Separate Ribosomal Pools in Sea Urchin Embryos: Ammonia Activates a Movement between Pools†

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ABSTRACT: Monoribosomes from unfertilized eggs of *Strongylocentrotus purpuratus* were shown to translate mRNA less efficiently than ribosomes derived from polyribosomes of embryos, as measured by globin synthesis in a ribosome-dependent rabbit reticulocyte lysate [Danilchik, M. V., & Hille, M. B. (1981) *Dev. Biol. 84*, 291-298]. Data presented in this paper show that monoribosomes from 16-cell and blastula embryos resemble monoribosomes from unfertilized eggs in translational capacity and are less active than the ribosomes associated with polyribosomes. Thus, we find two distinct populations of ribosomes in embryos. We define the less active monoribosome population as "naive" ribosomes and the more active, functioning polysome-derived ribosomes as "experienced" ribosomes. Naive and experienced ribosomes have the same elongation rates. The relationship between ionic triggers and the conversion of monoribosomes to experienced ribosomes was studied with the Ca^{2+} ionophore A23187, which releases intracellular Ca^{2+} stores, and NH_4Cl, which alkalinizes the cytoplasm. We found that ribosomes in the monoribosome populations from A23187-activated eggs or from NH_4Cl-activated eggs resembled naive monoribosomes from unfertilized eggs in their translational activity. In contrast, ribosomes derived from the polysomes of NH_4Cl-treated eggs were as active as the experienced polysome-derived ribosomes from normal embryos. Eggs activated with A23187 did not produce polyribosomes. The presence of significant amounts of experienced ribosomes in NH_4Cl-treated eggs implicates alkalinization of the cytoplasm as a stimulus for ribosome activation, which occurs slowly during initial development.

Unfertilized sea urchin eggs synthesize proteins at only a very low rate, despite the evident coexistence in the cytoplasm of all components necessary for translation (Denny & Tyler, 1964; Gross et al., 1964). The major block to protein synthesis in these eggs is the association of mRNA with the 40S preinitiation complex (Hille et al., 1981). The macromolecular change that prevents the association of ribosomes and mRNA into polyribosomes is not known, although it is known that the rates of protein synthesis after fertilization are increased (1) by the transient increase in the intracellular Ca^{2+} concentration from interal Ca^{2+} stores and (2) by the alkalinization of the cytoplasm through a Na^+/H^+ exchange (johnson et al., 1976; Steinhardt et al., 1977; Grainger et al., 1979; Dube et al., 1985). It has been suggested that masking of mRNAs by associated proteins is the major block in translation before fertilization [reviewed by Raff (1980)]. Recent work, however, has shown that sea urchin messenger ribonucleoprotein complexes (mRNPs) can be translated in vitro (Moon et al., 1982), that histone mRNPs, which are not translated until the two-cell stage (Wells et al., 1981), are stored in nuclei after the last meiotic division (Venezky et al., 1981; Showman et al., 1982; Angerer et al., 1984), and that mRNAs injected into sea urchin eggs compete with endogenous mRNAs for some rate-limiting component (Hille et al., 1985; Colin & Hille, 1986). None of these observations support the idea that messages are masked by proteins. If the stored cytoplasmic mRNPs are translatable in vivo, then which component of the initiation step limits the rate of protein synthesis in eggs? Monroy and co-workers previously suggested that ribosomes are activated after fertilization (Monroy et al., 1965). Several observations support this idea: (a) Salt washes of monoribosomes from sea urchin eggs contain inhibitors of protein synthesis (Metafora et al., 1971; Gambino et al., 1973; Hille, 1974). (b) These inhibitors are associated with the monoribosomes but not the polyribosomes of embryos (Hille, 1974). (c) The initial activity of monoribosomes from unfertilized eggs is lower, in vitro, than that of ribosomes derived from blastula polyribosomes (Danilchik & Hille, 1981). Observations on the phosphorylation state of the S6 ribosomal protein, however, cannot yet be correlated with ribosomal activity (Ballinger & Hunt, 1981; Ward et al., 1983; Takeshima & Nakano, 1983; Ballinger et al., 1984).

In this paper we continue our investigation of the role ribosomes might play in the early postfertilization rise in protein synthesis. We ask when, in vivo, does the conversion of less active monoribosomes of unfertilized eggs to polysomal-type ribosomes occur by comparing the activities of monoribosomes and polyribosome-derived ribosomes from early developmental stages. In addition, we investigate the relationships between the intracellular ionic changes and conversion of monoribosomes to the more active form. Finally, we ask whether the difference in the activities of monoribosomes and polysome-derived ribosomes is due to their respective elongation or initiation capacities.

Materials and Methods

Isolation of Sea Urchin Ribosomes. Sea urchin egg and embryo ribosomes tested in the reaction mixtures were obtained as previously described (Danilchik & Hille, 1981). Briefly, development was arrested by plunging embryo cultures into 16 volumes of wash medium (buffer A) at −3 °C. Buffer A contained 210 mM K^+, 5 mM Mg^{2+}, 80 mM Cl^-, 20 mM phosphate (pH 6.9-7.0 at 10 °C), and about 100 mM acetate.

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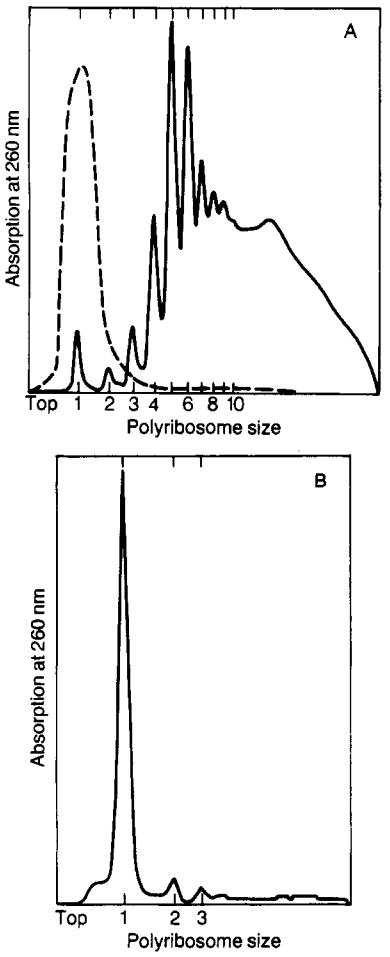

FIGURE 1: Sucrose gradient analysis of monoribosome and polyribosome preparation. (Panel A) Three A_{260nm} units of monoribosomes from unfertilized eggs was separated on preparative sucrose gradients as described under Materials and Methods and then recentrifuged on 15–40% sucrose gradients at 39 000 rpm for 80 min at 0 °C (--). A total of 5.1 A_{260nm} units of polyribosomes from hatched blastula embryos was pelleted through sucrose gradients, dissolved, and then recentrifuged as above (—). (Panel B) Polyribosomes in panel A were incubated in ribosome-depleted, initiation-deficient lysate, collected as a monoribosome peak on preparative sucrose gradients, and then recentrifuged as above.

Embryos were homogenized in 5 volumes of buffer A containing 1 mg/mL soybean trypsin inhibitor and 1 mM dithiothreitol. Polyribosomes from the postmitochondrial supernatants were pelleted through 10–30% sucrose gradients by centrifugation in a SW 27 Beckman rotor for 4.5 h at $92000g_{av}$. Fractions containing the monoribosomes that sedimented as a band in the lower third of the gradient were collected and the monoribosomes concentrated by pelleting in a type 65 Beckman rotor at $20000g_{max}$ for 20 h. The monoribosomal and polyribosomal pellets were resuspended in buffer A at 35–100 A_{260nm} units/mL and then cleared by a brief centrifugation. Analysis of these fractions by sucrose density gradient centrifugation is shown in Figure 1A. Polyribosomal ribosomes were released from mRNA by incubating a suspension of pelleted polyribosomes in a ribosome-depleted reticulocyte lysate, which contained no hemin and was initiation-deficient (Danilchik & Hille, 1981). These released ribosomes were collected on sucrose gradients as 80S particles, under conditions similar to those used above, and concentrated by centrifugation. An example of the ribosomes released from the polyribosomes shown in the sucrose density analysis in Figure 1B indicates that more than 93% of the ribosomes in this fraction sediment as monoribosomes.

Ribosomes from Artificially Activated Eggs. For some experiments, ribosomes were prepared simultaneously from cultures of unfertilized eggs, Ca^{2+}-ionophore-activated eggs, NH_4Cl-activated eggs, and two- and four-cell embryos. The cultures originated from a single spawning of several *Strongylocentrotus Purpuratus* females. Unfertilized eggs were incubated, with stirring, for 0.6 h at 12 °C, as a 2% (v/v) suspension in seawater containing 2 mM ATZ.[1] Eggs for Ca^{2+}-ionophore activation were washed 3 times with 10 volumes of Na-free seawater (484 mM choline chloride, 10 mM KCl, 27 mM $MgCl_2$, 29 mM $MgSO_4$, 11 mM $CaCl_2$, 2.4 mM $KHCO_3$, pH 8.0), resuspended as a 2% culture in Na-free seawater containing 2 mM ATZ, and then activated by the addition of 100 μL of 10 μM A23187 (Calbiochem) in Me_2SO to each 100 mL of the egg suspension. Virtually 100% of the eggs exhibited raised fertilization membranes. The suspension was incubated for 1.5 h at 12 °C. NH_4Cl-activated eggs were prepared by washing unfertilized eggs 3 times with 10 volumes of Ca-free seawater (500 mM NaCl, 10 mM KCl, 27 mM $MgCl_2$, 29 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 1 mM EGTA), resuspending them at 2% in Ca-free seawater containing 7.5 mM NH_4Cl and 2 mM ATZ, and then culturing them at 12 °C for 3 h. Embryos were prepared by fertilizing a 2% suspension of eggs in seawater containing 2 mM ATZ and then culturing the resulting zygotes at 12 °C for 3.5 h, by which time second cleavage was nearly complete. For convenience, polyribosomes for these studies were obtained by a modification of the previous centrifugation conditions. The postmitochondrial supernatants were layered on sucrose step gradients as follows, from bottom to top: 8 mL of 30%, 6 mL of 25%, 5 mL of 20%, and 9 mL of homogenate and overlayered with 8.5 mL of buffer A. The step gradients were centrifuged for 305 min at $95000g_{av}$ with no brake, at 0 °C, in a Beckman SW 27 rotor. After centrifugation, the supernatants and the top layer of sucrose were drawn off by aspiration. The bands corresponding to 80 S (near the interface between 25 and 30% sucrose) were collected and concentrated by centrifugation at $20000g_{max}$ for 20 h. Polyribosomal pellets were found in the step-gradient tubes containing homogenates of embryos and NH_4Cl-activated eggs. These pellets and the pelleted 80S fractions were resuspended at 13–48 A_{260nm} units/mL in buffer A containing 1 mM DTT and run off in the ribosome-free reticulocyte lysate, by the published method (Danilchik & Hille, 1981) except that monoribosomes were collected by the step-gradient method described above instead of linear gradients.

In Vitro Translation. Ribosome-dependent rabbit reticulocyte lysates, globin mRNAs, and polyribosome-bound initiation factors were prepared according to methods previously described (Danilchik & Hille, 1981). Sea urchin ribosomes were tested in the ribosome-depleted lysate, which was supplemented with the crude initiation factors and poly(A)+ mRNA from the reticulocyte polyribosomes and with [^{3}H]-leucine, nonradioactive amino acids, ATP, GTP, a nucleotide triphosphate regenerating system, Mg^{2+} to 1.5 mM, and K^+ to 105 mM and buffered with 20 mM Tris to pH 7.2–7.4 at 26 °C (Danilchik & Hille, 1981). Samples of 5 μL withdrawn at intervals were spotted on Whatman 3MM filter papers, treated for 3–5 min in hot 7.5% trichloroacetic acid, and

[1] Abbreviations: ATZ, 3-amino-1,2,4-triazole; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; Me_2SO, dimethyl sulfoxide; SDS, sodium dodecyl sulfate; SW, seawater; TCA, trichloroacetic acid; Tris, tris(hydroxymethyl)aminomethane. All seawater was filtered through nitrocellulose with 0.45-μm pores containing 100 mg/L streptomycin sulfate.

processed according to Danilchik and Hille (1981). Tritium incorporation was measured at an efficiency of approximately 40%.

An analysis of the products formed in the cell-free translation system was made by mixing samples of the translation mixtures with 2× gel sample buffer (Laemli, 1970), heating the mixture to 90 °C for 2 min, and separating the proteins by electrophoresis on 20% SDS–polyacrylamide gels. The gels were fixed, impregnated with the commercial scintillant En-Hance (New England Nuclear), dried, and exposed to Kodak XAR-5 X-ray film at −80 °C. The ^{3}H-labeled products of lysates incubated with egg monoribosomes or polysome-derived ribosomes from blastulae comigrated on the gels with endogenous rabbit globins. No significant amounts of sea urchin products were detected.

In Vivo Elongation Rates of Artificially Activated Eggs. The transit times in vivo were determined by the parallel line method of Fan and Penman (1970). Eggs were preloaded with [^{3}H]leucine in order to avoid possible errors from the continuous uptake of amino acids during the rate determinations (Brandis & Raff, 1978, 1979; Hille & Albers, 1979). Preloading is possible since echinoid eggs take up amino acids actively and irreversibly. Typically, 4–10% (v/v) cultures of eggs were incubated for 2 h in seawater containing 40 μCi of [^{3}H]leucine/mL of culture and 0.15 mM glycylglycine, pH 8.0. After incubation, the cultures were divided and washed 3 times in normal seawater, Ca-free seawater, or Na-free seawater. Eggs were activated as 2% cultures by sperm, NH_4Cl, or A23187 as indicated in Table I. To measure the kinetics of peptide synthesis, 1–2-mL culture samples were periodically removed and stopped in 20–40 volumes of 0.2 M KCl, 0.5 M triethanolamine, and 0.01 M $Mg(OAc)_2$, pH 7.8, and collected by gentle centrifugation. The cells were homogenized in a buffer designed by Goustin and Wilt (1981), which allows the pelleting of ribosomes and polyribosomes free of contaminating proteins and mRNPs. The Goustin buffer is 0.25 M NaCl, 50 mM $Mg(OAc)_2$, 25 mM EGTA, 25 mM Tris, 1 mM phenylmethanesulfonyl fluoride, and 1% Triton X-100.

The incorporation into total peptides was determined by TCA precipitation of the postmitochondrial supernatant and that into released peptides by TCA precipitation of the postribosomal supernatant. Samples were corrected to a constant number of eggs as determined by measurements of total proteins in the postmitochondrial supernatants by Bensadoun and Weinstein's (1976) modification of the Lowry method. The transit time was graphically determined as twice the time between the kinetic line for incorporation into total peptide and the line for incorporation into released peptides.

In Vitro Elongation Rates of Ribosomes. The transit times of the various preparations of ribosomes were determined by modifications of the method of Fan and Penman (1970). At several time intervals, 50-μL samples of the translation mixtures were rapidly diluted into 300 μL of polyribosome buffer (PB) at −3 °C. PB contained 100 mM KCl, 5 mM $Mg(OAc)_2$, and 20 mM Tris, pH 7.5 at 10 °C, and 100 μM emetine. Aliquots of the arrested reaction mixtures were precipitated with TCA to determine total incorporation into peptides. Other aliquots were layered on 15–50% linear sucrose gradients made up in PB, overlayered with PB, and centrifuged on a Beckman SW 50.1 rotor at $233000g_{av}$ for 2.5 h at 0 °C. The gradients were scanned at 260 nm to determine the positions of monoribosomes and polyribosomes. Fractions of the gradients were collected, precipitated with trichloroacetic acid, filtered, and analyzed for precipitated tritium to determine incorporation of [^{3}H]leucine into released (supernatant) and nascent (polyribosome region) globin chains. Total incorporation was also determined by summing the precipitated tritium in the gradient fractions. The transit times were calculated as above.

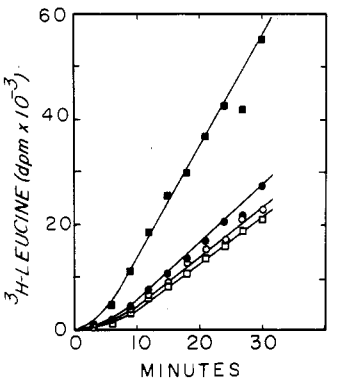

FIGURE 2: Time course for the translation of rabbit globin mRNA by ribosomes of unfertilized eggs and hatched blastulae. Incorporation of [^{3}H]leucine into trichloroacetic acid precipitable material was measured as described under Materials and Methods. Reaction mixtures were incubated at 26 °C, pH 7.20–7.25, and contained 4 A_{260nm} units/mL of sea urchin ribosomes, 20 μg/mL poly(A)-containing reticulocyte RNA, and other components as described under Materials and Methods. Polysome-derived ribosomes run off from hatched blastula polyribosomes by pretreatment in an initiation-deficient reticulocyte lysate (■); monoribosomes isolated from unfertilized eggs and that were not pretreated in an initiation-deficient lysate (●); monoribosomes isolated from unfertilized eggs (○) and hatched blastulae (□) that were pretreated with an initiation-deficient lysate.

RESULTS

Relative Translation Activities of Monoribosomes and Polyribosomal Ribosomes from Sea Urchin Embryos. To determine whether monoribosomes are activated immediately after fertilization of the type found in polyribosomes or they are activated gradually during development, we isolated ribosomes from several stages of development. Monoribosomes and polyribosomes were separated by centrifugation on sucrose density gradients. Ribosomes were released from polyribosomes by preincubation in ribosome-free initiation-deficient reticulocyte lysate (see Materials and Methods). Monoribosomes were also preincubated in reticulocyte lysate under identical conditions to ensure that this treatment did not modify the ribosomes. As shown in Figure 2, both unfertilized egg monoribosomes and polysome-derived ribosomes from hatched blastulae behaved as previously described when tested in the reticulocyte lysate assay system (Danilchik & Hille, 1981). Unexpectedly, however, monoribosomes from blastulae had kinetics identical with those of monoribosomes from unfertilized eggs. Similar results were obtained from monoribosomes of 16-cell embryos (see Figure 3). We conclude from these data that embryos contain two distinct populations of ribosomes: those in the monoribosome pool and those in the polyribosomes. A difference in the activities of ribosomes from these two pools persists to at least hatching blastula stage.

Two controls were done to ascertain that the difference between the two kinds of ribosomes was a function neither of how they were obtained nor of the run-off procedure:

(1) To test whether the run-off procedure per se had an effect on ribosome activity, some unfertilized egg monoribosomes were left untreated, while other aliquots were preincubated in the initiation-depleted reticulocyte lysate. We found that the run-off procedure, which was necessary to obtain polyribosome-derived ribosomes free from polyribosomal mRNA, did not significantly change the activity of nono-

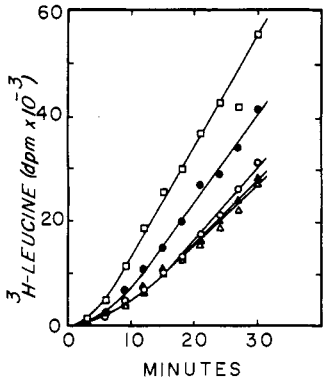

FIGURE 3: Time course of translation by monoribosomes from sea urchin unfertilized eggs and 16-cell embryos prepared in buffer A at −3 °C or SW at −3 °C. Conditions were as described for Figure 2. All ribosome fractions were pretreated with initiation-deficient reticulocyte lysate as described under Materials and Methods. Shown are data for blastula polysome-derived ribosomes prepared from cultures stopped in buffer A (□), monoribosomes from 16-cell embryos prepared in SW (●) or buffer A (○), and monoribosomes from unfertilized eggs prepared from cultures stopped in SW (▲) or buffer A (△).

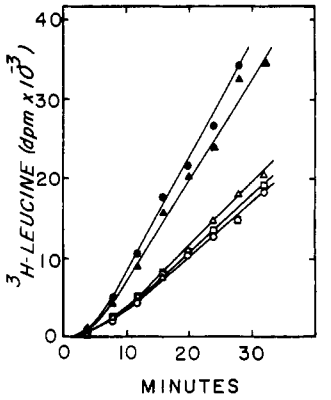

FIGURE 4: Translational activity of monoribosomes and polyribosome-derived ribosomes after treatment of eggs with NH_4Cl in Ca^{2+}-free SW and A23187 in Na^+-free SW. Incubation conditions were as described for Figure 2, except that 10 μg/mL mRNA was present. All ribosomes were pretreated with initiation-deficient reticulocyte lysate as described under Materials and Methods. Shown are data for polyribosome-derived ribosomes from two- to four-cell embryos (●) and from unfertilized eggs activated with NH_4Cl in Ca^{2+}-free SW (▲) and for monoribosomes from unfertilized eggs, treated with NH_4Cl in Ca^{2+}-free SW (△), treated with A23187 in Na^+-free SW (□), or untreated (○).

ribosomes in the reticulocyte lysate assay (Figure 2).

(2) To test whether in vitro run-off in the reticulocyte lysate produced artificially active ribosomes from polyribosomes, conditions were used that induced run-off in vivo. These conditions demonstrate that arresting embryonic metabolism in normal seawater is similar to slow cooling and oxygen depletion of cells, which cause ribosomes to run off polyribosomes (Davis, 1971). In this in vivo run-off experiment, development in 16-cell embryos was arrested by plunging the embryos into either −3 °C buffer A or −3 °C seawater just before homogenization. The total yield of ribosomes was the same under both arresting conditions; however, the polyribosomal pellet was roughly 40% smaller for seawater-arrested embryos than for embryos arrested in buffer A, as measured by absorbance at 260 nm. A total of 80–85% of the monoribosomes isolated under either of these conditions dissociates into subunits during centrifugation in 0.6 M KCl. Thus, after embryos were arrested in seawater, many ribosomes must have run off polyribosomes, resulting in cosedimentation, at 80 S, of polysome-derived ribosomes with naturally occurring monoribosomes. Monoribosomes that remain attached to mRNA will not dissociate into subunits under high-salt centrifugation conditions (Martin & Hartwell, 1970). Under low-salt centrifugation conditions, only a few ribosomes occur as subunits, presumably due to the low availability of initiation factors (Figure 1). The activities of monoribosomes run-off in vivo (by arresting embryos in seawater) were compared in ribosome-free lysates with monoribosomes arrested in buffer A and with ribosomes derived from polyribosomes of 16-cell embryos (Figure 3). Monoribosomes from the 16-cell embryos prepared with buffer A exhibited slow kinetics like those of unfertilized egg monoribosomes. In contrast, monoribosomes from embryos prepared in seawater exhibited overall kinetics intermediate between those of the monoribosomes and those for the polyribosome-derived ribosomes from hatched blastulae. These results confirm that ribosomes originating on polyribosomes, whether driven off polyribosomes in intact cells or in vitro, are more active then monoribosomes in vitro.

Role of Intracellular Ionic Changes on Ribosome Activation. Dramatic changes in the egg's intracellular ionic milieu follow fertilization and appear to be involved in the activation of the protein synthesizing machinery (Steinhardt & Epel, 1974; Paul & Epel, 1975; Shen & Steinhardt, 1978; Winkler et al., 1980; Epel et al., 1974; Dube et al., 1985). Conditions that uncouple the Ca^{2+} release and the alkalinization of the cytoplasm were used to determine whether either the Ca^{2+} flux, alone, or the cytoplasmic alkalinization, alone, mobilize monoribosomes. Cytoplasmic alkalinization without an increase in Ca^{2+} concentration was achieved by exposing eggs to NH_4Cl in calcium-free seawater. Conversely, the intracellular Ca^{2+} concentration was raised in the absence of a concomitant Na^+/H^+ exchange, and its resulting cytoplasmic alkalinization, by application of the calcium ionophore A23187 in sodium-free seawater. Figure 4 shows the time course for the translation of globin mRNA in the reticulocyte lysate by ribosomes obtained from eggs activated with NH_4Cl in Ca-free seawater, from eggs activated with A23187 in Na-free seawater, from unfertilized eggs, and from two- to four-cell embryos. Polyribosomes were obtained in as great a yield from eggs treated with NH_4Cl for 3 h at 12 °C as from embryos incubated for 3.5 h at 12 °C after fertilization, consistent with observations by Epel et al. (1974). Ribosomes derived from the polyribosomes of NH_4Cl-activated eggs were identical in activity with ribosomes derived from polyribosomes of two- to four-cell embryos (Figure 4). Polyribosomes were not detected in A23187-activated eggs. Monoribosomes derived from A23187-activated eggs and NH_4Cl-activated eggs exhibited translation kinetics like those of monoribosomes from unfertilized eggs. The absence of polyribosomes and the low level of activity of the monoribosomes of A23187-activated eggs indicate that an influx of Ca^{2+} without a concomitant intracellular pH change is not sufficient to convert ribosomes to the more active form. The presence of active ribosomes in NH_4Cl-treated eggs, equivalent to polyribosome-derived ribosomes from embryos, implicates alkalinization of the cytoplasm as a stimulus to ribosome activation.

Role of Intracellular Ionic Changes on in Vivo Transit Times. Brandis and Raff (1979) showed that the observed 2-fold increase in the efficiency of translation after the fertilization of *S. purpuratus* eggs is *not* due to the alkalinization of the cytoplasm as NH_4Cl does not increase elongation rates. Subsequent experiments by Winkler et al. (1980) suggested, but did not prove, that Ca^{2+} release concomitant with intracellular alkalinization does increase that elongation rate. We have tested this notion by measuring the transit times by the

Table I: Average mRNA Transit Times in Minutes[a]

	S. purpuratus, 12 °C[b]	
	expt I	expt II
unfertilized eggs	52 ± 5	60 ± 3
zygote	22 ± 1	32 ± 1
eggs in NH_3[c]		59 ± 3
eggs in A23187 plus NH_3[d]	26 ± 1	

[a] The methods for determining transit times are described under Materials and Methods. [b] Temperature refers to the in vivo incubation temperature. [c] Incubation was in calcium-free seawater with 10 mM NH_4Cl. This concentration of NH_4Cl gave optimal total incorporation into *S. purpuratus* and *Lytechinus pictus* eggs. [d] Incubation was in sodium-free seawater with 2.5 μM A23187 and 10 mM NH_4Cl.

parallel line method of Fan and Penman (1970). Table I shows the in vivo transit times for *S. purpuratus* eggs, zygotes, and NH_3-treated eggs with and without A23187 addition. These transit times refer to the synthesis at 12 °C of peptides with an average M_r of 48 600–54 300 (Brandis & Raff, 1978). The values are large relative to those for mammalian translational systems because translational rates are effected by temperature, changing 2–3-fold for every 10 °C change in temperature (Hille & Albers, 1979; Raff & Showman, 1983). The efficiency of translation increased 2-fold in the presence of both alkalinization and calcium ion release as shown for eggs treated simultaneously with A23187 and NH_4Cl. The transit times for this treatment were essentially the same as that for fertilized eggs and cleavage-stage embryos but different from that for eggs treated only with NH_4Cl. Alkalinization of the egg cytoplasm with NH_4Cl did not by itself increase the efficiency of translation. These experiments agree with and extend the experiments by Brandis and Raff (1978, 1979), Hille and Albers (1979), and Winkler et al. (1980).

In Vitro Transit Times of Sea Urchin Ribosomes on Globin mRNA. Since the in vivo transit time in sea urchin embryos has been found to accelerate about 2-fold when eggs are fertilized (Brandis & Raff; 1979; Stavy & Gross, 1969; Clegg & Denny, 1974; Ilan & Ilan, 1978; this paper), we tested whether the elongation rates of monoribosomes and polysome-derived ribosomes were similar or different. Transit times for globin synthesis (globin M_r 17 000) were measured for ribosomes from unfertilized sea urchin eggs, NH_4Cl-activated eggs, and two- and four-cell embryos at near steady-state conditions in reticulocyte lysates at 26 °C. Transit times in these reticulocyte lysates cannot be directly compared to those in sea urchins in vivo because of the higher temperature and the shorter length of the average peptides formed in the in vitro studies, both of which shorten the transit times, and because of the large dilution of the elongation factors in the in vitro assay, which must also lengthen the transit time. Both monoribosomes and polysome-derived ribosomes were tested in reticulocyte lysates to determine whether they had similar rates. Figure 5 shows the incorporation of [^{3}H]leucine into total and released peptides 30–50 min after the beginning of the incubation, at 26 °C, of sea urchin ribosomes in reticulocyte lysate depleted of reticulocyte ribosomes and containing free mRNA. The horizontal distance between the total and released incorporation is equivalent to half the transit time as described by Fan and Penman (1970). Thus measured, the transit times were between 9 and 11 min both for monoribosomes from unfertilized eggs (Figure 5A) and for monoribosomes from NH_4Cl-activated eggs (Figure 5C). Likewise, transit times were 9–11 min for ribosomes derived from polysomes of two- to four-cell embryos (Figure 5B) and NH_4Cl-activated eggs (Figure 5D). Hence, there is no significant difference in the transit times of monoribosomes and polyribosome-derived ribosomes, despite the fact that total incorporation is greater for polysome-derived ribosomes than for monoribosomes.

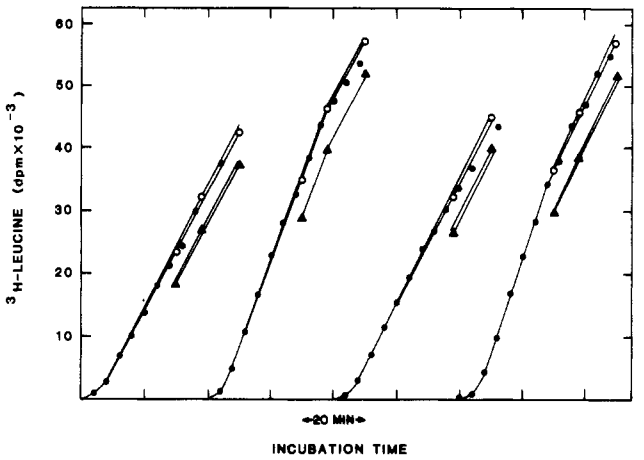

FIGURE 5: Elongation rates of monoribosomes from unfertilized eggs, polyribosome-derived ribosomes from two- to four-cell embryos, and monoribosomes and polyribosome-derived ribosomes from NH_4Cl-activated eggs. All ribosomes were pretreated with initiation-deficient reticulocyte lysate. Incubation conditions were as described under Materials and Methods. A total of 8 A_{260nm} units/mL of ribosomes and 10 μg/mL globin mRNA were used. Incubation pH was 7.20–7.21, at 26 °C. Samples taken from the reaction mixtures were centrifuged on sucrose gradients to separate released peptides from nascent peptides, as described under Materials and Methods. Each measurement begins at zero time but is sequentially displaced by 40 min for comparison. Nascent peptides sediment with polyribosomes, and released peptides remained in the supernatant fractions. (A) Monoribosomes from unfertilized eggs; (B) polyribosome-derived ribosomes from two- to four-cell embryos; (C) monoribosomes from NH_4Cl-activated eggs; (D) polyribosome-derived ribosomes from NH_4Cl-activated eggs. Shown in each panel is incorporation of [^{3}H]leucine into released (▲) and total (○, ●) globin chains. Total incorporation was determined by counting the trichloroacetic acid precipitated aliquots of the lysate (●) or by summation of the counts in the trichloroacetic acid precipitated fractions in the sucrose gradients (○).

Discussion

In this work, we extend our previous findings (Danilchik & Hille, 1981) to show that, in *S. purpuratus*, monoribosomes, whether from unfertilized eggs, 16-cell embryos, or hatched blastulae, are similar in activity. These results are thus consistent with previous findings, that monoribosomes of unfertilized eggs and zygotes have equivalent in vitro translation activity (Stavy & Gross, 1969; Clegg & Denny, 1974; Ilan & Ilan, 1978). Importantly, however, ribosomes from embryo polyribosomes are more active than monoribosomes from fertilized or unfertilized eggs. Thus, the difference between monoribosomes and polysome-derived ribosomes persists through blastula state, even when embryos are very active in protein synthesis and about 60–70% of the ribosomes are in polyribosomes (Infante & Nemer, 1967; Rinaldi & Monroy, 1969; Goustin & Wilt, 1981).

The question of possible functional differences between native monoribosomes and polysomal ribosomes has previously been considered in whole lysates from rabbit reticulocytes and HeLa cells (Adamson et al., 1969; Baglioni et al., 1969; Howard et al., 1970). Under steady-state conditions, a rapid exchange of ribosomal subunits occurs between subunit and polyribosomal pools, but free monoribosomes exchange only slowly with either pool (Falvey & Staehelin, 1970). An "antiassociation factor", presumably eIF-3 and eIF-6, prevents subunit reassociation but apparently does not actively promote

subunit dissociation (Kaempfer & Kaufman, 1972). Thus, in mammalian cells and cell extracts, free monoribosomes are not "inactive", as such. Rather, their relatively slow spontaneous dissociation strongly favors the recycling of just-terminated subunits from polyribosomes. On the other hand, when the overall rate of protein synthesis increases, for example, in the liver of young rats and mice (Staehelin et al., 1967) and in ascites cells stimulated by growth medium (Hogan & Korner, 1968a,b), free 80S ribosomes are quite capable of entering polysomes. An interesting calculation reveals another component to the bias favoring the recycling of ribosomes from polyribosomes: the maximum distance from a just-released ribosomal subunit to the 5′ initiation end of the same mRNA (globin) is estimated to be much less than the average distance to any neighboring monoribosome or free subunits (Baglioni et al., 1969; Howard et al., 1970). Statistically, then, reinitiation by a just-released subunit should be strongly favored over initiation by any other subunit that is free or generated from monoribosomes.

The difference in activity between monoribosomes and polysome-derived ribosomes of *S. purpuratus* eggs and embryos cannot be explained by a bias for released subunits as in the mammalian systems described above. First, ribosomes used in this study, whether polysome-derived or native monoribosomes, were delivered as predominantly free 80S ribosomes to the reaction mixture, which contained added free mRNAs and initiation factors. Thus, monoribosomes and polysome-derived ribosomes had an equal chance to interact with initiation factors and mRNAs. Steady-state conditions did not apply in the initial stages of the cell-free system. Differences in their in vitro behaviors must, therefore, be attributed to some aspect of their respective intrinsic activities as 80S ribosomes. Second, in vivo, ribosomes run off from polysomes do not return to their former, monosome-like state (Figure 3). Because both pools persist through early development, we refer to the ribosomes in the monoribosome pool as "naive", as opposed to the "experienced" ribosomes from the polysome pool. Naive monoribosomes may be unique to eggs and to embryos that are undergoing a translational control phase during early development. It seems unlikely that naive monoribosomes, as such, would normally be found in translationally active cells such as reticulocytes, where as many as 80% of the ribosomes may be found in functional polyribosomes (Falvey & Staehelin, 1970). They may also be absent in embryos that are synthesizing new ribosomes and undergoing a growth phase, though this fact remains to be shown.

Conditions that uncouple Ca^{2+} release from the subsequent cytoplasmic alkalinization were used to study the time course of ribosome activation. Exposure to Ca ionophore A23187, in the absence of Na^+, did not result in formation of polyribosomes or monoribosomes. However, eggs treated with NH_4Cl in Ca^{2+}-free seawater produced polyribosomes (Epel et al., 1974). Ribosomes derived from polysomes of these NH_4Cl-treated eggs were as active as ribosomes derived from polysomes of normal embryos. Thus, although a transient increase of intracellular Ca^{2+} appears necessary for full activation of the protein synthesizing machinery of unfertilized eggs, cytoplasmic alkalinization is sufficient to initiate the process of ribosome conversion with a time course consistent with that of normal development.

The last question addressed in this study is whether the low activity of monoribosomes could be due to their slow rate of elongation in vitro. Neither fertilization nor NH_4Cl treatment changed the in vitro elongation rate of monoribosomes. The absence of an NH_4Cl-induced change in the in vitro elongation rate is consistent with the observation that treatment of unfertilized eggs with NH_4Cl does not change the in vivo elongation rate (Hille & Albers, 1978). On the other hand, Ca^{2+} in the presence of ammonia stimulates an increase in the rate of peptide elongation in sea urchin eggs after fertilization as demonstrated in this paper. The activities of elongation factors, not ribosomes, are implied for this stimulation of the elongation rate by the postfertilization doubling of EF-1 activity concomitant with a small increase in EF-2 activity (Felicetti et al., 1972) and by the redistribution of EF-2 from monoribosomes and large detergent-soluble particles to a monomeric 5S form (Yablonka-Reuveni & Hille, 1983).

Thus, a gradual increase in the number of initiation-competent ribosomes, stimulated by alkalinization of the cytoplasm, may govern the rate of protein synthesis after fertilization. The mechanism of this change in ribosomal competence is not understood. Protein factors washed from monoribosomes at salt concentrations greater than 0.8 M KCl and as high as 1.4 M KCl–NH_4Cl are known to be inhibitory to cell-free translation systems (Metafora et al., 1971; Gambino et al., 1973; Hille, 1974; Hille et al., 1985; Hille & Danilchik, 1986). This factor preparation appears to block the initiation step as determined by the comparison of polyribosomal profiles of reticulocyte lysates incubated with monoribosomal salt washes, with emetine, and inhibitor of elongation, or with aurintricarboxylic acid, an inhibitor of initiation (A. T. Lewis and M. B. Hille, unpublished results). Possible mechanisms for the generation of initiation-competent ribosomes after fertilization include the modification and/or release of the inhibitor protein by enzymes sensitive to alkalinization. Studies to determine the mechanism are under way.

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