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COMPARISON OF MESSENGER RNA POOLS IN ACTIVE AND DORMANT *ARTEMIA FRANCISCANA* EMBRYOS: EVIDENCE FOR TRANSLATIONAL CONTROL

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Summary

In response to environmental anoxia, embryos of the brine shrimp *Artemia franciscana* enter a dormant state during which energy metabolism and development are arrested. The intracellular acidification that correlates with this transition into anaerobic dormancy has been linked to the inhibition of protein synthesis in quiescent embryos. In this study, we have addressed the level of control at which a mechanism mediated by intracellular pH might operate to arrest protein synthesis.

Two independent lines of evidence suggest that there is an element of translational control when protein synthesis is arrested in dormant embryos. First, as determined by *in vitro* translation techniques, there were no significant quantitative differences in mRNA pools in dormant as compared to actively developing embryos. In addition, fluorography of the translation products showed that there are no large qualitative changes in mRNA species when embryos become dormant. These data suggest that there was no net degradation of mRNA pools in dormant embryos and that protein synthesis may therefore be controlled more strongly at translation than at transcription. Second, polysome profile studies showed that dormant embryos possess reduced levels of polysomes relative to those found in cells of active embryos. The disaggregation of polysomes is an indication that the initiation step in protein synthesis is disrupted and is further evidence that the mechanism involved in protein synthesis arrest in dormant *Artemia* involves translational control.

Introduction

Numerous physiological studies have investigated the mechanisms that inhibit energy-producing pathways when an animal enters dormancy. However, the status and regulation of the energy-consuming biosynthetic processes in dormant animals have gone largely unstudied. Given that dormancy is ideally a reversible (i.e. survivable) condition, maintaining control of energy-consuming cellular activity could be vital if arousal from dormancy is to occur. To address this aspect of

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dormancy, we have been using the *Artemia* embryo as a model system to study the regulation of biosynthesis in an animal that undergoes reversible bouts of dormancy.

In response to adverse conditions, such as environmental anoxia, the embryos enter a dormant state where development is arrested (Busa and Crowe, 1983; Hofmann and Hand, 1990*a*) and become virtually ametabolic. During the first few days of anaerobic dormancy, the embryos reduce energy flows to 0.4 % of aerobic control values (Hand, 1990). It has become clear that acidification of embryo intracellular pH (pHi) either directly or indirectly mediates the metabolic arrest observed in anaerobic embryos (Busa *et al.* 1982; Busa and Crowe, 1983; for a review, see Hand, 1991). In addition, Carpenter and Hand (1986) made the observation that artificially acidified embryos that had access to oxygen maintained adenylate energy charges (AEC) nearly equivalent to those of control aerobic embryos (AEC=0.69 and 0.73, respectively), whereas anaerobic dormant embryos displayed a decrease in AEC to 0.42. This suggests that there are independent mechanisms controlling catabolic and anabolic events and that the energy-consuming pathways may not necessarily be constrained by embryo AEC. It was this particular insight that made *Artemia* an appealing study organism.

Recently we have demonstrated that the process of protein synthesis is coordinatedly arrested with energy metabolism in anaerobic and artificially acidified *Artemia* embryos (Hofmann and Hand, 1990*b*). Thus, a reduction in pHi is linked to inhibited protein synthesis when the embryos enter dormancy. With respect to general levels of control, the on/off switch in protein synthesis could be transcriptional or translational in nature. In the former case, acidic pHi promoted by anoxia would result in suppression of mRNA production as well as depletion of mRNAs. If control were at the level of translation, we would predict that cellular mRNA levels would be relatively unchanged upon entrance into dormancy and that alteration of some translational event(s) would act to inhibit protein synthesis.

In this study we have addressed the above hypotheses by posing questions that draw comparisons between dormant and active embryos in terms of the state of translation and transcription. How do levels of translatable mRNA compare in active and dormant embryos? Are there qualitative differences in proteins produced by active and dormant embryos that might suggest a change of mRNA species and, therefore, a role for transcriptional control? Since ribosome activity (as an indicator of translational activity) can be inferred from polysome profiles, how do polysome profiles from active and dormant embryos compare?

The results show (1) that active and dormant embryos contain similar quantities of translatable mRNA, (2) that there appear to be no qualitative differences in the set of proteins synthesized by active and dormant embryos, and (3) that polysomes are disrupted in dormant embryos compared to those observed in active embryos. The above observations suggest that the environmental control of gene expression in anaerobically dormant *Artemia* embryos occurs predominantly at the translational level rather than at transcription and that some control appears to occur at the initiation step of protein synthesis.

Materials and methods

Embryo hydration and incubation procedures

Dehydrated *Artemia franciscana* (Kellogg) embryos from the Great Salt Lake population were purchased from the Sanders Brine Shrimp Co. in 1989 and stored at −20°C. All embryos used in this study were taken from the same commercial batch. Embryos were hydrated for 4 h at 0°C and washed as previously described (Carpenter and Hand, 1986). After washing, embryos were stored overnight in 0.25 mol l^{-1} NaCl at 0°C for use in experiments the next day.

Hydrated embryos (1.5 g batches) were incubated at 23°C in 125 ml Ehrlenmeyer flasks containing 60 ml of 0.25 mol l^{-1} NaCl. Flasks were agitated on a rotary shaker at 1000 revs min^{-1} and aerated with the appropriate gas mixture using a flow-through gassing apparatus. The gas mixtures used in the experiments were as follows: aerobic development (60 % N_2:40 % O_2), anoxia (100 % N_2) and aerobic acidosis (60 % CO_2:40 % O_2). After incubation, embryos were filtered onto Durawipe filters, blotted dry, and 1.0 g samples were processed for RNA extraction or for polysome studies.

In experiments to determine levels of translatable mRNA, aerobic control embryos were incubated for 4 h and experimental treatment embryos received an additional 4 h of either anoxia or aerobic acidosis conditions. These short experimental treatments were chosen because entry into dormancy is a rapid process in *Artemia* embryos. After 1 h of anoxia, energy flow is suppressed to 8 % of aerobic control levels, and 1 h of aerobic acidosis suppresses energy flow to 25 % of control levels (Hand and Gnaiger, 1988). Similarly, the increase in cytochrome *c* oxidase levels (due to protein synthesis) seen after 2–4 h of aerobic treatment is fully blocked in this period by either anoxia or aerobic acidosis (Hofmann and Hand, 1990*a*). For the polysome experiments, aerobic development embryos were incubated for 4 h, while the experimental embryos were switched to either anoxia or aerobic acidosis for an additional hour.

RNA extraction

The procedure for extracting RNA from embryo tissue was modified from Clemens (1984) and Wallace (1987). After incubation, triplicate 1.0 g samples of embryos were homogenized with a ground-glass homogenizer in 15 volumes of extraction buffer (EB) consisting of 200 mmol l^{-1} Tris acetate, 50 mmol l^{-1} KCl, 10 mmol l^{-1} magnesium acetate, 350 mmol l^{-1} sucrose and 1.3 % Triton X-100, pH 8.5 at 4°C. The homogenate was centrifuged at 2000 ***g*** for 5 min at 4°C. SDS and EDTA were added to the supernatant to 1 % (w/v) and 2 mmol l^{-1}, respectively. The supernatant was mixed vigorously by hand at room temperature for 10 min with two volumes of phenol (EB-saturated; 0.1 % 8-hydroxyquinoline)–chloroform–isoamyl alcohol (24:24:1, v/v/v) and centrifuged in 30-ml Corex tubes for 10 min at 10 000 ***g*** at 4°C. The aqueous-phase supernatant was reserved, and the white interface and organic phase were re-extracted by the addition of an equal volume of TAE (100 mmol l^{-1} Tris acetate, 100 mmol l^{-1} sodium acetate,

2 mmol l^{-1} EDTA, pH 9.0), followed by mixing and centrifugation as above. The two aqueous-phase supernatants were then combined, extracted with an equal volume of phenol–chloroform–isoamyl alcohol, and mixed and centrifuged as above. After this step, 2.5 volumes of ethanol and 0.1 volumes of 3 mol l^{-1} potassium acetate (pH 5.5) were added to the aqueous phase, and the RNA was stored at −20°C for 18 h to foster precipitation. Precipitated RNA was collected by centrifugation at 10 000 *g* for 30 min. The resulting pellets were resuspended in 3 mol l^{-1} potassium acetate (pH 5.5) and centrifuged at 2000 *g* for 10 min. The RNA pellet was washed with 70 % ethanol, centrifuged as above and resuspended in sterile water. The ratio of absorbance (260 nm/280 nm) of this preparation was 1.78, which indicated low protein contamination.

The extraction efficiency of the phenol–chloroform method was determined by measuring the recovery of a ^{32}P-labeled RNA added to duplicate post-mitochondrial supernatants of 4 h *Artemia* embryos. Recovery of the labeled RNA was 80 and 84 % (duplicates) after phenol–chloroform extraction. After ethanol precipitation, 68 and 72 % of the total added label was recovered. The ^{32}P-labeled RNA was kindly provided by Dr Ravi Menon (MCD Biology, University of Colorado). The 1000-nucleotide RNA was synthesized *in vitro* using a linearized pG7Ex2-4 DNA template containing the human beta-casein exons 2-4 downstream from a SP6 promotor.

Poly(A)+RNA isolation

Polyadenylated RNA [poly(A)+mRNA] was prepared from total RNA using oligo(dT) cellulose affinity chromatography (Jacobson, 1987, with modifications). Prior to chromatography, the total RNA sample was heated at 65°C for 5 min, cooled on ice, and diluted 1:1 with sterile 1 mol l^{-1} NaCl. The sample was then applied at room temperature to a 2.0 ml column of oligo(dT) cellulose (Type III, Collaborative Research) in a chromatography buffer consisting of 10 mmol l^{-1} Tris–HCl, 0.5 mol l^{-1} NaCl and 1 mmol l^{-1} EDTA, pH 7.5. Absorbance was monitored at 260 nm. The ultraviolet-absorbing material that eluted in the void was reapplied to the column to increase the percentage of RNA binding. Poly(A)+RNA was eluted at 36°C with NaCl-free chromatography buffer. The column was regenerated by washing with 3 bed-volumes of 100 mmol l^{-1} NaOH, 5 volumes of distilled water and 5 volumes of binding buffer (Jacobson, 1987).

In vitro *translation assays*

Levels of translatable mRNA in dormant and active *Artemia* embryos were determined using *in vitro* translation in a rabbit reticulocyte lysate system (Bethesda Research Laboratories). Incorporation of [^{35}S]methionine into trichloroacetic acid (TCA)-precipitable protein was measured using either extracted total RNA or isolated poly(A)+RNA. The 30 μl reaction mixture was composed of the following: 10 μl of nuclease-treated rabbit reticulocyte lysate, 9.2 μl of sample and 10.8 μl of master mixture. The master mixture contained 19 amino acids (minus methionine) each at 139 μmol l^{-1} containing 5 μCi of [^{35}S]methionine

(Amersham; 1200 μCi mmol^{-1}), a mixture of ATP and GTP, and 38.5 mmol l^{-1} creatine phosphate. The K^+ and Mg^{2+} concentrations in the assays were 135 mmol l^{-1} and 1.2 mmol l^{-1}, respectively. With each set of translation assays, a rabbit globin mRNA assay (0.03 μg/assay) was run as an incorporation standard. Also, a zero-message control, which contained sterile distilled water in place of mRNA, was used to correct for background due to non-specific binding of [^{35}S]methionine to components of the reticulocyte lysate. Assays were incubated at 30°C for 1 h, and translation was terminated by placing the reaction tubes on ice. Each reaction mixture was then treated with 100 μg ml^{-1} pancreatic RNAase and incubated at 30°C for an additional 15 min. Duplicate 10 μl samples were pipetted onto Whatman GF/C filters and placed in cold 10 % TCA for 10 min. The filters were then washed twice in 5 % TCA at room temperature, followed by two washes in 95 % ethanol. The filters were allowed to dry in air, after which they were placed in fluor. Radioactivity in the precipitated protein was determined by liquid scintillation counting.

Fluorography

Proteins synthesized during *in vitro* translation of mRNA from aerobic and anaerobic dormant embryos were visualized using SDS–polyacrylamide slab gel electrophoresis combined with fluorography. Samples of translation assays were diluted 10-fold with sample buffer (60 mmol l^{-1} Tris–HCl, 5 % SDS, 1 % dithiothreitol, 20 % glycerol and 0.001 % Bromphenol Blue, pH 6.8) and incubated at 100°C for 2 min. Polyacrylamide separating gels (15 %) with a stacking gel (5 %) were prepared using the methodology of Jagus (1987), and 25 μl of diluted translation mixture was loaded per lane. The 1.5 mm gels were electrophoresed at 25 mA/gel for 8 h at 4°C. Following electrophoresis, gels were treated with a protein fixing solution (10 % trichloroacetic acid, 10 % glacial acetic acid and 30 % methanol) for a minimum of 1 h. Gels were then immersed in 100 ml of EN^{3}HANCE (New England Nuclear) and gently agitated for 1 h, after which the impregnated gels were soaked for 1 h in 4 % glycerol (300 ml/gel) with gentle agitation. Gels were dried under vacuum at 60°C in a Biorad model 483 slab gel drier. Finally, blue-sensitive Kodak XAR-5 X-ray film was exposed by contact with the dried gels for 48 h at −70°C.

Polysome profiles

To generate polysome profiles, 1.0 g samples of *Artemia* embryos were first homogenized in 5 volumes of gradient buffer composed of 50 mmol l^{-1} Tris–HCl, 10 mmol l^{-1} $MgCl_2$ and 100 mmol l^{-1} KCl, pH 7.8 at 4°C (Golub and Clegg, 1968). The embryo homogenate was centrifuged at 14 000 ***g*** for 30 min at 4°C, and 2 ml of the resulting post-mitochondrial supernatant was applied to 15 %–35 % sucrose density gradients (24 ml total volume) prepared with gradient buffer. The gradients were ultracentrifuged at 60 000 ***g*** for 4 h at 4°C in a Beckman SW 25.1 swinging bucket rotor. After ultracentrifugation, the gradients were fractionated,

and the presence of RNA in ribosomal material was assayed spectrophotometrically at 260 nm.

Results

Levels of translatable mRNA in active and dormant embryos

To determine whether the inhibition of protein synthesis in dormant *Artemia* embryos was due to a transcriptional or a translational mechanism, we measured the amount of total translatable mRNA in active and anaerobically dormant embryos. This method is useful because, while it does not reveal amounts of specific mRNA species, it does provide a relative index that can be used to compare messenger pools in the embryos experiencing different physiological conditions (Hershey *et al.* 1986).

Aerobic control embryos were incubated for 4 h and processed for RNA extraction, while experimental embryos were switched to either anoxia or aerobic acidosis conditions for an additional 4 h. The amounts of total extracted RNA were not significantly different between aerobic controls and anoxic embryos (Fig. 1A). The total extracted RNA in aerobic acidotic embryos was actually higher than control values (Fig. 1A). The biological significance, if any, of this

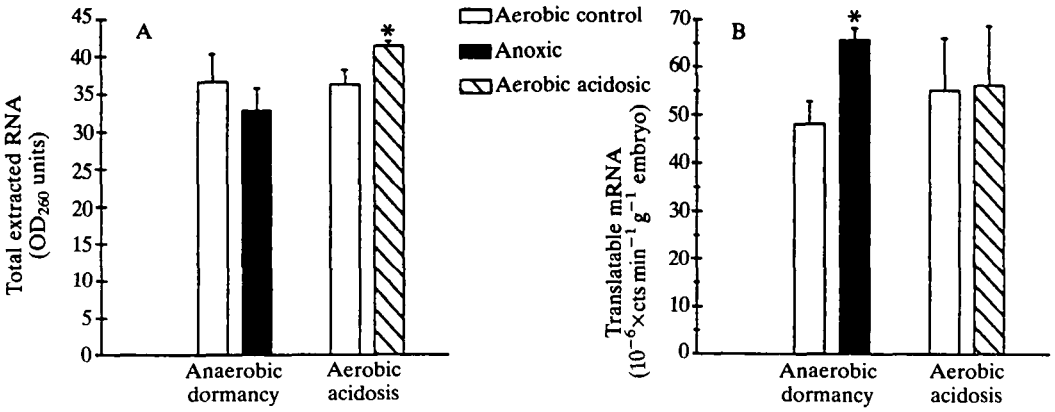

Fig. 1. (A) Levels of total extracted RNA in aerobic, anoxic and aerobic acidotic *Artemia franciscana* embryos. The separate experiments for anaerobic dormancy and aerobic acidosis each included a set of aerobic controls (N=6) and an experimental treatment (N=6). Each bar represents the mean+S.E. for six independent phenol extractions of 1.0 g samples of embryos. The asterisk indicates a significant difference from the control value (one-way ANOVA; P<0.05). (B) Total translatable mRNA in aerobic, anoxic and aerobic acidotic embryos. Equivalent amounts of phenol-extracted RNA were translated for control and experimental treatment embryos. Each bar represents the mean+S.E. for six independent phenol extractions. The asterisk indicates a significant difference from the control in a nested ANOVA design (P<0.05).

Table 1. In vitro *translation of poly(A)+mRNA isolated from* Artemia franciscana *embryos with oligo(dT) cellulose chromatography*

Experiment	Treatment	Translatable mRNA (10^{-6}×cts min^{-1} per 3 g embryo)
Anaerobic dormancy	60 % N_2:40 % O_2	28.0±0.19
	100 % N_2	28.5±1.56
Aerobic acidosis	60 % N_2:40 % O_2	39.9±1.72
	60 % CO_2:40 % O_2	36.4±3.34

Values are mean±S.E. of triplicate determinations on one batch of isolated poly(A)+mRNA.

increase is unclear. It should be noted that all cellular RNAs (e.g. rRNA, tRNA, mRNA) are present in the phenol-extracted preparation.

Equivalent amounts of the extracted RNA (as determined by absorbance at 260 nm) were translated *in vitro* in the rabbit reticulocyte cell-free system. Fig. 1B shows the amount of [^{35}S]methionine incorporated into TCA-precipitable protein, with the RNA samples from active and dormant embryos serving as the template. In the anaerobic dormancy experiment, the level of translatable mRNA was greater in the anoxic embryos as compared to the aerobic controls. The amounts of translatable mRNA in the aerobic acidotic embryos were not significantly different from those observed in the control embryos.

To remove any RNAs of low relative molecular mass that could have potentially inhibited translation (Piot *et al.* 1984; Slegers *et al.* 1989), polyadenylated mRNA [poly(A)+mRNA] was isolated from the total RNA using oligo(dT) cellulose affinity chromatography. The [^{35}S]methionine incorporation values (expressed per 3 g of embryo wet mass) appeared to be the same in control and experimental embryos in the anaerobic dormancy and the aerobic acidosis experiments (Table 1). These data suggest that the amount of translatable mRNA is similar in active and dormant embryos and that the transition into dormancy does not result in any net degradation of the mRNA pool.

SDS–PAGE analysis of translation products

In addition to these quantitative analyses of mRNA pools, qualitative changes in the messenger population were indirectly examined using SDS–polyacrylamide electrophoresis and fluorography of proteins produced by active and dormant embryos. It could be argued that, even though total translatable levels of poly(A)+mRNA do not decrease when embryos become dormant (see Fig. 1B), these quiescent embryos might synthesize a different set of mRNAs that code for a markedly different suite of proteins. Fig. 2 shows a fluorograph of [^{35}S]methionine-labeled proteins synthesized *in vitro* from mRNA from aerobic (lane B) and anoxic (lane C) embryos. There are no major differences between the two sets of proteins. These results suggest that there are no large changes in mRNA species when *Artemia* embryos become dormant.

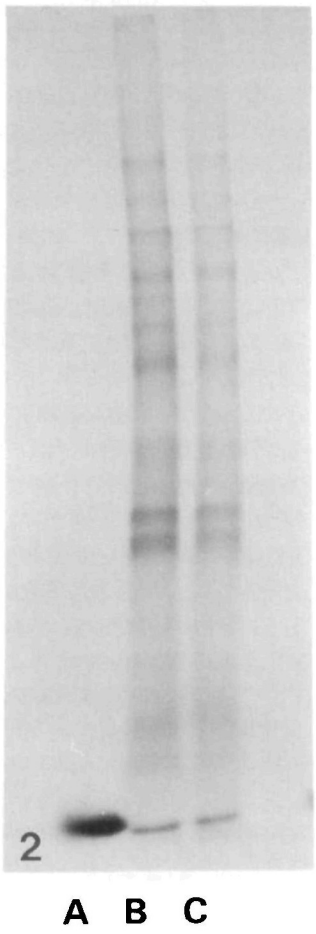

Fig. 2. Autofluorograph of translation products from aerobic and anoxic *Artemia franciscana* embryos. Reticulocyte lysate *in vitro* assays were programmed with phenol-extracted RNA from aerobic embryos and anaerobic dormant embryos. Equal volumes of the translation assay mixtures were electrophoresed on 15% polyacrylamide slab gels. Lane A, translation products encoded by rabbit globin mRNA (globin $M_r=16\times10^3$); lane B, translation products encoded by mRNA from 4 h aerobic *Artemia* embryos; lane C, translation products encoded by mRNA from anaerobically dormant *Artemia* embryos (i.e. 4 h of aerobic incubation followed by 4 h of anoxia).

Polysome profiles generated from active and dormant embryos

The main objective of this study was to determine the relative importance of transcriptional *versus* translational control in *Artemia* embryos entering dormancy. In addition to measuring translatable mRNA as an indicator of the site of control, we also examined the activity of ribosomes in dormant and active embryos using polysome profiles. Polysome profiles indirectly reflect the state of ribosome activity and can therefore serve as an indicator of protein synthesis activity in cells during different physiological states.

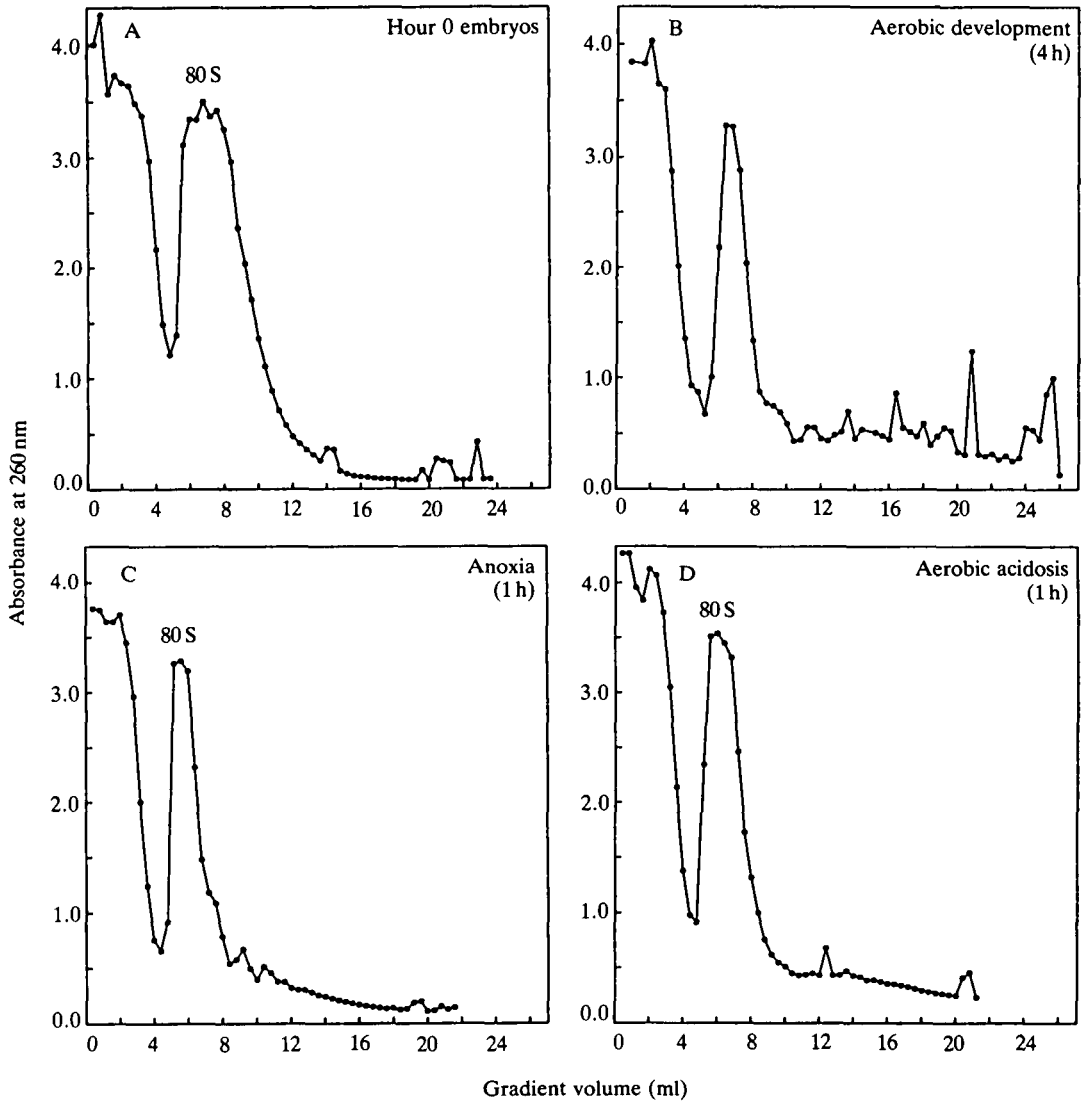

Fig. 3. A representative example of a sucrose density gradient analysis of polysomes in *Artemia franciscana* embryos during aerobic development and dormancy. Postmitochondrial supernatants were applied to 15 %–35 % sucrose density gradients, centrifuged, and absorbance profiles were determined at 260 nm. (A) Hydrated, dormant embryos (termed hour 0); (B) 4 h aerobic embryos; (C) anaerobic dormancy, 4 h aerobic embryos exposed to 1 h of anoxia (100 % N_2); (D) aerobic acidosis, 4 h aerobic embryos received an additional hour of incubation in 60 % CO_2:40 % O_2. Direction of sedimentation is from left to right.

Fig. 3 shows the polysome profiles generated from *Artemia* embryos under various physiological conditions. Cells from hour 0 embryos contain mostly single ribosomes (Fig. 3A), as demonstrated by the large 80 S monosome peak. These results corroborate those of Golub and Clegg (1968), who stated that hydrated,

Table 2. *Comparison of monosome and polysome peaks from* Artemia franciscana *embryos*

Experimental treatment	Monosome peak (% of total area)	Polysome region (% of total area)
Aerobic control (4 h)	38.3±3.66	61.7±3.66
Anoxia (1 h)	59.0±4.93*	41.0±4.93*
Aerobic acidosis (1 h)	68.3±2.03*	31.7±2.03*

Embryo extracts were applied to a 15 %–35 % sucrose density gradient and centrifuged for 4 h at 60000 *g* and 4 °C (see Materials and methods).

Each value represents the mean±1 S.E. for three separate experiments.

* Indicates a significant difference from aerobic controls in a *t*-test ($P<0.05$).

dormant (hour 0) embryos lack polysomes. After 4 h of aerobic development, bands of absorbance appear in the denser region of the sucrose gradient as a result of the presence of polysomes (Fig. 3B). If these aerobic embryos are switched to either anoxia (100 % N_2; Fig. 3C) or aerobic acidosis (60 % CO_2:40 % O_2; Fig. 3D) for 1 h, the polysome peaks become reduced and the area under the free ribosome peak increases. Qualitatively, this pattern was seen in three replicate experiments for each incubation treatment. There was variability in the degree of reduction in polysome levels during the early hours of dormancy. However, if the data are expressed as the percentage of total area under the 80 S monosome peak *versus* the polysome regions (Table 2), statistically significant reductions in polysome areas were observed under both experimental conditions.

Discussion

The results presented here provide evidence that the control of protein synthesis in dormant *Artemia* embryos is related to a translational mechanism as opposed to one that operates at transcription. First, there appear to be no significant quantitative changes in the pool of mRNA when an embryo enters a dormant state since the levels of translatable mRNA are not significantly different between active and dormant embryos (Table 1). In addition, there were no large qualitative differences between *in vitro* translated protein products from active and dormant embryos (Fig. 2). These results suggest that entry into dormancy, and the pHi changes associated with this transition, do not radically alter the mRNA pool present in the embryo. Second, polysomes were quantitatively reduced in profiles prepared from dormant embryos under both anoxic and aerobic acidotic incubation compared with levels in aerobic controls (Fig. 3). Clegg and Jackson (1989) reported polysome profiles for *Artemia franciscana* embryos (San Francisco Bay population) that changed more slowly under anoxia than those described here. In addition to potential inter-populational differences, their embryos were aerobic for 2 h prior to anoxic exposure, while the embryos in our study were given 4 h of

aerobic treatment. Polysome abundance has been observed to increase across the first 4 h of aerobic incubation (Golub and Clegg, 1968; G. E. Hofmann and S. C. Hand, unpublished observations), and this feature could make the relative differences between aerobic and anaerobic treatments more apparent in our study.

Two important observations can be made from the above data: (1) dormant embryos (i.e. those with an acidic pHi) are not as active in protein synthesis as are aerobic embryos and (2) the reduction of the polysome peaks in dormant embryos indicates that the process of initiation maybe disrupted (Duncan and Hershey, 1984). The results of the polysome experiments support the proposal that some type of translational control acts to arrest protein synthesis when *Artemia* embryos become quiescent. However, it is appropriate to note that the reduced levels of polysomes in cells of dormant embryos might reflect a direct effect of pH (or other factors) on ribosome stability. The reduction in pHi in dormant embryos may act either to disaggregate pre-existing ribosomes or to prevent further reassociation of the 40 S and 60 S subunits during initiation.

Given that pHi does have some role in the control of translation in dormant *Artemia* embryos, it would be interesting to know at what step(s) in translation this mechanism operates. The possible mechanisms of translational control can be categorized into two basic types: (1) alterations in translational capacity that involve direct effects on the translational machinery (Hershey, 1991) and (2) regulation at the level of the message, where mRNA is altered such that it is 'masked' and no longer directs protein synthesis (Spirin, 1969; Rosenthal and Wilt, 1987; Winkler, 1988). These two types of mechanisms are not necessarily mutually exclusive and, at least in the sea urchin egg, translational control operates at multiple levels (Winkler *et al.* 1985; Colin *et al.* 1987; Hansen *et al.* 1987; Lopo *et al.* 1988; Winkler, 1988).

Previous evidence for pHi control operating at the level of the translational machinery was found in a study where ribosomes isolated from unfertilized sea urchin eggs were less competent in translation (*in vitro* using a ribosome-free reticulocyte system) than were ribosomes from fertilized eggs that had been run off polysomes (Danilchik and Hille, 1981). Additional studies have shown that other components of translation involved in initiation, such as eukaryotic initiation factor 4F (eIF4F; Lopo *et al.* 1988), guanine nucleotide exchange factor (GEF; Colin *et al.* 1987) and 43 S pre-initiation complex formation (Winkler *et al.* 1985) were altered in the sea urchin egg upon fertilization.

During initiation, pHi plays a regulatory role by affecting 5′ cap recognition. In the rabbit reticulocyte translation system, a step in cap recognition was affected by pH changes over the range 6.5–7.5, such that *in vitro* protein synthesis was suppressed fivefold at the lower range of pH (Rhoads *et al.* 1983). Such a phenomenon could partly explain the observed inhibition of protein synthesis under the acidic pHi condition of dormant *Artemia* embryos. Similarly, evidence for the influence of pHi on phosphorylation of ribosomal proteins can be found in starved *Tetrahymena thermophila*, where a pHi change of 0.8 units was correlated with the reversible phosphorylation of a 40 S ribosomal protein (Goumard *et al.*

1990). This mechanism is appealing because there is abundant evidence for covalent modification of eIF2 in other systems (Hershey, 1989) including *Artemia* (Thoen *et al.* 1986), and such a mechanism would allow for rapid, reversible regulation without dramatic changes in the concentration of cellular components involved in protein synthesis.

With respect to the second broad category of translational control mechanisms, we are currently investigating the role of mRNA masking in the observed inhibition of protein synthesis when *Artemia* embryos enter anaerobic dormancy. This type of mechanism is of interest because the embryos are known to contain large quantities of messenger ribonucleoprotein particles (mRNPs; Slegers *et al.* 1981) and the mRNAs in these complexes could be differentially masked during transitions into and out of dormancy. Our preliminary experiments show that anaerobic incubation results in mRNPs that are at least 20% less active in translation assays than are mRNPs isolated from aerobic embryos prior to the transfer to anoxia. The formation of mRNP complexes could have a dual role in the *Artemia* system – to inhibit translation during dormancy and to stabilize the mRNA during the period when the embryo is quiescent. In general, it is not uncommon to find such mRNP complexes in systems that must store mRNA during a quiescent state for later use (Woodland, 1982; Rosenthal *et al.* 1980; Rosenthal and Wilt, 1987; Grainger and Winkler, 1987). Various studies have provided data regarding mechanisms for mRNA masking (Tate and Marshall, 1991) that we feel could be operative when the *Artemia* embryo becomes quiescent. For example, an M_r 38000 poly(A)+ binding protein (P38) has been isolated from nonpolysomal mRNPs in dehydrated, dormant *Artemia* embryos and has been shown to inhibit protein synthesis in rabbit reticulocyte lysates (see Slegers *et al.* 1989, for a review). However, there is currently no information available showing that such a mechanism operates during active to dormant transitions.

In summary, the pHi changes correlated with the entry of pre-emergence *Artemia* embryos into dormancy do not result in large changes in mRNA pools but do appear to alter the activity of ribosomes. Thus, we believe the data indicate that the mechanism of pHi influence over protein synthesis involves at least a translational component. Experiments concerning pHi-mediated translational regulation of gene expression in quiescent *Artemia* are currently under way in our laboratory.

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