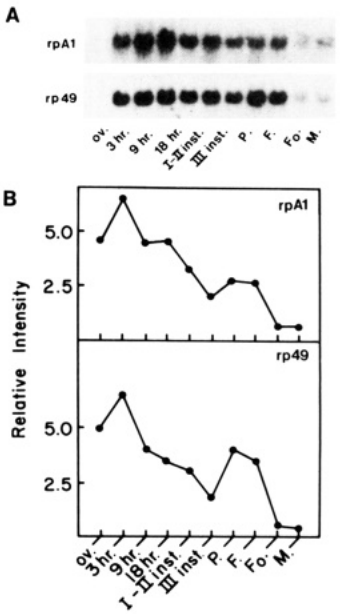

FIGURE 5: Changes in ribosomal protein mRNA abundance as a function of development. An equal mass (15 μg) of polyadenylated RNA from different developmental stages was analyzed by electrophoresis in a denaturing agarose gel followed by hybridization of ^{32}P-labeled probes to the corresponding blot. (A) Autoradiograms. (B) The signals on the autoradiograms in (A) were quantitated by scanning densitometry. The cloned r-protein rpAl and rp49 probes were used. The source of RNAs was as follows: ov., ovaries; 3 h, 9 h, and 18 h, embryos of the indicated ages; I–II inst., larvae at the first- to second-instar transition; III inst., third-instar larvae; P., pupae; F., whole adult females; Fo., adult females from which ovaries were removed; M., adult males. Hybridization of the blots with a rRNA probe (results not shown) verified that no significant differences in the level of contaminating rRNA existed among the samples.

However, comparison of the "strategies" adopted for regulation of r-protein gene expression in two developing systems—*Drosophila* and *Xenopus*—is of particular interest. Both organisms do not synthesize rRNA during early embryogenesis. In *Xenopus*, lack of rRNA synthesis in early embryos is accompanied by the disappearance of most r-protein mRNAs (Weiss et al., 1981; Pierandrei-Amaldi, 1982). In contrast, in early *Drosophila* embryos, r-protein mRNA abundance remains high,but this class of mRNAs is specifically inhibited from association with polysomes (cf. translational regulation).

In adult flies, r-protein mRNAs occur in considerably lower abundance. Unlike early development, pretranslational, possibly transcriptional regulation may play a major role in regulating r-protein gene expression in the mostly differentiated and nongrowing tissues of the adult. It should be noted that if mRNA content per animal rather than abundance were plotted in Figure 5, the profile would be radically different. The r-protein mRNA content would increase significantly as a function of development because compared to embryos, the content of polyadenylated RNA per organism is about 20-fold higher in third-instar larvae and pupae, about 10-fold higher in adult males, and about 100-fold higher in adult females (our unpublished experiments). However, representation of r-protein mRNAs relative to the total RNA population is low in the adult, as indicated in Figure 5.

Few clues are available for understanding how selectivity of r-protein mRNA translation is achieved during early *Drosophila* development. The possibility that the observed pattern is due to a generalized drop in protein synthesis that affects small mRNAs more severely than large mRNAs is unlikely. Several nonribosomal mRNAs, including histone mRNAs, that share common properties with r-protein mRNAs (they code for small proteins, polysomal–postpolysomal distribution during oogenesis is similar to that of rpAl mRNA) do not follow the regulatory pattern of the r-protein mRNAs (Fruscoloni et al., 1983; Ruddell & Jacobs-Lorena, 1985). Stable, covalent modification of r-protein mRNAs also seems unlikely because postpolysomal r-protein mRNAs from early embryos are efficiently translated in a cell-free system (Fruscoloni et al., 1983). Regardless of the actual mechanism, it is ultimately the nucleotide sequence of the r-protein mRNAs that must be responsible for their special properties. The availability of techniques to modify genes in vitro and to reintroduce them into the whole organism (Rubin & Spradling, 1982) will allow further progress in understanding the basis for selective translational regulation of r-protein mRNAs in *Drosophila*.

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Single-Strand-Specific Degradation of DNA during Isolation of Rat Liver Nuclei†

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ABSTRACT: We have investigated structural change in rat liver DNA produced by different isolation procedures and specifically compared the integrity of DNA derived by phenol extraction from isolated and purified nuclei with preparations extracted immediately from a crude liver homogenate containing intact nuclei. As indicated by stepwise elution from benzoylated DEAE-cellulose, most structural change in DNA was evident following nuclei isolation. Damage principally involved generation of single-stranded regions in otherwise double-stranded DNA fragments; totally single-stranded DNA was not detected by hydroxylapatite chromatography. Caffeine gradient elution suggested formation of single-stranded regions extending for up to several kilobases. In neutral sucrose gradients, differences in sedimentation rates of respective DNA samples consequent upon S1 nuclease digestion could be detected after isolation of nuclei, though not in other circumstances. The observed single-strand-specific nuclease digestion of DNA could apparently be reduced if steps were taken to reduce autodigestion during nuclei isolation by reduction of temperature and covalent cation concentration. The results are discussed in terms of the use of exogenous and endogenous nucleases in chromatin fractionation studies involving isolated nuclei and possible artifactual findings that may be generated by single-strand-specific autodigestion.

Digestion of chromatin with DNase I or micrococcal nuclease under carefully defined conditions is considered to permit fractionation of DNA on the basis of its association with transcribed or nontranscribed genes (Garel & Axel, 1976; Bloom & Anderson, 1978; Tata & Baker, 1978; Anderson et al., 1983). Typically, this procedure involves isolation of nuclei prior to digestion after which the respective fractions are separated by centrifugation. During isolation, chromatin may

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