Haschemeyer, A. E. V., and Rich, A. (1967), *J. Mol. Biol. 27*, 369.
Kirtley, M. E., and Koshland Jr., D. E. (1967), *J. Biol. Chem. 242*, 4192.
Momparler, R. L., and Fisher, G. A. (1968), *J. Biol. Chem. 243*, 4298.
Rossi, M., Geraci, G., and Scarano, E. (1967), *Biochemistry 6*, 3640.
Rossi, M., and Scarano, E. (1969), *4th Meeting Federation European Biochem. Soc., Madrid.*
Scarano, E., Geraci, G., and Rossi, M. (1967a), *Biochemistry 6*, 192.
Scarano, E., Geraci, G., and Rossi, M. (1967b), *Biochemistry 6*, 3645.
Smith, M., and Khorana, H. G. (1958), *J. Amer. Chem. Soc. 80*, 1142.

Multiple Ribonucleic Acid Polymerases and Ribonucleic Acid Synthesis during Sea Urchin Development*

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ABSTRACT: Ribonucleic acid (RNA) synthesis during early sea urchin development has been examined at three levels of biological organization. The multiple RNA polymerases which are present in isolated nuclei have been solubilized and separated by chromatographic procedures at various stages of development. The total level of polymerase I, presumed to be associated with ribosomal RNA synthesis, increases in proportion to cell division so that the level per cell is nearly constant. In contrast, the levels of polymerases II and III per embryo increase only slightly; thus, the levels per cell actually decline severalfold. Polymerases II and III account for the bulk of the activity and at least one of these (polymerase II) is believed to be associated with the synthesis of deoxyribonucleic acid like (DNA-like) RNA. The decline in the level of total polymerase per cell parallels the decline in the apparent rate of RNA synthesis in the intact embryo. Optimal conditions have been established for the measurement of template-bound RNA polymerase activity in isolated nuclei. Under all of the metal ion and ionic strength conditions tested and at all stages of development examined, the RNA synthesized is DNA like as determined by partial base ratio and nearest-neighbor frequency analyses; and in this respect, the RNA resembles that synthesized *in vivo* during short labeling periods. The low ionic strength (intact nuclei) activity declines severalfold from the early blastula to the late gastrula stages on a per nucleus basis, in general agreement with the observations on the rates of RNA synthesis per cell in intact embryos. Rates of RNA synthesis *in vivo* were calculated after determining the uridine triphosphate specific activity and the incorporation of radioactivity into RNA during short labeling periods with [^{3}H]uridine. The apparent rate per cell declines about two- to threefold from the early blastula to the late gastrula stages. These observations suggest the involvement of the multiple RNA polymerases in the regulation of ribonucleic acid synthesis. Additional indirect observations suggest that other factors may be involved in the control of the activity of these enzymes.

The patterns of RNA synthesis during sea urchin development have been qualitatively described. The synthesis of DNA-like RNA[1] begins very early in development (Nemer and Infante, 1965) and is apparent at all stages examined thereafter (Glisin and Glisin, 1964; Gross *et al.*, 1964; Emerson and Humphreys, 1970). In contrast, earlier studies (Nemer, 1963; Comb *et al.*, 1965; Giudice and Mutolo, 1967) suggested that ribosomal RNA synthesis is not initiated until gastrulation. Recently, however, Emerson and Humphreys (1970) have demonstrated ribosomal RNA synthesis at the blastula stage and have, furthermore, suggested that the rate of accumulation of rRNA per cell might be constant from the blastula to the postgastrula stages, while the rate of accumulation of DNA-like RNA decreases severalfold.

It is apparent that there are changes in the relative rates of synthesis and accumulation of the major classes of RNA during development, as well as changes in the populations of DNA-like RNA molecules formed during this interval (Whiteley *et al.*, 1966; Glisin *et al.*, 1966). Elucidation of the mechanisms regulating the synthesis of the various species of RNA remains a formidable problem. Studies on the chromatin found in the cells of higher organisms suggest

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[1] Abbreviations used are: DNA-like RNA, RNA having a base composition similar to that of DNA; TGMED buffer is 0.05 M Tris-HCl (pH 7.9), 25% (v/v) glycerol–5 mM $MgCl_2$–0.1 mM EDTA–0.5 mM dithiothreitol.

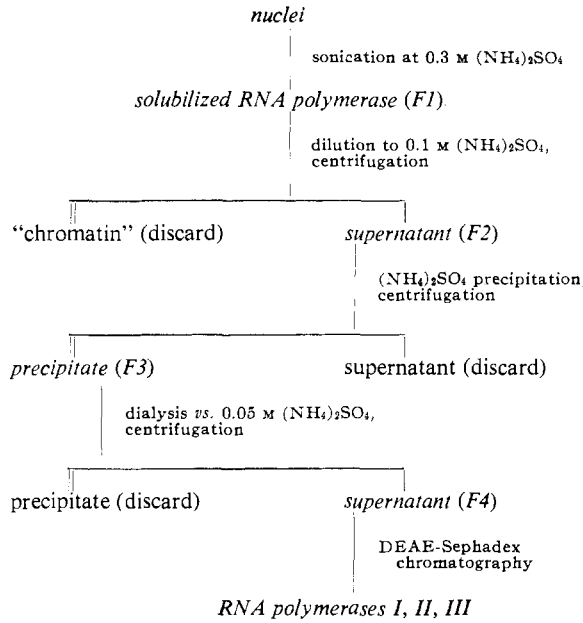

FIGURE 1: Scheme for the purification and analysis of RNA polymerase in isolated nuclei. The fractions containing the activity are in italics. Details of the procedure are given in Methods.

that modifications of this nucleoprotein complex might control RNA synthesis (Bonner *et al.*, 1968; Huang and Huang, 1969; Bekhor *et al.*, 1969; Paul and Gilmour, 1968). However, it is also possible that the DNA-dependent RNA polymerase might be involved in a regulatory capacity.

We have recently discovered multiple forms of RNA polymerase in the nuclei of sea urchins and rat liver (Roeder and Rutter, 1969b). These enzymes have different molecular and catalytic properties. Their apparent specific localizations within the nucleolus and nucleoplasm (Roeder and Rutter, 1970) suggest that the enzymes may have specific functions; for example, in the synthesis of large classes of RNA.

The aim of the present studies is a quantitative description of RNA synthesis during the early period of sea urchin development at various levels of biological organization. The apparent rates of RNA synthesis have been estimated in the cells of the intact embryo and in isolated nuclei under conditions where the enzymes remain associated with the endogenous templates; in addition, the levels of the solubilized nuclear RNA polymerases have been determined at various stages of sea urchin development. These sets of data have then been correlated with each other and with the patterns of RNA synthesis described earlier.

Methods

Culture Conditions. Eggs and sperm of the sea urchin, *Strongylocentrotus purpuratus*, were obtained by injection of 0.55 M KCl into the coelomic cavity. After filtering through No. 155 Nitex nylon cloth, the eggs were washed two times by settling from 50 volumes of artificial sea water (Tyler, 1953). Settled eggs were resuspended in 50 volumes of artificial sea water, and fertilization was effected by the addition of undiluted sperm to a final concentration of 0.001%. The cells were then washed twice, as above, to remove excess sperm. Embryos were cultured at concentrations of about 10,000/ml, in the presence of 0.25 mg/ml of streptomycin sulfate at 11–13° with stirring. Only cultures with greater than 97% fertilization and normal morphological development were used. Embryos were harvested at the appropriate stage by either allowing them to settle or by mild centrifugation. With embryos prior to the hatching stages, the fertilization membranes were removed prior to harvesting. This was accomplished by forcing the embryo suspensions (at concentrations of 10,000/ml or less) through a large syringe fitted with a Swinney adapter (Millipore Corp.) containing two layers of No. 69 or 64 (depending on the egg size) Nitex nylon cloth (Tobler, Ernst and Trabler, Inc., New York) over a stainless steel grid. With the appropriate nylon mesh size, greater than 90% of the membranes were removed by a single passage with little visible damage to the embryos.

Isolation of Nuclei. Modifications of the procedures of Hinegardner (1962), Loeb *et al.* (1967), and Blobel and Potter (1966) were used. All operations were carried out at 0–2°. Embryos were washed once in 5 volumes of artificial sea water containing 0.25 mg/ml of streptomycin, and then washed 3–4 times in 5 volumes of 0.55 M KCl by resuspension and centrifugation for 3 min at 500*g*. The pelleted embryos were resuspended in 5 volumes of 0.05 M Tris-HCl (pH 7.6), 1 mM EDTA, and 0.25 M sucrose and quickly centrifuged for 1 min at 1000*g*. The pellet was rapidly resuspended in 3 volumes of the same buffer and homogenized (usually about 10 strokes) with a motor-driven glass–Teflon homogenizer. The homogenate was mixed with 2 volumes of 2.3 M sucrose, 0.01 M Tris-HCl (pH 7.6), 0.025 M KCl, and 0.010 M $MgCl_2$. Aliquots of 50 ml were placed in centrifuge tubes and each underlaid with 10 ml of 1.9 M sucrose, 0.01 M Tris-HCl (pH 7.6), 0.025 M KCl, and 0.005 M $MgCl_2$. After centrifugation at 25,000 rpm (Spinco 25.2 rotor) for 45 min, the supernatants were discarded; and the nuclear pellets were resuspended in 0.01 M Tris-HCl (pH 7.9), 5 mM $MgCl_2$, and 1.0 M sucrose to a final DNA concentration of about 2 mg/ml. For studies in which the nuclei were assayed for activity without prior solubilization of the enzyme (*in situ* assays), the suspensions were used immediately. For the studies in which the enzyme activity was first solubilized, the suspensions of nuclei were made 5 mM in dithiothreitol and were quick-frozen and stored at −90° until use.

RNA Polymerase Solubilization and Initial Fractionation. Suspensions of nuclei (stored at −90°) were thawed and the enzyme activity solubilized and partially purified as described previously (Roeder and Rutter, 1970). A schematic representation of the isolation procedure is shown in Figure 1. Aliquots of the fractions (F1-F4) which were to be assayed for activity were adjusted to an ammonium sulfate concentration of 0.09–0.10 M by dilution with TGMED buffer. Protein was determined according to the method of Lowry *et al.* (1951) and DNA according to the method of Burton (1956).

Chromatographic Resolution of Multiple Polymerases. Columns (0.8 × 12 cm) of DEAE-Sephadex (A-25) (Pharmacia) were equilibrated with TGMED buffer containing 0.05 M ammonium sulfate. After application of the sample the columns were washed with TGMED buffer containing 0.05 M ammonium sulfate. The enzyme activities were subsequently eluted with linear gradients (45 ml) of ammonium sulfate (0.05–0.5 M) in TGMED buffer. Fractions of 1.2 ml

were collected in tubes containing 0.05 ml of 20 mg/ml of crystalline bovine serum albumin. Aliquots were assayed immediately for enzyme activity.

Assay for RNA Polymerase Activity in Solubilized RNA Polymerase Preparations. The standard reaction mixture contained in a final volume of 125 μl: 7 μmoles of Tris-HCl (pH 7.9), 0.75 μmole of NaF, 0.5 μmole of phosphoenolpyruvate, 2.5 μg of pyruvate kinase (desalted), 0.2 μmole of 2-mercaptoethanol, 0.075 μmole each of GTP, CTP, ATP, 0.0125 μmole of unlabeled UTP, 1 μCi of [^{3}H]UTP (13.8 Ci/mmole), 30–40 μg of native calf thymus DNA, 1 μmole of KCl, and 50 μl of the enzyme preparation in TGMED buffer. The final concentrations of Mn^{2+}, Mg^{2+}, and ammonium sulfate present for each experiment are given in the appropriate table or figure legend. Each reaction was initiated by adding the solubilized enzyme preparation (at 0°) to the other components (at 0°) and transferring to a 30° bath. After incubation for 10 min, reactions were terminated and acid-insoluble radioactivity determined by the glass-fiber filter method as previously described (Roeder and Rutter, 1970).

Assay for RNA Polymerase Activity in Situ in Nuclear Preparations. The components present in the reaction mixtures (125 μl) were as described for the solubilized polymerase assays (above) except that the calf thymus DNA and KCl were omitted. The metal ion (Mn^{2+} and Mg^{2+}) and ammonium sulfate concentrations in each experiment are indicated in the appropriate figure and table legends. Reactions were initiated as above. Incubation, unless otherwise noted, was carried out for 10 min at 15°. Reactions were terminated by the addition of 0.1 ml of cold 0.1 M sodium pyrophosphate (adjusted to pH 7 with HCl) containing 4 mg/ml of RNA, 2 mg/ml of bovine serum albumin, followed by 2 ml of cold 10% trichloroacetic acid (w/v) in 95% ethanol. The suspensions were sonicated briefly (2–3 sec) with a Branson S-125 Sonifier (microtip attachment, power setting No. 1) and centrifuged for 5 min at 1000*g*. The pellets were then washed 4 times (by resuspension and centrifugation) with cold 5% trichloroacetic acid containing 0.04 M sodium pyrophosphate. The final pellet was hydrolyzed with 0.5 ml of 5% trichloroacetic acid at 95° for 15 min. The hydrolysate was dispersed in 15 ml of a dioxane-based scintillation solution containing 5 g of Omnifluor (New England Nuclear) and 100 g of naphthalene per l. of solution and counted in a Packard Tri-Carb scintillation counter. Efficiencies (generally about 20%) were determined by the external standard method.

Determination of ^{32}P Distribution among the 2′(3′)-Mononucleotides of Alkaline Hydrolysates of RNA. [^{3}H]UTP in the reaction mixtures was replaced by [α-^{32}P]UTP (1 μCi). Nuclei were incubated and subjected to the cold trichloroacetic acid washes as described above (*in situ* assays). The pellets were further washed twice with cold 10% KOAc in 95% ethanol. The dried pellets were hydrolyzed in 0.2 ml of 0.3 N KOH at 37° for 22 hr. The distribution of radioactivity in the 2′(3′)-mononucleotides was then determined according to the procedure described by Sebring and Salzman (1964).

Labeling of Embryos in Vivo with [^{3}H]Uridine. Embryos at the appropriate stage were lightly centrifuged and resuspended to concentrations of about 10^5 embryos/ml in artificial sea water containing 0.25 mg/ml of streptomycin. Embryos were maintained in suspension by gently bubbling air into the culture. [^{3}H]Uridine was added to a final concentration of 1–5 μCi/ml. The total uridine concentration was adjusted to the level indicated for each experiment by the addition of unlabeled uridine. Aliquots (usually about 3×10^6 embryos) were removed at periodic intervals and centrifuged quickly. The supernatants were removed by aspiration and the pellets frozen immediately in a Dry Ice–acetone bath and kept at −20° for subsequent analysis.

Determination of UTP Specific Activity and Radioactivity in RNA following in Vivo Labeling. Each frozen pellet was thawed and homogenized extensively (at 0°) after adding 5 ml of 0.6 N perchloric acid. Aliquots were then removed for determinations of DNA and radioactivity incorporated into RNA (see below). After centrifugation of the remainder of the homogenate for 5 min at 10,000*g*, the supernatant was removed and the pellet was reextracted with 2 ml of 0.2 M perchloric acid. After centrifugation, the supernatants were combined and filtered through a Millipore filter (HA, 0.45 μ pore size) using positive pressure. The filtrate was neutralized with 6 N KOH. After 1 hr at 0°, the $KClO_4$ was removed by centrifugation (5 min, 10,000*g*). Each supernatant was passed through a 1×40 cm column of Sephadex G-50 (medium, bead form) equilibrated with 0.01 M Tris-HCl (pH 7.0). Fractions (1 ml) were collected and 10-μl aliquots counted in 10 ml of Kinard (1957) scintillation fluid. This procedure effectively separates the high molecular weight cloudy material from the radioactive nucleotides. Samples from the latter 80% of the radioactivity peak were combined and concentrated to about 4–5 ml by ultrafiltration with a UM-2 membrane (Amicon Corp.). The sample was then concentrated to about 1 ml by flash evaporation. Aliquots were then subjected to thin-layer chromatography on polyethyleneimine–cellulose according to the method (procedure 3) described by Randerath and Randerath (1967). The separated ribonucleoside triphosphates were eluted from the spots and an aliquot of each counted in 10 ml of Kinard scintillation fluid; another aliquot was used for optical density measurements.

For the measurement of labeled RNA, aliquots of the original homogenate were diluted to 2 ml with cold 5% trichloroacetic acid after adding 0.1 mg of RNA. After centrifugation (10 min, 1000*g*) the pellets were washed 4 times in cold 5% trichloroacetic acid and twice in 10% KOAc in 95% ethanol. The pellets were hydrolyzed in 0.3 ml of 0.3 N KOH for 24 hr at 37°. The hydrolysates were cooled, neutralized with concentrated perchloric acid, and $KClO_4$ was removed by centrifugation. Each supernatant was counted in 10 ml of Kinard scintillation fluid.

Chemicals. Chemicals and biochemicals were obtained from the following vendors: unlabeled ribonucleoside triphosphates, P-L Biochemicals; [α-^{32}P]- and [^{3}H]Ribonucleoside triphosphates (1–10 Ci/mmole), Schwarz BioResearch; [^{3}H]uridine (15.1 Ci/mmole), New England Nuclear; phosphoenolpyruvate (A grade, trisodium), calf thymus DNA (Type I), Sigma Chemical Co.; pyruvate kinase (A grade, 150 eu/mg), Worthington Biochemical Co.; polyethyleneimine, K & K Laboratories; DEAE-Sephadex (A-25), Pharmacia.

When necessary, pyruvate kinase was desalted by centrifugation of the ammonium sulfate suspension, resuspension of the precipitate in 0.05 M Tris-HCl (pH 7.9), and passage through a Sephadex G-25 column.

TABLE I: Yields of Soluble RNA Polymerase Activity from Embryonic Sea Urchin Nuclei.

Experiment[a]	Fraction[b]	Total Units Activity[c]		Per Cent Activity		Specific Activity[d] (Units/μg of Protein)
		Mn^{2+}	Mg^{2+}	Mn^{2+}	Mg^{2+}	
A (16 hr)	F1	32,400	9,600	100	100	0.35
	F2	27,000	10,200	83	106	0.50
	F3	33,900		104		0.85
	F4	34,100	9,130	105	95	1.00
B (27 hr)	F1	40,800	26,200	100	100	0.31
	F2	36,300	25,300	89	96	0.41
	F3	53,800		131		0.74
	F4	48,300	28,500	118	109	0.99
C (34 hr)	F1	39,800	22,600	100	100	0.17
	F2	39,300	22,800	99	101	0.20
	F3	55,500		139		0.42
	F4	45,600	27,800	115	122	0.58
D (52 hr)	F1	24,000	16,100	100	100	0.21
	F2	20,000	15,300	83	95	0.37
	F3	32,600		136		0.54
	F4	29,000	18,700	121	116	0.80

[a] The stages of development at the various time points are as follows: A (16 hr) 150 cells (average); B (27 hr) swimming blastula; C (34 hr) mesenchyme blastula; D (52 hr) postgastrula (small triradiate spicules present). The solubilization was performed on quantities of nuclei containing 8.0, 16.2, 18.3, and 15.0 mg of DNA, respectively, in experiments A, B, C, and D. [b] Fractions are those shown in Figure 1. [c] Mn^{2+} activity was determined in the presence of 1.6 mM Mn^{2+}, 2 mM Mg^{2+}, and 0.06–0.07 M ammonium sulfate; Mg^{2+} activity was determined at 6 mM Mg^{2+}–0.04 M ammonium sulfate. One unit of activity represents the incorporation of 1 pmole of UMP/10 min. [d] Based upon the Mn^{2+} activity.

Results

Solubilized RNA Polymerase Activity in Sea Urchin Nuclei. SOLUBILIZATION AND PARTIAL PURIFICATION OF THE RNA POLYMERASE ACTIVITY PRESENT IN EMBRYONIC NUCLEI. The procedure utilized for the solubilization and partial purification of the nuclear RNA polymerases is shown schematically in Figure 1. The details of this procedure can be found in Roeder and Rutter (1970). Although the specific activity of fraction 4 is only 3–4 times greater than that of fraction 1 (Table I), fraction 4 contains nearly all of the initial activity and less than 1% of the initial DNA. This is crucial for the subsequent purification and analytical procedures.

Some of the catalytic properties of the multiple sea urchin RNA polymerases have previously been reported (Roeder and Rutter, 1969b). Figure 2 shows the effects of temperature upon the multiple polymerases. The reactions at 30° show an initial burst of activity which has nearly plateaued by 20 min. The reactions at 15° proceed at much lower initial rates but continue for longer periods of time. The apparent thermal inactivation of the enzymes at 30° may be not totally unexpected since the organisms normally live at low (8–10°) temperatures. As a matter of convenience, assays of the solubilized polymerases have routinely been performed at 30° for 10 min since the UMP incorporation is linear for at least this time period.

LEVELS OF THE MULTIPLE RNA POLYMERASES IN EMBRYONIC NUCLEI. In order to make quantitative estimates of the relative amounts of the multiple enzymes present at various stages of development, it is necessary to show that the recoveries of the total activity are nearly quantitative at all stages examined. The results of four experiments (with nuclei from different stages) are presented in Table I. Activity is monitored under two sets of ionic conditions. In the presence of Mn^{2+} at 0.065 M ammonium sulfate, purified polymerases I, II, and III show, respectively, approximately 70, 80, and 95% maximal activity. In the presence of Mg^{2+} at 0.04 M ammonium sulfate, polymerase I is maximally active while polymerase II (which has an extreme Mn^{2+} dependence) and polymerase III would be expected to show less than 10 and 50%, respectively, of their maximal activity. These estimates are based on the catalytic properties of the purified enzymes (Roeder and Rutter, 1969b), but it is, of course, possible that the ionic requirements of the enzymes in the crude fractions are not the same as those of the purified enzymes. Nevertheless, the yields of activity are quite comparable, regardless of the assay conditions, and suggest that no selective losses have occurred.

To assess the relative proportions of the multiple enzymes in these soluble preparations, aliquots were subjected to DEAE-Sephadex chromatography as shown in Figure 3. Although the total activity in the crude fractions is quite stable, the purified enzymes eluted from DEAE-Sephadex are rather unstable (especially I and III). For this reason fractions were collected in the presence of bovine serum albumin and assayed immediately after elution. When this precaution is taken, determination of the relative distribution of the enzymes is quite reproducible. The apparent hetero-

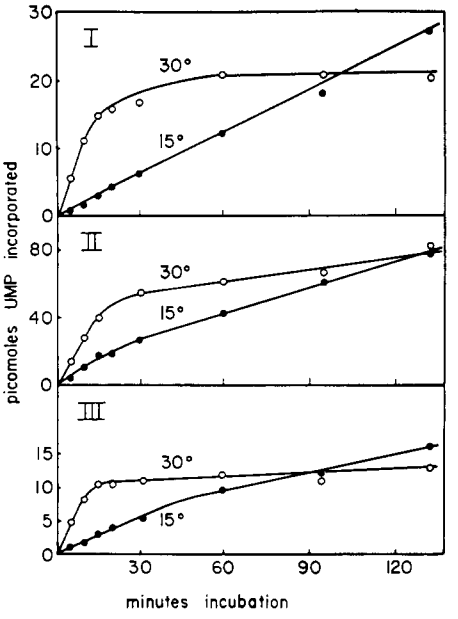

FIGURE 2: Effect of temperature upon the activity of the purified sea urchin RNA polymerases. Standard assay conditions (Methods) were employed except that the reaction volumes were increased to 250 μl, and the radioactive UTP content was elevated to 20 μCi per reaction. The total concentration of UTP was kept at 0.10 mM. The assay concentrations of ammonium sulfate were 0.04, 0.064, and 0.074 M for polymerases I, II, and III, respectively. The reaction mixtures for I, II, and III contained 75, 37, and 42 μg of DEAE-Sephadex purified enzyme protein, respectively; and, in addition, each contained 100 μg of bovine serum albumin. At periodic time intervals, 20-μl aliquots were removed from each and the trichloroacetic acid insoluble radioactivity determined as in Methods. The incorporation represents UMP incorporation per 20-μl aliquot. Values shown are the average of duplicate determinations: (—●—●—) 15°; (—○—○—) 30°.

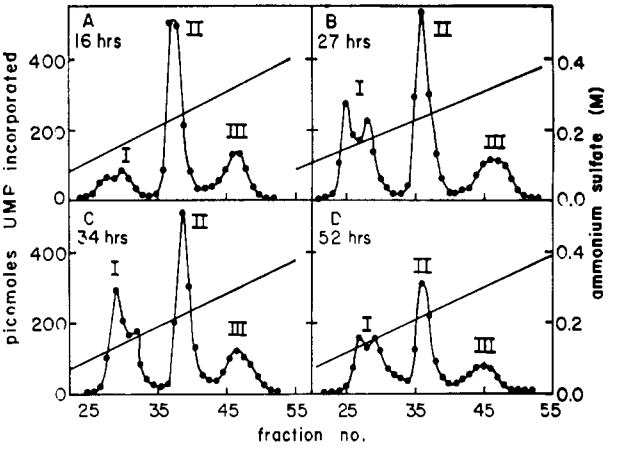

FIGURE 3: DEAE-Sephadex chromatography of solubilized RNA polymerase preparations from sea urchin nuclei at different stages of development. Samples of soluble, partially purified RNA polymerase preparations were chromatographed as described in Methods. The preparations used were those shown in Table I (fraction 4 in each case). For A, B, C, and D the input samples contained respectively, 8100, 10,200, 9180, and 6090 units of activity, measured at 0.1 mM UTP, 1.6 mM Mn^{2+}, 2 mM Mg^{2+}, and 0.06 M ammonium sulfate. Fractions of 1.1 ml were collected in tubes containing 0.05 ml of 20 mg/ml of crystalline bovine serum albumin. Aliquots of 50 μl were assayed for activity in the presence of 0.01 mM UTP, 1.6 mM Mn^{2+}, and 2 mM Mg^{2+} at the ammonium sulfate concentration resulting from dilution of this volume to the final assay volume of 125 μl. This resulted in assay concentrations of salt which were nearly optimal for peak II and peak III fractions, but which were slightly suboptimal for peak I. The activities shown represent total picomoles incorporated per fraction in 10 min. When the peak tubes from each experiment were reassayed at 0.091 mM UTP, the absolute activities were found to be 2.3, 3.8, and 2.3 times higher, respectively, for peaks I, II, and III. For a given peak, the ratio of activity at the two UTP concentrations was nearly constant for all stages of development examined: (—●—●—) activity; (——) ammonium sulfate molarity.

geneity in peak I, which was reported earlier (Figure 1a in Roeder and Rutter, 1969b), is even more obvious in Figure 3. Although these experiments were performed with frozen enzyme preparations, the splitting of peak I was also observed when freezing was not employed at any time. Whether this splitting represents true heterogeneity or a modification(s) in polymerase I which occurs during the isolation is not clear.

Table II summarizes the data from Table I and from Figure 3. The relative proportions of the polymerases were calculated from the DEAE elution profiles as described in the legend. At the three later stages of development, the relative proportions of the polymerases remain essentially constant. At 16-hr development, however, the proportions are significantly different from the later stage values, with polymerase II representing a higher and polymerase I a lower percentage of total activity.

The total activity present in fraction I at the various stages of development has been normalized on a DNA basis (Table II). These data demonstrate that the total activity per cell, measured in the presence of Mn^{2+} at an ammonium sulfate concentration (0.065 M) where all polymerases show most of their activity, decreases significantly during development. The levels of activity of the polymerases (on a DNA basis) were calculated from the total activity present in the crude fraction and the relative proportions determined by DEAE chromatography. The cellular levels of polymerase II and polymerase III decrease with time of development; the largest decline occurs between the 16-hr (early blastula) and the 27-hr (hatching blastula) stages. In contrast, the cellular level of polymerase I shows a much smaller change (no more than 50%) throughout the stages examined.

For a comparison of the net increases of the RNA polymerase activities, the levels per embryo have been calculated. These data are shown in the last three columns of Table II (see the legend for the calculation). While the levels of polymerases II and III increase only about 50–60%, the level of polymerase I increases much more dramatically (about 5 times).

Template-Bound RNA Polymerase Activity in Isolated Nuclei. CONDITIONS FOR THE MEASUREMENT OF RNA POLYMERASE ACTIVITY. The effects of temperature on the RNA polymerase activity displayed by isolated nuclei are shown in Figure 4 at two different concentrations of ammonium sulfate. At low ionic strength, where the nuclei remain intact during the incubation, the activity at 30° has nearly plateaued by 10 min, whereas at 10 and 15° the activities are linear for at least 20 min. The activity curves at 0.3 M ammonium sulfate, where the nuclei are lysed and form a viscous gel, are all qualitatively similar at 10, 15, and 30° with the activity being progressively higher with increased temperatures.

TABLE II: Levels and Relative Proportions of Sea Urchin RNA Polymerases I, II, and III During Development.

Experiment[a]	Cells per Embryo[b]	Activity Distribution: Relative Proportions on DEAE[c] I	II	III	Units/mg of DNA[d] Total	I	II	III	Units/10^6 Embryos[e] I	II	III
A (16 hr)	150	0.12	0.70	0.18	4050	490	2830	728	113	654	168
B (27 hr)	350	0.29	0.55	0.16	2025	730	1390	403	393	748	217
C (34 hr)	450	0.29	0.55	0.16	2170	630	1190	347	436	824	239
D (52 hr)	600	0.31	0.53	0.16	1600	495	847	254	534	913	263

[a] Experiments A–D are the same as those described in Table I and Figure 3. [b] Estimated from the data of Hinegardner (1967) except for expt A where direct cell counts were made. [c] The data are taken from Figure 3. The total activity in each peak, measured at 0.01 mM UTP, was converted into activity at 0.09 mM UTP by using the conversion factors given in the legend to Figure 3. The relative proportions of polymerases I, II, and III at each stage examined were then calculated from these data. [d] Total activity represents that present in fraction 1 as shown in Table I (assayed in the presence of Mn^{2+} at 0.065 M ammonium sulfate). The data for I, II, and III were calculated by multiplying the total activity (column 6) by the relative proportion of each enzyme determined by DEAE analysis (columns 3, 4, and 5). [e] Calculated from the estimated cell number per embryo (column 2) and the activity levels in columns 7, 8, and 9. A value of 1.54 $\mu\mu$g was used for the diploid cell DNA content (Tyler and Tyler, 1966).

The early loss of activity seen at 30° is not due to degradation of labeled RNA, as shown by pulse-chase experiments (unpublished experiments) and, therefore, probably represents inactivation of some component in the enzyme–template complex or the loss of other contributory factor(s). Because of the near linearity of all reactions at 15°, this temperature has routinely been used for polymerase assays in nuclei.

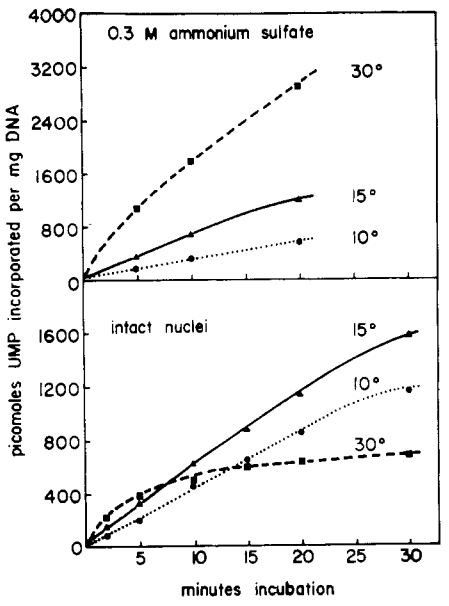

FIGURE 4: Effect of temperature on the RNA polymerase activity in embryonic sea urchin nuclei. Nuclei were from 32-hr (mesenchyme blastula) embryos. The nuclei present in each reaction contained 0.110 mg of DNA and in all cases the metal ion concentration was 6 mM Mg^{2+}. The concentration of ammonium sulfate present for the assay of intact nuclei was 0.005 M. Each value represents the average of duplicate determinations: (··●···●··) 10°; (—▲—▲—) 15°; (--■--■---) 30°.

The effect of ionic strength upon the RNA polymerase activity in nuclei at various divalent metal ion conditions is shown in Figure 5. At 5.8 mM Mg^{2+} and at 1.5 mM Mn^{2+} (concentrations which are nearly optimal for each ion) the curves are biphasic. At the higher concentration of each metal ion, the biphasic nature of each curve (especially for Mn^{2+}) is less apparent, due principally to selective repression of the activity at the lower ionic strengths. At ammonium sulfate concentrations greater than 0.1 M, the activity is less sensitive to changes in the concentration of the metal ions.

It is reported elsewhere (Roeder, 1969) that the incorpora-

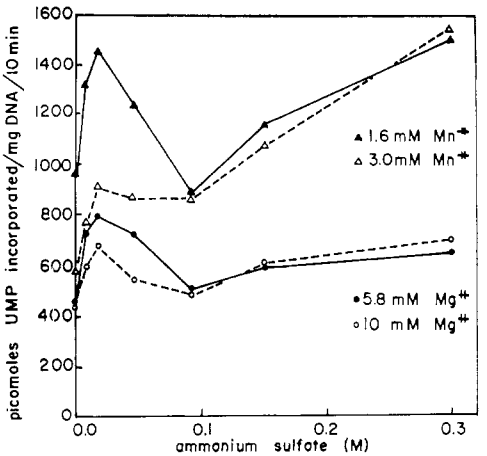

FIGURE 5: Effect of ammonium sulfate and divalent metal ions on the RNA polymerase activity in embryonic sea urchin nuclei. Nuclei were from 32-hr (mesenchyme blastula) embryos. The nuclei present in each reaction mixture contained 0.175 mg of DNA. Except for the metal ion and salt concentrations, incubation conditions were as described in Methods. Each incubation was for 10 min at 15°. Each value is the average of duplicate determinations: (—●—●—) 5.8 mM Mg^{2+}; (--○--○--) 10 mM Mg^{2+}; (—▲—▲—) 1.6 mM Mn^{2+}, 2 mM Mg^{2+}; (--△--△--) 3.0 mM Mn^{2+}, 2 mM Mg^{2+}.

TABLE III: RNA Polymerase Activity in Embryonic Sea Urchin Nuclei at Various Stages of Development.

Assay Conditions			pmoles of UMP Incorporated/mg of DNA per 10 min[a]				
			hr Development[b]				
M $(NH_4)_2SO_4$	mM Mg^{2+}	mM Mn^{2+}	15	25	39	47	55
0.00	6.0	0	538	557	774	517	260
0.00	2.0	1.6	921	1050	987	510	303
0.02	6.0	0	891	886	1120	700	390
0.02	2.0	1.6	1438	1550	1288	831	450
0.29	6.0	0	908	726	775	590	584
0.29	2.0	1.6	2060	1675	1680	1375	1345

[a] Values represent pmoles of UMP incorporated per mg of DNA in 10 min at 15°. Each value represents the average of triplicate determinations. [b] The embryos used at each stage of development were from the same large culture which was obtained by fertilization of a batch of eggs from a single female. The stages of development at the various time points were as follows: 15 hr, 150 cell (average); 25 hr, hatching blastula; 39 hr, late mesenchyme blastula; 47 hr, midgastrula; 55 hr, postgastrula (skeletal primordia visible).

tion of UTP into acid-insoluble material under various ionic conditions is inhibited by pretreatment of the nuclei with deoxyribonuclease or actinomycin D. Furthermore, the incorporation is dependent upon the presence of all four ribonucleoside triphosphates, and the isolated products are degraded by ribonuclease but not by deoxyribonuclease. Thus, it is apparent that under the ionic conditions tested incorporation of radioactivity from [^{3}H]UTP into acid insoluble material represents DNA-dependent RNA polymerase activity.

RNA POLYMERASE ACTIVITY IN NUCLEI AT VARIOUS STAGES OF DEVELOPMENT. The polymerase activity of isolated nuclei from various stages of development was determined under several sets of ionic conditions (Table III). We have earlier shown that sea urchin polymerase I, presumably involved in the synthesis of ribosomal RNA (see Discussion), is maximally active at less than 0.04 M ammonium sulfate and is equally active with Mg^{2+} or Mn^{2+}. Sea urchin polymerase II, presumably involved in the synthesis of DNA-like RNA (see Discussion), is optimally active at 0.1 M ammonium sulfate and has a Mn^{2+}:Mg^{2+} activity ratio of 10. It was anticipated, therefore, that it might be possible to select conditions under which the nuclei would show some specificity in transcription and that if the relative rates of synthesis of various classes of RNA (*e.g.*, ribosomal and DNA like) changed with development, these might be detected. For these reasons, the measurements in nuclei were performed at high and at low salt concentrations in the presence of Mg^{2+} alone (6 mM) and in the presence of 1.6 mM Mn^{2+} (in addition to 2 mM Mg^{2+}) (Table III).

Maximal activity under conditions where the nuclei remain intact is obtained at 0.02 M ammonium sulfate in the presence of 1.6 mM Mn^{2+} (Figure 5). This activity drops severalfold during development (Table III). The activity measured at 1.6 mM Mn^{2+} in the absence of ammonium sulfate also drops severalfold during development. The activities measured at 6 mM Mg^{2+} in the absence of ammonium sulfate and at 6 mM Mg^{2+}–0.02 M ammonium sulfate show slight increases at the mesenchyme blastula stage (38 hr) and subsequently decline two- to threefold. The activities at 0.3 M ammonium sulfate decline only slightly during development. Under these latter conditions, where the nuclei are lysed, the template is presumably rendered nonlimiting by the removal of most of the chromosomal proteins; but the RNA polymerase may also be partially dissociated (see Discussion).

NATURE OF THE RNA PRODUCTS OF THE RNA POLYMERASE REACTIONS IN ISOLATED NUCLEI FROM VARIOUS STAGES OF DEVELOPMENT. The G:U ratios for ribosomal RNA (1.87 for 28 S and 1.53 for 18 S) differ markedly from the deoxyguanosine:thymidine ratio of DNA (0.63) (Emerson and Humphreys, 1970) so that measurements of the relative

TABLE IV: Relative Incorporation of GMP and UMP by Embryonic Sea Urchin Nuclei During Development.

Assay Conditions			GMP:UMP Incorporation Ratio[a]			
			hr Development[b]			
M $(NH_4)_2SO_4$	mM Mg^{2+}	mM Mn^{2+}	17	33	48	55
0.00	6.0	0	0.65	0.60	0.54	0.56
0.02	6.0	0	0.72	0.60	0.64	0.54
0.30	2.0	1.6	0.74	0.57	0.63	0.61

[a] Values represent the GMP:UMP incorporation ratio after 10-min reaction at 15°. Incubations were conducted as described in Methods except that the concentrations of UTP and GTP were both changed to 0.3 mM. The absolute activity values for the UTP incorporation were comparable with those reported in Table III. Each value represents the average of triplicate determinations. [b] The stages of development at the various time points were as follows: 17 hr, 150 to 200 cells; 33 hr, early mesenchyme blastula; 48 hr, early to midgastrula; 55 hr, postgastrula (skeletal primordia present).

TABLE V: Nearest-Neighbor Frequencies of the RNA Synthesized *in Vitro* by Embryonic Sea Urchin Nuclei at Various Stages of Development.[a]

Stage (hr)[b]	M $(NH_4)_2SO_4$	Per Cent of ^{32}P cpm Recovered in				Gp:Up
		Cp	Ap	Gp	Up	
14	0.005	17.3	28.5	20.7	34.1	0.61
	0.30	16.8	28.1	20.8	34.4	0.61
33	0.005	18.3	28.0	19.3	34.3	0.56
	0.30	19.2	27.5	20.2	33.1	0.61
48	0.005	19.0	27.3	20.0	33.6	0.60
	0.30	19.8	26.9	21.0	32.3	0.65
61	0.005	19.6	27.1	20.2	33.1	0.61
	0.30	19.7	27.5	20.6	32.2	0.64
67	0.005	19.7	26.4	21.1	32.8	0.64
	0.30	20.1	27.4	20.0	32.6	0.61

[a] Incubations were carried out for 10 min at 15° in the presence of 6 mM Mg^{2+} and with 1–2 μCi of [α-^{32}P]UTP present per assay. Other experimental details describing the alkaline hydrolysis of the acid-insoluble products and the subsequent electrophoretic resolution of the resultant 2′(3′)-mononucleotides can be found in Methods. The recovery of input cpm in the resolved electrophoretic spots was always greater than 90%. Each value shown represents the average of duplicate determinations for two separate experiments. [b] The developmental stages at the various times indicated were as follows: 14 hr, 100 to 150 cells; 33 hr, mesenchyme blastula; 48 hr, early gastrula; 61 hr, postgastrula (skeletal primordia present); and 67 hr, early prism.

incorporation of GMP and UMP (from GTP and UTP, respectively) into RNA provide a convenient way of looking at the nature of the products of the polymerase reactions. The GMP:UMP incorporation ratios were determined at low ionic strengths in the presence of Mg^{2+} alone and in the presence of Mn^{2+} at high ionic strength in nuclei from various stages of development. As shown in Table IV, under all the ionic conditions tested and at all stages examined after 17 hr, the GMP:UMP ratio is nearly equal to or less than that expected for DNA-like RNA. At 17 hr the ratios are slightly greater, but still very much closer to the G:U ratio of DNA-like RNA than that of rRNA.

The nature of the products synthesized at high and at low ionic strengths (in the presence of Mg^{2+} only) were also examined by labeling with [α-^{32}P]UTP and subsequently determining the distribution of the labeled α-phosphate in the 2′(3′)-mononucleotides in alkaline hydrolysates of the products. The data in Table V show that the products at both ionic strengths and at all stages of development examined are indistinguishable by this method. While the relative proportions of radioactivity in the 2′(3′)-nucleotides cannot necessarily be equated with product base compositions, the distributions will reflect the base compositions of the RNAs if all the nucleotide sequences are distributed randomly. The very good correlation of the Gp:Up ratio (last column in Table V) from this experiment with the actual G:U ratio of the products (Table IV) suggests that this may be so. The Gp:Up ratios obviously are close to the deoxyguanosine:thymidine ratio of DNA. These results indicate that under the conditions employed, the isolated nuclei do not synthesize rRNA in sufficient quantities to skew the distributions to any significant degree at the stages of development examined.

Apparent Rates of RNA Synthesis per Cell. The incorporation of [^{3}H]uridine into UTP and RNA in intact embryos has been followed during short labeling periods (Table VI). The radioactivity which enters RNA during the latter part of the labeling period has been converted into picomoles of UMP using the average UTP specific activity during this same period. Details of the calculations are given in the table legend. This assumes that all the radioactivity in RNA is in UMP. Some radioactivity would actually be expected to be in CMP, however, because of the possibility of UTP being converted into CTP during the labeling period. The CTP specific activity, however, was never greater than 15% that of UTP. Since most of the RNA synthesized at any stage appears to be DNA-like with a C:U ratio of 0.61 (Emerson and Humphreys, 1970), neglecting the CTP contribution should not introduce an error of more than 10%.

The apparent rates of synthesis, normalized to DNA, are summarized in Table VI. It is obvious that the rates of synthesis per cell *decrease* with time of development. The method is likely not accurate to more than ±20–30%, but differences between the 12-hr (and possibly the 17-hr) determinations and those of the later stages are certainly significant. The validity of the data also, of course, rests on the assumption that there are no large errors resulting from compartmentation of the UTP pool or from very rapid turnover of the newly synthesized RNA. Large errors due to turnover seem unlikely due to the short labeling periods used, but there is, as yet, no information about compartmentation of UTP in these cells. The data presented here are in general agreement with that recently published by Kajima and Wilt (1969). These workers estimated the rates of synthesis from measurements of the GTP specific activity and of the radioactive GMP in RNA following short labeling periods.

Discussion

Levels of the RNA Polymerases and RNA Synthesis. The occurrence of multiple forms of RNA polymerase in em-

TABLE VI: Incorporation of [^{3}H]Uridine into UTP and RNA at Various Stages of Development.[a]

Expt	Stage	Extracellular [^{3}H]Uridine (μM)	dpm/μmoles of UTP			dpm (RNA)/μg of DNA			pmoles of UMP Incorporated/μg of DNA per min[b]
			4 min	8 min	12 min	4 min	8 min	12 min	
1	12 hr (32–64 cells)	0.066	43	70	77	620	2400	4000	5.2
			5 min	10 min	15 min	5 min	10 min	15 min	
2	17 hr (early blastula)	0.066	75	73	69	824	1690	2970	3.6
3	33 hr (mes.[c] blastula)	0.066	136	107	71	1680	2710	4050	3.0
4	47 hr (gastrula)	0.066	90	59	51	984	1680	2200	1.9
5	60 hr (late gastrula)	0.066	73	93	54	664	1510	2210	1.9
			3 min	6 min	9 min	3 min	6 min	9 min	
6	12 hr (32–64 cells)	0.97	15	19	21	154	384	732	5.7
7	39 hr (mes.[c] blastula)	0.97	15	29	32	42	116	277	1.8
8	64 hr (early prism)	0.97	12	21	31	37	136	300	2.1

[a] Embryos were labeled with [^{3}H]uridine and the UTP specific activity and radioactivity on RNA determined as described in Methods. [b] The apparent rate was calculated using the average UTP specific activity between the last two time points analyzed for each experiment and the radioactivity incorporated into RNA between these same time points. [c] mes. = mesenchyme.

bryonic sea urchins (Roeder and Rutter, 1969b, and this paper) suggested that variations in the synthesis of large classes of RNA during early sea urchin development might be related to changes in the levels of these enzymes. Recent studies on the cognate polymerases in rat liver nuclei suggest that the various forms may indeed have distinct transcriptive functions. Rat liver polymerase I is localized primarily within the nucleolus while polymerase II and another form possibly analogous to the sea urchin polymerase III are found in the nucleoplasm (Roeder and Rutter, 1970). It has also been shown that α-amanitin, the bicyclic polypeptide toxin from *Amanita phalloides*, specifically inhibits polymerase II activity (a possible inhibition of polymerase III has not yet been tested) while exerting no effect on polymerase I activity (Lindell *et al.*, 1970). In isolated nuclei treated with α-amanitin, the RNA synthesized is G + C rich (ribosomal like) (F. Weinberg and W. J. Rutter, unpublished observations). These results suggest the involvement of polymerase I in the synthesis of ribosomal RNA and polymerase II (and perhaps polymerase III) in the synthesis of DNA-like RNA. The molecular and catalytic properties of forms I and II of the sea urchin embryo are similar to those of their counterparts (I and II) in rat liver (Roeder and Rutter, 1969b) and in calf thymus (S. P. Blatti, R. F. Weaver, and W. J. Rutter, unpublished observations) and suggest that the respective sea urchin enzymes have similar functions. The observations presented in this report are in agreement with that inference and support the contention that the sea urchin polymerases are separate, independently regulated entities.

From the early blastula to the postgastrula stages, the total extractable RNA polymerase activity decreases about two- or threefold on a per cell basis. This is largely caused by decreases in polymerase II and polymerase III activities; the level of polymerase I per cell remains essentially constant. These changes are qualitatively consistent with the changes in the pattern of RNA synthesis occurring in the intact embryo. The decrease in total RNA polymerase per cell corresponds to the decline in the apparent rate of (total) RNA synthesis in the embryo (Table VI, Kajima and Wilt, 1969). However, Emerson and Humphreys (1970) have demonstrated that during the period of development studied here, ^{32}P-pulse-labeled RNA is DNA like. Thus the rate of total RNA synthesis reflects primarily the rate of DNA-like RNA synthesis. The decline in the level of polymerase II (which accounts for the majority of nuclear activity) is consistent with the presumed function of this enzyme in the synthesis of DNA-like RNA. In contrast, the relative increase in polymerase I corresponds with the increase in ribosomal RNA synthesis which occurs during development (Nemer, 1963; Comb *et al.*, 1965; Giudice and Mutolo, 1967). In fact these earlier studies had failed to detect any ribosomal RNA synthesis until near gastrulation. However, Emerson and Humphreys (1970) have pointed out that the relatively higher rate of DNA-like RNA synthesis during the early stages of development could have obscured a constant rate of ribosomal RNA synthesis per cell. Our finding that polymerase I is present during these early stages emphasizes the possibility of rRNA synthesis during this early embryonic period. (In fact the level of RNA polymerase I stays constant on a per cell basis.) This does not mean that the rate of RNA synthesis will be a direct function of the enzyme level; in fact, it seems likely that the activity of the enzyme will be regulated within the cell. For example, polymerase I accounts for up to 30% of the total solubilized activity, whereas the level of ribosomal RNA synthesis *in vivo* is a much smaller percentage of the total RNA synthesis (less than 5%). The activities of the different polymerases can be altered by the template structure and by the metal ion and ionic strength conditions (Roeder

and Rutter, 1969b) as well as by the temperature; furthermore, the conditions employed in our *in vitro* experiments probably do not coincide with those that prevail in the intact cell. The development of nucleolar structure may provide another means of regulation. The initiation or acceleration of ribosomal RNA synthesis has been generally correlated with the appearance of definitive nucleoli (Brown, 1966). During sea urchin development, the nucleoli first appear near the beginning of gastrulation (Cowden and Lehman, 1963; Karasaki, 1968). It seems possible that polymerase I may become specifically sequestered within the nucleolar structure with a resultant intensification of ribosomal RNA synthesis. Finally, the rate of accumulation of ribosomal RNA may be regulated by changes in the rate of its turnover. For example, the absence of specific methylating enzymes during early development might result in an increased turnover of ribosomal RNA, since it has been shown in HeLa cells that the rRNA precursor is degraded within the nucleus when methylation is blocked (Vaughan *et al.*, 1967).

The range of transcriptive specificity for the various polymerases remains a point of major significance. Coordinate transcription of specific groups of genes by each of the enzymes is certainly a possibility. For example, if polymerase I is involved in rRNA synthesis, it might also be responsible for the transcription of the genes coding for 5S RNA and ribosomal proteins, since these molecules are synthesized coordinately with rRNA during amphibian development (Brown and Littna, 1966; Hallberg and Brown, 1969).

Activity of Template-Associated RNA Polymerase in Isolated Nuclei. Isolated nuclei have been widely employed for studies of RNA synthesis during transitions in cellular activity (for a review, see Tata, 1966). These systems seem particularly advantageous since the rates of RNA synthesis can be determined at constant precursor levels, whereas *in vivo* studies are often complicated by the possibility of variable precursor levels. For the nuclear analyses to be relevant to the whole cell system, obviously the qualitative and quantitative nature of RNA synthesized by these nuclei must be the same *in vitro* and *in vivo*. That is, the natural enzyme–template relationships must be maintained. RNA synthesis by isolated sea urchin nuclei is markedly affected by the divalent metal ions present, as well as by the ionic strength. The biphasic curves of activity versus ammonium sulfate concentration (Figure 5) are similar to those reported by Johnson *et al.* (1969) (for rat liver nuclei). The complexity of the activity–ionic strength profiles of RNA synthetic activity in sea urchin nuclei is not surprising since the three RNA polymerases are differentially affected by the ionic environment (Roeder and Rutter, 1969b). In addition, nucleoprotein templates (chromatin) are also altered by changes in the ionic strength. High salt concentrations increase the template activity by dissociation of the chromosomal proteins (Chambon *et al.*, 1965; Marushige and Bonner, 1966; Breuer and Florini, 1966; Georgiev *et al.*, 1966). Thus, measurable differences in the RNA synthetic capacity of isolated nuclei from cells in various states of metabolic activity might be due to alterations in either the enyzme or template.

Under conditions for the optimal measurement of RNA polymerase activity in *intact* sea urchin nuclei (0.02 M ammonium sulfate and 1.6 mM manganese) the level of RNA synthesis decreases about threefold during the period of development examined. This is in qualitative agreement with the decline in the levels of the extractable polymerase and the *in vivo* rates of RNA synthesis. However, in the presence of manganese at higher salt concentrations (0.3 mM ammonium sulfate) there is only a slight change in the level of activity during this period. Under these latter conditions, the template is presumably no longer rate limiting and the activity of the nondissociable-RNA polymerase is measured (Chambon *et al.*, 1965). This activity may still not represent the total enzyme level of the nuclei, since there is no convincing evidence that all of the template-associated enzyme remains bound during the assay. Thus, there need not be a direct correlation between the RNA synthesis in isolated nuclei and that in the whole cell. Some degree of specificity must, however, be retained in the isolated nuclei. The RNA synthetic pattern of isolated sea urchin nuclei, for example, contrasts sharply with that of rat liver nuclei. In the former, ribosomal RNA synthesis is not detectable at any stage in development, even at low ionic strength and in the presence of magnesium–conditions which are relatively selective for sea urchin polymerase I (Roeder and Rutter, 1969b). Similar conditions in rat liver nuclei result in the synthesis of RNA with a base ratio similar to that of ribosomal RNA (Widnell and Tata, 1966; Pogo *et al.*, 1967). Thus, regardless of the artificial nature of the assay conditions, the RNA synthesized by the isolated nuclei appears qualitatively similar (DNA-like) to that RNA which is preferentially produced by the nuclei in their respective cellular environments. Isolated nuclei may, therefore, be an excellent vehicle for an experimental study of transcriptive regulation.

Regulation of RNA Polymerase Activity. There is indirect and tentative evidence that the nucleus may contain other factors which modify the activity of the polymerases. The apparent rate of RNA synthesis within the cells of the sea urchin embryo is more than an order of magnitude greater than that observed in isolated nuclei, or with the extracted polymerases (*cf.* Tables II, III, and VI). A similar situation is found in rat liver: the estimated *in vivo* rates of synthesis (Bucher and Swaffield, 1969) are 10–15 times greater than the maximum activity measured in isolated nuclei (Roeder and Rutter, 1970). Of course, this discrepancy might simply be a result of lack of duplication of the *in vivo* ionic conditions. However, it is also possible that specific regulatory factors, perhaps analogous to those described for the bacterial enzymes (Burgess *et al.*, 1969; Bautz *et al.*, 1969; Bautz and Dunn, 1969; Davidson *et al.*, 1969; Travers, 1969; Pene, 1969) are normally operative *in vivo* and have been lost or inactivated during the isolation of the nuclei. The present studies emphasize that RNA synthesis in eukaryotic cells may be controlled, at least in part, by mechanisms which operate at the level of the RNA polymerase.

References

Bautz, E. K. F., Bautz, F. A., and Dunn, J. J. (1969), *Nature (London) 223*, 1022.

Bautz, E. K. F., and Dunn, J. J. (1969), *Biochem. Biophys. Res. Commun. 34*, 230.

Bekhor, I., Kung, G. M., and Bonner, J. (1969), *J. Mol. Biol. 39*, 351.

Blobel, I. G., and Potter, V. R. (1966), *Science 154*, 1662.

Bonner, J., Dahmus, M. E., Fambrough, D., Huang, R. C., Marushige, K., and Tuan, D. Y. H. (1968), *Science 159*, 47.

Breuer, C. B., and Florini, J. R. (1966), *Biochemistry 5*, 3857.

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