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Early Stimulation of RNA Synthesis by Erythropoietin in Cultures of Erythroid Precursor Cells

(fetal-mouse liver/gel electrophoresis/differentiation/protein synthesis)

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ABSTRACT **The effect of erythropoietin on cultured erythroid precursor cells from 13-day mouse-fetal livers was examined. Within 1 hr, erythropoietin causes a 2- to 3-fold stimulation of uridine incorporation into RNA by these cells. The types of RNA preferentially stimulated by erythropoietin during the first hour of exposure of the cells to the hormone include ribosomal RNAs and their precursors, as well as 4–5S RNA. No unique RNA species, not present in control cells, could be detected by sucrose gradient sedimentation or gel electrophoresis. Inhibition of protein synthesis for up to 1 hr does not abolish the stimulatory effect of erythropoietin on RNA synthesis, suggesting that the effect of the hormone on RNA synthesis is not mediated by a newly synthesized protein.**

Erythropoiesis constitutes a model for the study of the regulation of cell differentiation (1, 2). *In vivo*, the rate of erythropoiesis is regulated by erythropoietin, a glycoprotein hormone (3). Cultures of hematopoietic tissues of adult and fetal animals respond to erythropoietin with cell proliferation (4–6), increased RNA synthesis (1, 7, 8), and increased heme and hemoglobin synthesis (9, 10). The first detectable effect of erythropoietin on macromolecular synthesis in cultures of either rat bone marrow or fetal-mouse liver cells is stimulation of RNA synthesis (8, 9). The nature of the RNA synthesized in cultures of erythroid cells in response to erythropoietin has been the object of several studies (7, 8, 11). Interpretation of these studies, however, is hindered by the presence, in the cultures, of large numbers of cells that have passed the erythropoietin-dependent stage and that do not require erythropoietin for completion of their maturation. The present study is designed to examine the pattern of RNA synthesis in a fractionated population of hormone-responsive erythroid-cell precursors that do not synthesize globin or contain globin messenger RNA activity (4, 12). We examined the RNA synthesized in response to erythropoietin in cultures of these erythroid precursor cells. We demonstrate that, within 1 hr, erythropoietin causes a 2- to 3-fold stimulation of RNA synthesis. The RNA species produced preferentially in response to the hormone are, mainly, ribosomal 28S and 18S RNA and their precursors as well as 4–5S RNA. No unique RNA species could be detected by density gradient sedimentation and gel electrophoresis. Inhibition of protein synthesis by cycloheximide or pactamycin does not abolish the early stimulation of RNA synthesis induced by erythropoietin.

MATERIALS AND METHODS

Preparation of Cell Cultures. Fetuses were obtained from hormonally primed C57 BL/6J pregnant mice (13). The livers of 13-day-old fetuses were disaggregated mechanically and filtered through a nylon filter of 10-μ m pore size (Nitex NC-10). Cells cultured at this stage are referred to as "unfractionated cells." Populations of immature precursor erythroid cells, devoid of hemoglobinized cells, were obtained by treating the above cells with rabbit antiserum to adult mouse erythrocytes and guinea pig complement, essentially as described by Cantor *et al.* (4). These purified, erythropoietin-responsive, immature, erythroid cells are referred to as "erythroid precursor cells." After antibody lysis, the cells were washed with culture medium and then suspended in medium containing 100 μg/ml of deoxyribonuclease (bovine pancreas, Worthington, electrophoretically purified) and incubated at room temperature for 20 min. The cells were subsequently centrifuged for 5 min at 1000 rpm in an International refrigerated centrifuge, model PR-2, and washed three times before final suspension. This DNase treatment and subsequent low-speed centrifugation, results in a reduction in the number of naked nuclei present in the cell pellet. Their number decreases from an average of 3.5 per intact cell after antibody treatment to 0.5 after DNase treatment. This DNase treatment has no effect on the cells, as judged either from a comparison of the rates of RNA synthesis during the first 24 hr in culture or from the morphologic maturation of these cells in the presence of erythropoietin for 48 hr. For culturing, the concentration of cells was adjusted to 2×10^6 cells per ml. For light microscopy, the cells were stained with benzidine and Wright–Giemsa stain (14). The cells were cultured in Waymouth's Medium MB 752/1 (GIBCO, Grand Island, N.Y.) containing 10% fetal-bovine serum (Microbiological Associates, Inc., Bethesda, Md.), 3% chick-embryo extract (GIBCO), 15 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES, Microbiological Assoc., Bethesda, Md.), and 50 units of penicillin and streptomycin per ml. Human urinary erythropoietin (N.I.H., Pool H-5-TaLSL, specific activity 58.8 units/mg) was used at a final concentration of 0.17 units/ml of culture. Cells were incubated at 37° without CO_2. At the end of the incubation period the cell suspensions were transferred to cooled centrifuge tubes, centrifuged, and washed five times with cold Waymouth's medium.

Cell Labeling. RNA was labeled either with 2–40 μCi/ml of [5-^{3}H]uridine (specific activity, 25 Ci/mmol) or with 0.2–1 μCi/ml of [2-^{14}C]uridine (specific activity, 50 Ci/mol). In double-labeling experiments, [5-^{3}H]uridine was diluted with unlabeled uridine so that the total uridine concentration was the same in both control and erythropoietin-treated cells.

TABLE 1. *Effect of erythropoietin on uridine incorporation in cultures of erythroid precursor cell*

Cells in culture	Total incubation time	Erythropoietin addition at 0 time	[^{3}H]Uridine incorporation: Nucleoside added at	Incorporation (cpm/culture)	Stimulation* (%)
Unfractionated cells	30 min	−	0 min	27850 (±1985)	
	30 min	+	0 min	36760 (±4159)	132
	70 min	−	40 min	34710 (±2891)	
	70 min	+	40 min	52400 (±5121)	151
	120 min	−	0 min	121000 (±11000)	
	120 min	+	0 min	193600 (±20020)	160
	270 min	−	240 min	30140 (±1918)	
	270 min	+	240 min	52150 (±6448)	173
	21 hr	−	20.5 hr	7118	
	21 hr	+	20.5 hr	24910	350
Precursor cells	70 min	−	40 min	41000 (±3963)	
	70 min	+	40 min	109900 (±13990)	268
	270 min	−	240 min	38770 (±4021)	
	270 min	+	240 min	126000 (±16600)	325
	21 hr	−	20.5 hr	21060 (±2992)	
	21 hr	+	20.5 hr	60030 (±6314)	285

* (cpm in erythropoietin-treated culture/cpm control culture) × 100.

Proteins were labeled with L-[4, 5-^{3}H]leucine, specific activity 5 Ci/mmol. All radiochemicals were purchased from New England Nuclear Corp., Boston, Mass.

Inhibitors. Pactamycin was a gift from the Upjohn Co., Kalamazoo, Mich. Acti-dione (cycloheximide) was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Actinomycin-D was purchased from Merck & Co., Inc., Rahway, N.J.

Total Cell Radioactivity. For measurements of total radioactivity incorporated, the cells were suspended in 30% medium, lysed by freezing and thawing, and then precipitated in 5% cold trichloroacetic acid. When protein synthesis was measured, the preparations were heated at 95° for 10 min. The precipitates were collected on Millipore filters, dried, and counted in Aquasol scintillation solution (New England Nuclear Corp., Boston, Mass.). In experiments in which the incorporation of labeled uridine into alkali-stable counts was to be measured, an aliquot of the cell suspension was made 0.3 M in KOH, incubated at 37° for 18 hr, neutralized with HCl, and then precipitated with cold trichloroacetic acid and treated as above.

RNA Extraction. Total cellular RNA was extracted as described by Perry *et al.* (15). Cells to be fractionated into nuclear and cytoplasmic fractions were suspended in 2 ml of 10 mM Tris–10 mM NaCl–5 mM $MgCl_2$ (pH 8.5), and 2 mM adenosine 2′- and 3′-, uridine 2′- and 3′-, cytidine 2′- and 3′-, guanosine 2′- and 3′-monophosphoric acid (Sigma Chemical Co., St. Louis, Mo.). The 2′- and 3′-mononucleotides, being the products of RNase reaction, were added in order to retard the action of RNases released during cell disruption. The cells were left in the above buffer for 2 min at 0° and then homogenized with a Dounce glass homogenizer with 10 strokes. The nuclei were separated by centrifugation at 1000 × *g* for 2 min; their purity was checked by phase microscopy. The RNA of the cytoplasmic fraction was extracted in a buffer containing a final concentration of 10 mM EDTA–10 mM acetate buffer (pH 8.5)–0.5% Na dodecyl sulfate. A relatively high pH was used in order to reduce the lysosomal ribonuclease activity (16). An equal volume of phenol, heated to 55°, was added and the mixture was shaken with a Vortex mixer. Extraction with phenol was repeated three times. The RNA was extracted from the nuclear pellet as described by Penman (17). The aqueous phase of all preparations was adjusted to 0.2 M in NaCl and the RNA was precipitated with two volumes of −20° 95% ethanol, overnight.

Polyacrylamide Gel Electrophoresis. Gels of 2.2% acrylamide concentration were used. Acrylamide (Eastman Kodak Co., Rochester, N.Y.) was recrystallized from chloroform. Ethylene diacrylate (K and K Laboratories, New York) was used as cross-linking agent (18). Ammonium persulfate and *N,N,N′,N′*-tetramethylethylenediamine were purchased from Eastman Kodak Co. The electrophoresis buffer was the following: 40 mM Tris–2 mM EDTA–20 mM acetate (pH 7.4)–0.2% Na dodecyl sulfate–10% glycerol (hexane-extracted). Gels were polymerized in the above buffer without Na dodecyl sulfate (19). The gels were prepared in Plexiglas tubes of 6 mm inner diameter. For radioactivity measurements, the gels were frozen in a dry ice–hexane bath and sliced into 1-mm slices with a gel slicer (Metaloglass, Inc., Boston, Mass.) The slices were hydrolyzed with 1.5 ml of 10% NH_4OH at 60°, and counted in 10 ml of Aquasol. The data from double-labeled experiments were analyzed with a digital computer program developed by Yund *et al.* (20).

RESULTS

Stimulation of Uridine Incorporation by Erythropoietin. Erythropoietin causes an increase in the incorporation of [^{3}H]uridine into RNA in cultures of either "unfractionated erythroid cells" or "erythroid precursor cells." This stimulation can be observed as early as 30 min after addition of the hormone (Table 1). The relative stimulation of uridine incorporation by preparations of precursor cells is higher than

TABLE 2. *Effect of inhibitors of protein synthesis on leucine and uridine incorporation in cultures of precursor cells*

Inhibitor*	EPO and label added at (min)	EPO addition	Labeled precursor	Cells harvested at (min)	Incorporation in the absence of inhibitor (cpm/10^6 cells)	Stimulation by EPO† (%)	Incorporation in the presence of inhibitor (cpm/10^6 cells)	Stimulation by EPO (%)	Inhibition by the inhibitor§ (%)
Cycloheximide 6×10^{-4} M	25	−	[^{3}H]Leu	55	2740 ± 190		356 ± 40		87
	25	+	[^{3}H]Leu	55	3680 ± 285	134	454 ± 43	127	88
	25	−	[^{3}H]Leu	85	6850 ± 582		959 ± 61		86
	25	+	[^{3}H]Leu	85	9400 ± 715	137	1320 ± 91	137	86
	25	−	[^{3}H]U	55	19800 ± 1990		17200 ± 1300		13
	25	+	[^{3}H]U	55	24500 ± 2620	124	20600 ± 1350	119	16
	25	−	[^{3}H]U	85	31500 ± 2460		26300 ± 1570		30
	25	+	[^{3}H]U	85	49400 ± 3850	157	33600 ± 1700	128	32
Pactamycin 10^{-6} M	30	+	[^{3}H]Leu	60	4050 ± 370	N.A.‡	364 ± 31	N.A.	91
	45	+	[^{3}H]Leu	75	4200 ± 348	N.A.	379 ± 39	N.A.	91
	30	+	[^{3}H]U	60	52700 ± 3210	N.A.	50600 ± 3010	N.A.	4
	45	+	[^{3}H]U	75	55000 ± 3980	N.A.	31300 ± 1780	N.A.	43

* Inhibitor was added after 15 min of incubation.
† (cpm in EPO-treated culture/cpm control culture) × 100.
‡ Not applicable.
§ 100 − [cpm in the presence of inhibitor/cpm in the absence of inhibitor] × 100.
EPO, erythropoietin.

that of the unfractionated cells during the first hours of incubation. This, presumably, reflects the fact that the erythroid precursor cell population is enriched in erythropoietin-target cells, the erythropoietin-responsive cells.

The subcellular distribution of trichloroacetic acid-precipitable [^{3}H]uridine incorporated by erythroid precursor cells was examined after 60 min of incubation in the presence of erythropoietin. More than 80% of the radioactivity is confined to the nucleus; of this, 7% appears as alkali-resistant radioactivity. These results are comparable to previous observations in other erythroid systems (21).

Preincubation of the erythroid cells in the absence of erythropoietin results in a progressive decline in the rate of RNA synthesis (to 60% of the initial rate by 5 hr and to 20% by 8 hr). This decline may reflect either an arrest in the cell cycle due to a loss of proliferative capacity or a cell deterioration in the absence of erythropoietin. Despite this progressive decline in RNA synthesis, the erythropoietin-responsive cells retained the ability to respond to the hormone; the relative stimulation of [^{3}H]uridine incorporation by erythropoietin remained relatively constant between 30 min and 8 hr of culture.

Effect of Inhibition of Protein Synthesis on Stimulation of RNA Synthesis by Erythropoietin. Stimulation of RNA synthesis is an early response of the erythropoietin-responsive cell to the hormone. Experiments were designed to determine whether this RNA synthesis is dependent upon prior protein synthesis. Two inhibitors were used: cycloheximide, which inhibits the translocation step (22), and pactamycin, which, at low concentration, selectively blocks initiation of protein synthesis (23, 24). The effect of the inhibitors on [^{3}H]leucine and [^{3}H]uridine incorporation by precursor cells was examined in the presence and in the absence of erythropoietin (Table 2). At both 55 min and 85 min, erythropoietin induced a stimulation of protein synthesis comparable in magnitude to that of RNA synthesis. Cycloheximide inhibited protein synthesis by more than 85% both in the presence and in the absence of erythropoietin. RNA synthesis, however, was inhibited by only 15%, 40 min after exposure to cycloheximide. This inhibition increased to about 30% by 70 min. There was no substantial difference between cells grown with or without the hormone. The stimulation of [^{3}H]uridine incorporation by erythropoietin was demonstrable in the presence of cycloheximide despite the suppression of protein synthesis. Similar results were obtained with pactamycin (Tables 2 and 3). At levels of pactamycin inhibiting protein synthesis by more than 90%, RNA synthesis was inhibited by less than 5% in 45 min and by 43% in 60 min. The effect of erythropoietin on RNA synthesis was still demonstrable under conditions in which pactamycin inhibited RNA synthesis to 56% of the control rate (Table 3). Inhibition of protein synthesis does result in a progressive decrease in overall RNA synthesis, and

TABLE 3. *Effect of pactamycin on the stimulation of [^{3}H]-uridine incorporation by erythropoietin in cultures of precursor cells*

EPO addition at (min)	Uridine incorporation: No pactamycin (cpm/culture)	Uridine incorporation: + Pactamycin (cpm/culture)	Inhibition by pactamycin (%)
—	8680 ± 312	4880 ± 185	44
65	10000 ± 450	5600 ± 212	44

Pactamycin (2 μM) was added after 50 min of incubation. [^{3}H]Uridine was added to all cultures after 105 min of incubation, and the cells were harvested after 120 min of incubation.

The difference in [^{3}H]uridine incorporation between erythropoietin and control cultures with and without pactamycin, is statistically significant ($P < 0.005$).

EPO, erythropoietin.

TABLE 4. *Effect of [^{3}H]uridine specific activity on total incorporation in cultures of precursor cells*

Specific activity of [^{3}H]uridine (mCi/mg)	EPO addition	Uridine incorporation: Incorporation (cpm/culture)	Uridine incorporation: Stimulation by EPO (%)
2	−	297200 ± 16550	
2	+	462700 + 18500	156
1	−	149700 ± 8977	
1	+	236000 ± 16020	158

Cells were incubated as described in *Methods* but fetal-calf serum and chick-embryo extract were dialyzed before use. Cultures were incubated for 1 hr.

EPO, erythropoietin.

both erythropoietin-treated and control cultures are affected proportionally.

[^{3}H]Uridine incorporation into trichloroacetic acid-precipitable counts can only be equated to RNA synthesis if there is no appreciable change in the intracellular pool of RNA precursors. Relative pool size was determined by incubation of hormone-treated and control cultures at two levels of [^{3}H]-uridine specific activity. The results (Table 4) are consistent with a constant intracellular pool.

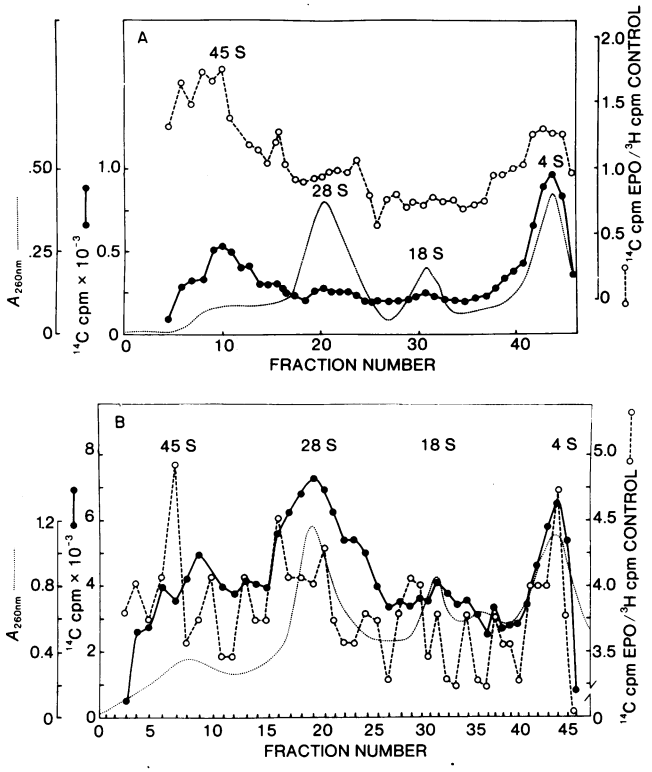

FIG. 1. Density gradient sedimentation pattern of RNA from precursor erythroid cells cultured with and without erythropoietin. Erythropoietin (*EPO*)-stimulated cultures were labeled with [^{14}C]uridine, and control cultures with [^{3}H]uridine. Total cellular RNA was extracted and applied on a 15–30% linear sucrose gradient with a 60% sucrose cushion at the bottom. The gradient was made in 0.1 M NaCl–10 mM Tris·HCl (pH 7.4)–1 mM EDTA–0.5% Na dodecyl sulfate. Centrifugation was in a SW-27 rotor at 23,000 rpm for 14 hr at 20°. (*A*) Cells were incubated with and without erythropoietin for 17 min. (*B*) The cells were incubated with and without erythropoietin for 60 min. Radioactive uridine was added at the beginning of each culture period.

TABLE 5. *Effect of actinomycin-D on [^{3}H]uridine incorporation in cultures of unfractionated cells*

Actinomycin-D (μg/ml)	No erythropoietin (cpm/10^6 cells)	+ Erythropoietin (cpm/10^6 cells)
0	27350	45180
0.013	7050	7120
0.33	390	430
3.3	230	210

Actinomycin-D was added at the beginning of the incubation; [^{3}H]uridine was added at 30 min; and the cells were harvested at 180 min.

Types of RNA Synthesized in Erythropoietin-Stimulated and Control Cultures. To determine the types of RNA whose synthesis is preferentially stimulated by erythropoietin, the RNA synthesized by precursor erythroid cells, cultured in the pres-

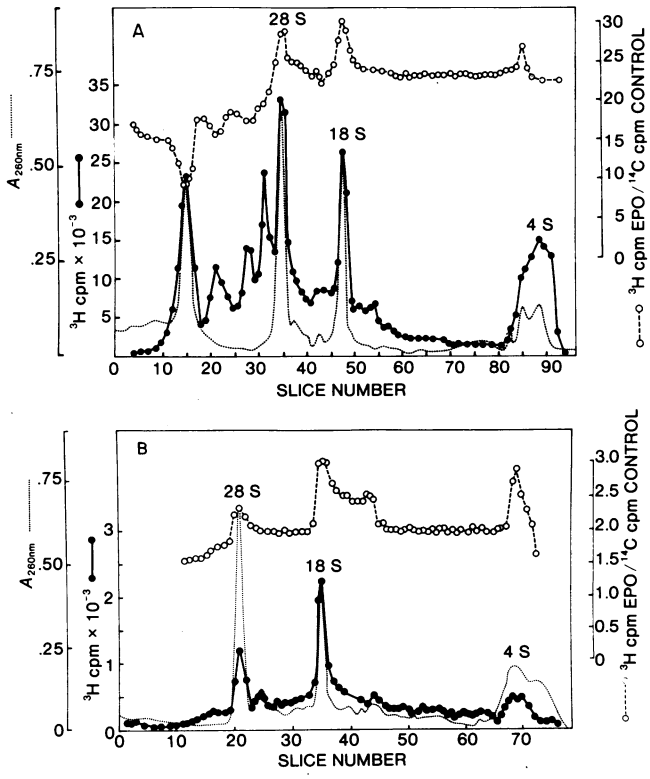

FIG. 2. Polyacrylamide gel electrophoresis of RNA from precursor erythroid cells cultured with and without erythropoietin. Erythropoietin (*EPO*)-stimulated cultures were labeled with [^{3}H]uridine while control cultures were labeled with [^{14}C]-uridine. Erythropoietin was added at 0 time. (*A*) Total cellular RNA extracted from cultures that were labeled for 1 hr. Then a 100-fold excess of unlabeled uridine was added, and the incubation was continued for another hour before harvesting. (*B*) Cytoplasmic RNA extracted from cells labeled during the first hour of incubation.

ence or absence of erythropoietin, was analyzed by sucrose gradient centrifugation and polyacrylamide gel electrophoresis. In double-label experiments the ratio of radioactivity in erythropoietin-stimulated RNA and in control RNA was computed for each fraction or slice. The absolute value of the ratio depends on the particular conditions of each experiment but relative increase of the ratio in a fraction indicates preferential synthesis of that particular RNA species by the erythropoietin-stimulated culture relative to control.

Differences in the pattern of RNA synthesis from erythropoietin-treated and control cultures can be seen within 17 min after exposure to the hormone (Fig. 1*A*). The ratio of incorporation of [^{14}C]uridine (with erythropoietin) to [^{3}H]uridine (control) indicates that there are several types of RNA preferentially synthesized by the hormone-treated precursor cells. They include heavier-than 45S, 45S, 32S, and 4–5S RNA. By 60 min in culture (Fig. 1*B*) the differences between the erythropoietin-stimulated and control RNA are more pronounced. The 4–5S and the 45S and 32S RNA fractions are the most stimulated species. Analysis of the RNA on polyacrylamide gels is shown in Fig. 2. In the pattern of RNA extracted from cells labeled with uridine for 1 hr and harvested after a second hour of chase (Fig. 2*A*), the first radioactive peak (corresponding to slice no. 15) associated with a decreased ratio of ^{3}H to ^{14}C, is due to DNA. Treatment with DNase abolishes this radioactive peak and normalizes the ratio. The 28S, 18S, and 4–5S RNA show an increased ^{3}H/^{14}C ratio, indicating increased synthesis by the erythropoietin-stimulated cells. The peaks between the DNA and the 28S peak, including the 45 and 32S RNA, show a decrease in the ^{3}H/^{14}C ratio indicating that the specific activity of these species is lower in the erythropoietin-stimulated than in the control cultures. This finding may indicate a more rapid processing of ribosomal precursor RNA or may be due to faster dilution of the label in these ribosomal RNA precursor species because of the increased rate of synthesis in the erythropoietin stimulated cultures.

The pattern of cytoplasmic RNA labeled for 1 hr (Fig. 2*B*) shows that 28S, 18S, and 4–5S RNA are preferentially synthesized by the stimulated cells, as is RNA corresponding to 12–14 S. This latter RNA species has been previously detected in erythroid cells (28, 29). There is no indication, in either preparation, of the synthesis of 9–10S RNA, which might correspond to messenger RNA for hemoglobin.

Analysis of the RNA synthesized by erythroid precursor cells in response to erythropoietin indicates that the main RNA species stimulated by the hormone is ribosomal RNA and 4–5S RNA. These observations are consistent with the high sensitivity of the fetal-mouse liver erythroid cells to actinomycin-D (Table 5). Inhibition of RNA synthesis by actinomycin-D at concentrations that suppress only the formation of ribosomal RNA (25) abolishes the stimulatory effect of erythropoietin on uridine incorporation.

DISCUSSION

We have shown that erythropoietin stimulates the synthesis of RNA in erythroid cell precursors within 17 min of culture. These purified populations of precursor cells do not synthesize hemoglobin and have no detectable messenger RNA activity for globin (12). This population is enriched in erythropoietin-responsive erythroid precursor cells, relative to the unfractionated cell population. Although stimulation of RNA synthesis is the earliest detectable effect of erythropoietin on macromolecules in cultures of fetal hepatic erythroid cells (26), this does not necessarily mean that it is the primary effect. The results obtained with both cycloheximide and pactamycin indicate that the hormone-induced stimulation of RNA synthesis is detectable when protein synthesis is inhibited by 90%, and that even when overall RNA synthesis is inhibited to almost 50% of control the relative stimulation of RNA synthesis by the hormone remains unchanged. These findings suggest that either no new protein is required for the erythropoietin-induced stimulation of RNA to occur or, if a new protein is indeed required, it is normally made in large excess. Gross and Goldwasser (27), using rat bone-marrow cultures, have concluded that stimulation of RNA synthesis by erythropoietin does not require prior protein synthesis.

We observed that erythropoietin induced a stimulation of protein synthesis, comparable to that of RNA synthesis, even in the presence of more than 85% inhibition of protein synthesis. In earlier studies, it was shown that stimulation of RNA synthesis by the hormone precedes detectable stimulation of protein synthesis. (1). These observations, taken together, suggest that the hormone-induced increase in protein synthesis may be secondary to and coordinated with RNA synthesis. The fact that ribosomal RNA synthesis accounts for a major portion of the RNA formed during the initial exposure to the hormone and that, as previously reported, there is no detectable increase in globin synthesis for at least 5 hr, suggests that the major portion of this increased protein synthesis is due to synthesis of ribosomal proteins.

Analysis of the RNA species synthesized by erythroid precursor cells in the presence and in the absence of erythropoietin did not provide evidence for the synthesis of a unique, distinct RNA species in response to the hormone. Rather, the synthesis of several types of RNA is stimulated, including 45S, 32S, 28S, 18S, 14S, and 4–5S RNA. There is no indication that the hormone causes an early, preferential synthesis of an RNA species migrating at the 9–10S region, the expected position of globin messenger RNA. Terada *et al.* (12), using the same culture system as the one used in these studies, could first detect biologically active globin messenger RNA after 10 hr of incubation of precursor cells in the presence of erythropoietin. The experiments reported here, together with previous data, suggest that erythropoietin acts to initiate a coordinated developmental program in the erythropoietin-sensitive cell, which includes the synthesis of various requisite species of RNA, cell division (4, 30), and the production of cell-specific messenger RNA for globin (12). Erythropoietin action on its target erythroid precursor cell may be analogous to the action of other hormones on their respective target cells. Growth hormone has an early stimulatory effect on ribosomal RNA in rat-liver nuclei (31). Similarly, in the same nuclei, hydrocortisone stimulates nucleolar RNA polymerases, and, consequently enhances synthesis of ribosomal RNA (32). The emerging picture for the mode of action of erythropoietin is consistent with a scheme in which erythropoietin acts on a cell already differentiated to the extent of being able to respond to the hormone by, initially, increasing nuclear RNA synthesis. The first detectable species of RNA preferentially stimulated by the hormone, as determined by the present techniques, is principally ribosomal RNA.

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