only when antigen is present in large excess, when $[PL_2] >> [PL_1]$, and when the cell population is homogeneous. Even in this specific and restricted case, one measures the ratio between the *square* of the number of sites per surface area of the cell—not the ratio of the absolute number of sites.

A comparison of relative antibody affinities using the same cell system is valid as long as $[PL_2]$ is the predominant species.

The determination of the total number of antigenic sites per cell is subject to a possible error by a factor of 2 depending upon the relative association constants K_1 and K_2 as well as the range of $[P_T]/C$ used in the experiment. Monovalent Fab binding to cell surfaces provides an unequivocal result. However, experimentally this can be done only if the antibody affinity is high and the number of antigenic sites per cell is at least 10^5. At low antibody affinity and s values, the number of cells/liter required for measurable interaction becomes prohibitively high.

Finally, all attempts to compare soluble and membrane-bound forms of an antigen as described by Williams (1977) are shown in section 7 to be fundamentally invalid.

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Changes in Poly(adenylic acid) Polymerase Activity during Sea Urchin Embryogenesis†

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ABSTRACT: Between fertilization and the two-cell stage there is a doubling of the poly(adenylic acid) [poly(A)] content of sea urchin embryos. This net increase in poly(A) is due to cytoplasmic polyadenylation of stored underadenylated messenger RNA molecules. The present work was initiated to determine the mechanisms responsible for the changes in the rate of poly(A) synthesis during early sea urchin development. The total poly(A) polymerase activity in *Strongylocentrotus purpuratus* is constant during development, from the unfertilized egg until the prism stage. As development proceeds, however, there is a rearrangement in the subcellular localization of the enzyme. In the unfertilized egg, the enzyme activity is almost entirely localized in the 100000g supernatant fraction. As embryogenesis proceeds, there is a progressive increase in the percent of enzyme activity which is associated with the nuclear fraction, along with a concomitant decrease in the supernatant activity. A substantial proportion of the poly(A) polymerase activity is present in enucleated as well as nucleated merogons prepared from *S. purpuratus* eggs indicating that the soluble poly(A) polymerase activity observed during early development is due to an actual cytoplasmic localization rather than nuclear leakage. The post-fertilization increase in polyadenylation, therefore, need not be accomplished by de novo synthesis of poly(A) polymerase.

Fertilization or artificial activation of sea urchin eggs initiates increases in protein, DNA, and RNA synthesis (Gross, 1967). During the period of increasing protein synthesis, the poly(A)[1] content of the embryo increases to more than twice the level present in unfertilized eggs (Slater et al., 1972; Wilt, 1973). This net increase in polyadenylation occurs on underadenylated messenger RNAs stored in the egg cytoplasm and is essentially complete by the time of the second cleavage (Wilt, 1973; Slater et al., 1973; Slater & Slater, 1974). The fact that polyadenylation occurred to the same extent in parthenogenetically activated enucleated egg fragments (Wilt, 1973) implied that a cytoplasmic mechanism for polyadenylating messenger RNA exists. Since this demonstration, nonmitochondrial cytoplasmic polyadenylation has been detected in mammalian cell lines (Brawerman & Diez, 1975; Sawicki et al., 1977), and important roles for the poly(A) segment in controlling messenger RNA stability in the cytoplasm have been suggested (Nudel et al., 1976; Huez et al., 1978).

The mechanisms by which protein, DNA, RNA, and poly(A) syntheses increase after fertilization are not known.

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[1] Abbreviations used: poly(A), poly(adenylic acid); poly(G), poly(guanylic acid); poly(C), poly(cytidylic acid); Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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The present study was undertaken to characterize the poly(A) polymerase reaction in vitro and quantitate enzyme activity during sea urchin embryogenesis in order to define those factors which regulate enzyme activity.

Experimental Procedures

Embryo Culture Conditions. Eggs and sperm from the sea urchin, *Strongylocentrotus purpuratus*, were obtained by injection of 0.5 M KCl into the coelomic cavity. The eggs were fertilized and cultured by conventional methods (Hinegardner, 1967) in a 1% (v/v) suspension at 15 °C in the presence of 50 μg/mL of streptomycin. One milliliter of packed *S. purpuratus* eggs is equivalent to 2×10^6 eggs. Only cultures with greater than 95% fertilization and normal development were used.

All experiments were repeated with the sea urchin, *Lytechinus pictus* (Pacific Biomarine), and except as noted all results were the same. All experiments detailed in this paper were performed with *S. purpuratus* unless otherwise stated.

Subcellular Fractionation. Unfertilized eggs or embryos were collected by centrifugation and washed once in either 1.0 (eggs) or 1.5 M (embryos) glucose. The pelleted samples were resuspended in 7 vol of 50 mM Tris-HCl, pH 7.5, containing 5 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.5 mM EDTA, and 25% (vol/vol) glycerol and homogenized in a stainless steel Dounce homogenizer. The homogenate was either centrifuged at 12000*g* for 20 min to obtain a 12000*g* supernatant and pellet or, for more detailed subcellular distribution experiments, the homogenate was successively centrifuged at 1000*g* for 10 min, 5000*g* for 10 min, 12000*g* for 20 min, and 100000*g* for 60 min. All pellets were resuspended in an appropriate volume of homogenization buffer.

Preparation of Nucleated and Enucleated Merogons. Merogons were prepared by layering 2.0 mL of a 15% suspension of eggs over a 30-mL linear gradient constructed from 1.0 M sucrose (prepared in distilled water) and a mixture of 3 parts of Millipore-filtered sea water plus 1 part of 1.0 M sucrose. The gradients were centrifuged at 12 °C in an SW 25 rotor at 10000*g* for 5 min, followed by centrifugation at 19000*g* for 10 min. After centrifugation, the nucleated and enucleated merogons, which were separated by at least 6 mm, were collected by puncturing the side of the cellulose nitrate tube with a syringe and carefully withdrawing each separated band. The merogons were diluted with approximately 4 vol of homogenization buffer and centrifuged at 10000*g* for 10 min to pellet the samples. The pelleted samples were then resuspended in approximately 7 vol of homogenization buffer and homogenized in a stainless steel Dounce homogenizer. As judged by phase microscopy of fixed samples of the gradient bands, there was no cross-contamination of the nucleated and enucleated fractions.

Poly(A) Polymerase Assay. Poly(A) polymerase activity was measured in a reaction mixture containing 50 mM Tris-HCl, pH 8.0, 0.65 mM $MnCl_2$, 1 mM dithiothreitol, 75 μg of poly(A)$_{\overline{11}}$ primer (saturating primer concentration), 1 mM ATP containing 0.5 μCi of [8-^{3}H]ATP (Schwarz/Mann), and 50–200 μg of enzyme protein in a final volume of 0.1 mL. After incubation at 37 °C for 20 min, the reaction was terminated by the addition of 0.1 mL of cold 25% trichloroacetic acid. The precipitates were collected onto glass fiber filters and washed with 30 mL of 5% trichloroacetic acid and then 10 mL of methanol, and the acid-insoluble radioactivity was determined by liquid scintillation counting in Omnifluor–toluene scintillation fluid containing 4% NCS solubilizer (Amersham/Searle). The resulting values were corrected for blanks incubated without enzyme. Both ATP and poly(A)

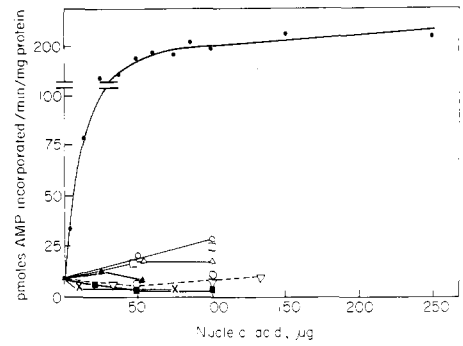

FIGURE 1: Priming ability of different nucleic acids. *S. purpuratus* egg homogenate was assayed for poly(A) polymerase activity, with the following nucleic acids used as primer at the indicated concentrations: (●—●) nuclease-digested high molecular weight poly(A); (○—○) high molecular weight poly(A); (△—△) yeast RNA; (▲—▲) poly(C); (□—□) *E. coli* transfer RNA; (■—■) ribosomal RNA; (X—X) $(Ap)_5A$; (○---○) poly(G); (▽---▽) denatured calf thymus DNA.

primer were used at saturating concentrations and all reactions were linear with respect to time and enzyme concentration. When either specific or total activity per milliliter of eggs from different females was compared, there was no more than a twofold variation. Some losses in enzyme activity occurred after freezing and thawing of the samples; therefore, all fractions were assayed immediately.

Preparation of Poly(A) Primer. Primer was prepared by nuclease digestion of commercial high molecular weight (>100 000) poly(A) essentially by the method of Sibler et al. (1972), using 50 μg of micrococcal nuclease (Worthington Biochemical Corp.) for 1000 A_{260} units of poly(A) (Sigma Chemical Co.).

Protein Determination. Protein was determined by the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard.

Results

Characterization of Poly(A) Polymerase Activity. Optimum conditions for measuring poly(A) polymerase activity were determined using unfractionated egg and embryo preparations as the enzyme source. Reaction characteristics and requirements were the same for all developmental stages examined. The pH optimum of the enzyme was 8.0 in either Tris-HCl or Hepes buffer. The sea urchin enzyme is a Mn^{2+}-requiring enzyme, with the optimal concentration of Mn^{2+} being dependent upon the ATP concentration present. The ratio of Mn^{2+}:ATP producing the greatest increase of activity is 0.65 (data not shown).

The poly(A) polymerase reaction was highly dependent upon the presence of a priming RNA molecule. Enzyme activities were increased approximately 5- to 20-fold by the inclusion of saturating amounts of primer, indicating that the polymerase is probably not saturated with respect to primer in vivo. Although most poly(A) polymerases are able to use a wide variety of RNAs (Edmonds & Winters, 1976) or DNA (Mans & Huff, 1975) as primer, this is not true for the sea urchin enzyme. As shown in Figure 1, of the nine nucleic acid molecules tested for priming ability, only a nuclease-treated preparation of the high molecular weight poly(A) was an efficient primer. In order to ensure that the differences in priming ability of the nucleic acids were not due to their differential degradation by ribonucleases in the homogenate, the recovery of [^{14}C]tRNA used as primer was measured after the standard incubation. As judged by both acid-precipitable radioactivity and migration on 10% polyacrylamide gels, the

Table I: Poly(A) Polymerase Activity in *S. purpuratus* during Early Cleavage

hours post-fertilization	poly(A) polymerase act. (nmol of AMP incorp./(min·mL of eggs))[a]
unfertilized egg	30.4
1 h (fertilized egg)	28.1
4 h (8-cell)	31.3
6 h (32-cell)	27.2

[a] One milliliter of eggs = 2×10^6 eggs.

[^{14}C]tRNA was not degraded during the incubation. In addition, even after partial purification of the enzyme activity by ion-exchange chromatography, only the nuclease-treated poly(A) could be effectively used as a primer.

The priming poly(A) was prepared by treating the high molecular weight poly(A) with micrococcal nuclease to produce oligonucleotides with a 3′-phosphate and then with alkaline phosphatase to yield free 3′-OH groups. Comparison of the effectiveness of different nuclease-treated poly(A) primer preparations to increase AMP incorporation using the same enzyme preparation showed that increases in enzyme activity varied between 5- and 11-fold. The presumed difference between the various primer preparations was probably chain length. This suggests that the sea urchin poly(A) polymerase not only recognizes rather specific structural features of the nucleic acid primer but also is selective on the basis of chain length. This necessitated the preparation of nuclease-treated primer on a large scale so that a uniform primer preparation could be used for all experiments. Characterization of the primer by polyacrylamide gel electrophoresis (Nudel et al., 1976) and 3′-end group analysis (Reyes, 1972) showed that the preparation was homogeneous with an average chain length of 10–12 adenylate residues.

An ATP concentration of 1 mM was determined to be saturating and other ribonucleoside triphosphates were either not or only minimally (<10%) incorporated compared with the rate of AMP incorporation.

Under conditions of saturating primer, the enzyme catalyzed the addition of 2 AMP residues to the primer during the standard 20-min incubation. When the amount of primer added was less than saturating (7.5 vs. 75 μg), the enzyme catalyzed the addition of 6–7 AMP residues to the primer.

Sea urchin poly(A) polymerase has also been similarly characterized by Hyatt (1967a,b) and Slater et al. (1978).

Changes in Poly(A) Polymerase Activity and Subcellular Localization during Development. In order to determine if the total poly(A) polymerase activity measured in vitro correlated with the increase in the rate of poly(A) synthesis observed during embryogenesis, total poly(A) polymerase activity was measured in whole homogenates during different developmental stages. Whole homogenates were chosen as the enzyme source since varying amounts of activity were lost during either aging or partial purification of the enzyme. In addition, mix-match experiments using homogenates and/or subcellular fractions from different developmental stages gave the calculated additive result (for example, see Table II), indicating the absence of measurable development-specific activators or inhibitors. As shown in Table I, total poly(A) polymerase activity measured in vitro did not change significantly during the first 6 h of development. These times were chosen so as to include the time of the greatest increase in net poly(A) accumulation in vivo, as well as times when no net poly(A) synthesis is occurring. These results indicate that the unfertilized egg contains sufficient poly(A) polymerase for early development. Figure 2 shows a more extensive developmental analysis as well as a subcellular fractionation of enzyme activity during development. Although total poly(A) polymerase activity per embryo remained constant during the first 48 h of development, the subcellular localization of the enzyme did change. In the unfertilized egg, approximately 80% of the total enzyme activity was located in the 100000*g* supernatant fraction, while less than 5% was associated with the 1000*g* pellet. This distribution of enzyme remained the same until approximately the hatching blastula stage (19 h), when the enzyme underwent an apparent redistribution between the supernatant and pellet fractions. The loss in activity from the 100000*g* supernatant fraction from the unfertilized egg until the prism stage (48 h) was almost entirely accounted for by the increase in activity associated with the 1000*g* pellet fraction over this time period. Activity associated with each of the other subcellular fractions was at most 12% of the total. In order to ensure that the observed distributions were not due to some peculiarity of the glycerol-containing medium, an experiment was performed in which eggs and embryos were fractionated in 50 mM Tris-HCl, pH 7.5, containing 0.25 M sucrose. This medium gave similar results for the distribution of both total protein and enzyme activity as did the glycerol-containing medium (data not shown). The enzyme activity associated with large particulate material sedimenting at 1000*g* was further characterized by preparation of highly purified nuclei by the method of Hinegardner (1962). This method results in nuclei with minimal cytoplasmic contamination and they have been well characterized (Aronson et al., 1972). When nuclei were prepared from 25-h embryos, at least 50% of the activity associated with the 1000*g* pellet was present in the highly purified nuclear preparation. Whether the other 50% of the activity in the 1000*g* pellet represents enzyme that is either only loosely associated with or released from nuclei during preparation is not known. Rose et al. (1977) have observed that poly(A) polymerase of rat liver nuclei is present in both a bound form localized in the chromatin and an easily extractable form localized in a nuclear sap fraction.

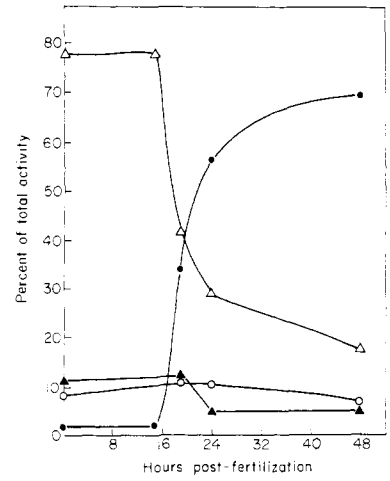

FIGURE 2: Changes in subcellular distribution of poly(A) polymerase activity during development. *S. purpuratus* eggs and embryos were harvested at the indicated times after fertilization and subjected to differential centrifugation, and each of the resulting subcellular fractions was assayed for poly(A) polymerase activity. The results are expressed as percent of the total poly(A) polymerase activity where total activities were equal to 15.1, 19.8, 16.5, 20.5, and 19.2 nmol of AMP incorporated/(min/mL of eggs) for 0 (unfertilized egg), 15 (prehatching blastula), 19 (hatched blastula), 24 (early gastrula), and 48 (early prism) h after fertilization, respectively. (●—●) The 1000*g* pellet, nuclei; (○—○) 12000*g* pellet; (▲—▲) 100000*g* pellet; (△—△) 100000*g* supernatant.

Table II: Effect of Mixing Unfertilized Egg and Gastrula Homogenates on the Distribution of Enzyme Activity[a]

stage	poly(A) polymerase act.[b] (nmol/(min·mL of eggs))		enzyme act. in supernatant (% of total)
unfertilized egg	homogenate	26.2	83.6
	supernatant	21.9	
	pellet	6.4	
gastrula	homogenate	48.6	24.3
	supernatant	11.8	
	pellet	34.1	
unfertilized egg + gastrula	homogenate	37.7	50.1
	supernatant	18.9	
	pellet	18.3	

[a] *Lytechinus pictus* eggs and gastrula stage embryos were homogenized separately in 7 vol of buffer, and then an equal portion of each of the two homogenates was mixed. The three samples were then centrifuged at 12000*g* for 20 min to obtain a 12000*g* supernatant and pellet. The poly(A) polymerase activity of the resulting fractions was then measured as described in the Experimental Procedures. The optimum conditions for *L. pictus* poly(A) polymerase were the same as those for *S. purpuratus*. [b] The differences in enzyme activity seen between the unfertilized egg and gastrula samples are examples of the variation encountered in enzyme activity between different animals. When a single batch of *L. pictus* eggs is used, enzyme activity remains constant during development as it does for *S. purpuratus*.

Adventitious binding of soluble enzyme to nuclei is not thought to be a major source of pellet-associated activity for two reasons: (1) when equal amounts of egg and gastrula homogenates were mixed and then centrifuged to produce a 12000*g* pellet and supernatant, the distribution of enzyme activity was equal to the average of the values for the two developmental stages alone (Table II); (2) the distribution of enzyme activity remains unchanged for the first 15 h of development when the number of nuclei has increased 200-fold (Figure 2).

Recent work on polyadenylation of maternal messenger RNA in vivo has indicated that there is a rapid and extensive turnover of the poly(A) on polysomal maternal messenger RNA (Dolecki et al., 1977; Wilt, 1977). In order to determine if changes in ribonuclease activity could be masking real changes in poly(A) polymerase activity, the stability of [^{3}H]poly(A) was studied at different developmental stages. Incubation conditions were essentially identical with the standard poly(A) polymerase assay system, except that input ATP was unlabeled and [^{3}H]poly(A) was added. At no developmental stage was more than approximately 5% of the [^{3}H]poly(A) rendered trichloroacetic acid soluble during a 20-min incubation period (data not shown). These results indicate that, under the assay conditions used to measure poly(A) polymerase activity, ribonuclease activity directed against poly(A) is minimal and support the conclusion that poly(A) polymerase activity is constant throughout embryogenesis.

Verification of Cytoplasmic Localization of Enzyme Activity in Early Development. It was necessary to determine that the soluble poly(A) polymerase activity seen during early developmental stages was due to an actual cytoplasmic location and not extracted from nuclei during homogenization. Nuclear leakage of poly(A) polymerase has been described for other systems and is dependent upon the method used for tissue fractionation (Rose et al., 1976; Winters & Edmonds, 1973; Tsiapalis et al., 1975). Rose et al. (1976) found that homogenization of rat liver in hypertonic (2.2 M) as opposed to isotonic (0.25 M) sucrose minimized nuclear losses. When isotonic and hypertonic sucrose-containing buffers were used to prepare nuclei and post-nuclear supernatant fractions from eggs and early embryos, no difference in the subcellular distribution of poly(A) polymerase activity was seen (data not shown).

Table III: Subcellular Distribution of [^{3}H]Thymidine, [^{3}H]Guanosine, and Poly(A) Polymerase Activity in Hatching Blastulas[a]

fraction	[^{3}H]-thymidine (%)	[^{3}H]-guanosine (%)	poly(A) polymerase act. total act. (nmol of AMP incorp./(min·mL of eggs))	poly(A) polymerase act. distribution (%)
homogenate	100	100	20.5	100
1000*g* pellet	90.3	90.3	6.9	33.7
5000*g* pellet	0.3	0.3	1.6	7.8
12000*g* pellet	0.0	0.3	1.5	7.3
100000*g* pellet	0.2	0.3	2.8	13.6
100000*g* supernatant	0.7	1.0	7.3	35.6
% recovery	91.5	92.2		98.0

[a] A culture of fertilized *S. purpuratus* eggs was divided into thirds and cultured until the hatching blastula stage (~18-h post-fertilization), when they were then processed for differential centrifugation as described in Experimental Procedures. One-third of the sample was cultured in the presence of 0.2 μCi/mL of [^{3}H]thymidine from fertilization until hatching. Another one-third of the sample was pulse labeled with 4.0 μCi/mL of [^{3}H]-guanosine for 1.5 min just prior to harvesting. Incorporation of [^{3}H]guanosine was terminated by diluting the embryos into an excess of 1.5 M glucose cooled to −10 °C in a NaCl-ice-water bath. Embryos were processed for differential centrifugation as described in Experimental Procedures.

As another test for possible nuclear leakage, a culture of embryos was divided into thirds and each was grown to the hatching blastula stage, where at least 50% of the poly(A) polymerase activity was known to be localized in the 12000*g* supernatant. One-third of the culture was grown in the presence of [^{3}H]thymidine to label DNA and act as a marker for nuclei. Another one-third was pulse labeled with [^{3}H]-guanosine for 1.5 min at the hatching blastula stage and then processed for subcellular fractionation immediately. During the pulse, the guanosine is incorporated into acid-insoluble RNA which is localized in the nucleus, the time of the pulse being too short for transport of the labeled molecules to the cytoplasm (Kedes & Gross, 1969). The last third of the original culture was used to determine the distribution of poly(A) polymerase activity. As shown in Table III, fractionation of the [^{3}H]guanosine-labeled homogenate revealed that greater than 97% of the recovered acid-insoluble radioactivity was associated with the nuclear fraction (as indicated by the sedimentation of [^{3}H]thymidine) and less than 1.5% of the recovered acid-insoluble radioactivity was associated with the cytoplasmic fraction at a time when 50% of the enzyme activity was located in the 12000*g* supernatant. These data suggest that nuclear leakage is minimal.

In order to have a more definitive indication of the cytoplasmic location of the enzyme, unfertilized *S. purpuratus* eggs were divided into nucleated and enucleated merogons. Initial centrifugation of eggs in a density gradient at low speed, followed by centrifugation at an increased relative force, causes the eggs to stratify and separate into an upper, lighter fragment that contains a nucleus and a lower, dense fragment that lacks a nucleus (Harvey, 1956). If the enzyme activity residing in the supernatant fraction of homogenates prepared from eggs were due to nuclear leakage, then the enucleated fragment should be devoid of enzyme activity. As shown in Table IV,

Table IV: Distribution of Poly(A) Polymerase Activity in *S. purpuratus* Eggs[a]

fraction	sp. act. (pmol of AMP incorp./(min·mg of protein))
whole eggs[b]	284
nucleated merogon	350
enucleated merogon	175

[a] Nucleated and enucleated egg merogons were prepared as described in Experimental Procedures. Poly(A) polymerase activity was assayed using the total homogenate prepared from these fractions as the enzyme source. Results reported are for one of three separate fractionation experiments performed. [b] Whole eggs refer to a sample of the original egg preparation not subjected to the centrifugation procedure.

however, a considerable proportion of the poly(A) polymerase activity appeared in the enucleated fragment as well as in the nucleated fragment. These experiments do not allow precise quantitation of the amount of activity present in nuclei and cytoplasm. Since enucleated merogons contain more yolk than lighter nucleated merogons, comparison of enzyme specific activity may be misleading. These results and those of Slater et al. (1978) leave no doubt that much of the enzyme activity in the egg has a cytoplasmic localization.

Inhibitor Studies. In order to further characterize the polyadenylation reaction and to eliminate the possibility that AMP incorporation was due to contributions from other enzyme activities, the sensitivity of the in vitro reaction to various inhibitors was monitored (Table V). The failure of both actinomycin D and α-amanitin, used at concentrations sufficient to inhibit RNA polymerases II and III in sea urchins (Morris & Rutter, 1976), to suppress the polyadenylation reaction of either the egg or prism-stage enzyme preparation suggests that RNA polymerase is not responsible for the AMP incorporation observed. In addition, the inclusion of all four ribonucleoside triphosphates to the assay buffer resulted in an inhibition of enzyme activity, rather than a stimulation, as would be expected if RNA polymerase were contributing to the incorporation. The reaction was strongly inhibited by pyrophosphate and the addition of phosphate to the assay buffer increased homogenate incorporation of AMP at all stages of development (data not shown). These observations suggest the absence of polynucleotide phosphorylase activity. Deoxy-ATP did not inhibit AMP incorporation suggesting the absence of terminal deoxyribonucleotidyl transferase activity. Cordycepin did not suppress in vitro polyadenylation, a result that has been observed by others (Haff & Keller, 1975; Winters & Edmonds, 1973). Incorporation of labeled AMP was inhibited by each of the other nucleoside triphosphates when they were present in the assay buffer at an equimolar concentration to ATP. Rose & Jacob (1976) recently reported that in vitro polyadenylation, using an enzyme preparation from rat liver nuclei, was inhibited by polyamines, particularly spermine and spermidine. When used at concentrations which were sufficient to produce almost total inhibition of the rat liver enzyme, spermidine produced an approximate twofold increase in incorporation of AMP with both sea urchin enzyme preparations.

Table V: Effect of Various Inhibitors on Poly(A) Polymerase Activity

addition	concn	poly(A) polymerase act.[a] (% of control) unfertilized egg	prism stage
actinomycin D	25 μg/mL	99.1	106
α-amanitin	1 μg/mL	100	95.0
	10 μg/mL	103	102
	100 μg/mL	102	97.7
cordycepin	1 mM	101	98.0
	2 mM	103	91.0
sodium	0.1 mM	80.2	
pyrophosphate	1 mM	22.2	13.6
	10 mM	11.2	3.7
UTP	1 mM	88.8	97.0
GTP	1 mM	58.6	62.3
CTP	1 mM	80.3	86.2
UTP, GTP, CTP	1 mM	82.5	83.3
dATP	1 mM	94.5	95.6
spermidine	1 mM	143	128
	10 mM	200	191

[a] Poly(A) polymerase activity was measured in a 12000*g* supernatant prepared from unfertilized eggs and a resuspended 12000*g* pellet prepared from prism-stage embryos. Activity is expressed as the percent of the total activity as assayed for both preparations in the absence of any additions. Total activity (100%) was 312 and 261 pmol of AMP incorporated/(min·mg of protein) for the egg and prism preparations, respectively. Results reported are the mean of two separate experiments.

Discussion

Sea urchin poly(A) polymerase activity was first described by Hyatt (1967a,b) who studied the reaction characteristics in isolated nuclei but did not quantitate enzyme activity with respect to development. With the exception of having rather specific primer requirements, the enzyme described here appears to be similar to that reported by Hyatt (1967a,b) and to most of the other poly(A) polymerases characterized in other organisms (Edmonds & Winters, 1976).

Poly(A) polymerase is present in unfertilized *S. purpuratus* eggs and total enzyme activity per embryo remains constant from the unfertilized egg until the prism stage. In addition, poly(A) polymerase activity, as measured in vitro, is sufficient to account for the approximate twofold net increase in poly(A) which occurs during the first 2 h after fertilization. During this time there is a net synthesis of approximately 1.8 μg of poly(A) per mL of embryos (Wilt, 1973) and, as calculated from the in vitro enzyme assays, poly(A) polymerase can catalyze the addition of 5.2–10.4 μg of AMP/(min·mL of embryos). This is probably sufficient enzymatic capacity to account for both the net poly(A) synthesis as well as poly(A) turnover occurring during this time. Since the unfertilized egg appears to have sufficient poly(A) polymerase for early development, regulation of enzyme activity in vivo must be effected by substrate and/or primer availability or the presence of activators or inhibitors rather than increases in the number of polymerase molecules. The fact that poly(A) polymerase activity can be increased up to 20-fold in vitro by the addition of exogenous primer suggests that the enzyme is not saturated with primer in vivo. If there are endogenous activators or inhibitors of poly(A) polymerase activity present in the egg or embryo, it is possible that homogenization and preparation of samples for assay disrupt their association with the polymerase or dilute their concentration to an ineffectual level, since mix-match experiments with enzyme preparations from different developmental stages failed to detect their presence.

The finding of a constant level of poly(A) polymerase activity throughout sea urchin development is in agreement with the work of Morris & Rutter (1976) but contrasts with the recent results of Slater et al. (1978). The latter authors described a cytoplasmic Mn^{2+}-requiring poly(A) polymerase activity having similar substrate and primer preferences and antimetabolite resistance to the enzyme activity reported here. Slater et al. (1978) compared enzyme activity in eggs and four-cell embryos and found that at the four-cell stage poly(A) polymerase activity, as assayed either in the presence or

absence of exogenous primer, was about 50% of the level found in unfertilized eggs; other developmental stages were not examined. Although not presented in this paper, we found that poly(A) polymerase activity assayed without the addition of exogenous primer was also constant throughout the developmental stages examined. The general level of enzyme activity in the unfertilized egg (both nmol of AMP incorporated per mg of protein and per mL of eggs) found by Morris & Rutter (1976), Slater et al. (1978), and that reported in the present study are all similar. Since the addition of emetine, a protein synthesis inhibitor, to cultures of embryos prevented the decrease in poly(A) polymerase activity assayed in vitro, Slater et al. (1978) suggested that polymerase activity may be subject to negative translational control. In any given batch of eggs or embryos from either *L. pictus* or *S. purpuratus*, we have never encountered changes in activity greater than variability between replicate samples (±15%) and we are unable to explain the differences between the present findings and those of Morris & Rutter (1976) with the results of Slater et al. (1978).

In contrast to the constancy of total poly(A) polymerase activity during development, the intracellular location of the enzyme changes. Initially, the enzyme is located in the 100000*g* supernatant fraction; however, beginning at approximately the hatching blastula stage, the enzyme becomes progressively associated with the nucleus. The mixing experiments argue strongly against adventitious binding of enzyme activity to nuclei and the experiments on purified nuclei showed that half of the activity is retained after extensive purification. Furthermore, approximately 25% of the activity associated with the 1000*g* pellet could not be extracted by high salt, though it was released by treatment with 0.25% Triton X-100.

The studies reported here were also performed on eggs and embryos from the sea urchin, *Lytechinus pictus*, and similar results were obtained. The only major difference between the two species appears to be the timing of the change in enzyme localization. In *L. pictus* enzyme activity becomes associated with the nucleus as early as the eight-cell stage, and by the hatching blastula stage the majority of enzyme activity is associated with this fraction (unpublished observations).

Experiments on the regulation of DNA (Fansler & Loeb, 1969) and RNA (Maroun, 1973; Morris & Rutter, 1976) polymerase activity during sea urchin development have also revealed that the total activity of these polymerases does not change with development, while the subcellular localization does. Loeb & Fansler (1970) have shown that the observed change in the localization of *S. purpuratus* DNA polymerase activity reflects a physical migration to the nucleus of the polymerase molecules that were synthesized during oogenesis and stored in the cytoplasm of the egg. It is interesting that, if the movement of poly(A) polymerase activity also reflects migration of enzyme formed during oogenesis, then the preformed poly(A) and DNA polymerases partition into the nucleus on different time schedules.

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