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Research Article

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Sequential Activation of Splenic Nuclear RNA Polymerases by Erythropoietin

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ABSTRACT The spleen of the ex-hypoxic polycythemic mouse was employed to study the effect of erythropoietin on nuclear RNA polymerase activity. On the basis of ionic strength requirements and sensitivity to the fungal toxin α-amanitin, two major forms (I and II) of nuclear RNA polymerase were identified. Within 0.5 h after administration of erythropoietin, at a time when no morphologically identifiable erythroblasts were present in the spleen, there was an increase in the activity of polymerase II. By 2 h, polymerase II activity had declined to control levels. At 3 h, polymerase I activity began to increase, rising to a peak, 88% above control levels, by 12 h. During this period, early erythroblasts began to appear in the spleen. At 12 h, a second increase of similar magnitude occurred in polymerase II activity. Polymerase I activity fell to control levels by 18 h while polymerase II declined more slowly. These data indicate that stimulation of transcription is an early effect of erythropoietin. Multiple forms of RNA polymerase are involved and activation of these is sequential. Nuclear RNA polymerase activity is maximal during the period of early erythroblast proliferation and declines as these cells mature.

INTRODUCTION

The earliest observed effect of erythropoietin on target cells is an increase in the incorporation of precursors into RNA. Accumulation of [^{3}H]uridine in mouse spleen cell nuclei has been demonstrated by autoradiography within 1 h after administration of erythropoietin (1), and by 2 h, there was a net increase in the production of both nuclear and cytoplasmic RNA (2). In rat bone marrow cultures, an increase in uridine incorporation into RNA occurred within 15 min after exposure to erythropoietin, and the RNA species formed had sedimentation constants of 150, 55-65, 45, 28, 18, 9, 6, and 4S (3). By contrast, in fetal liver cultures, consisting only of proerythroblasts and basophilic erythroblasts, within 1 h after exposure to the hormone, an increase in uridine incorporation occurred in 45, 32, 28, 18, 14, and 4-5S RNA (4). In none of these studies, however, is it clear whether incorporation of labeled precursor into RNA represents a net change in the rate of RNA synthesis or is a reflection of a hormone-mediated change in transport or pool size (5). In addition, it is not known if there is activation of RNA synthesis in erythropoietin-sensitive cells before the proerythroblast stage of development.

In order to resolve these questions, we have investigated the effect of in vivo administration of erythropoietin on the activity of nuclear DNA-dependent RNA polymerase in the spleen of the ex-hypoxic polycythemic mouse. The results indicate that erythropoietin stimulates transcription and that multiple forms of nuclear RNA polymerase are involved. Activation of these is sequential, and an increase in transcriptional activity occurs before the appearance of proerythroblasts in the spleen.

METHODS

Preparation of polycythemic mice. Female Swiss Webster mice weighing 20–25 g were housed in dimethyl silicone membrane enclosures (General Electric Co., Schenectady, N. Y.) for a period of 2 wk (6). The venous hematocrit was determined 5 days after removal from the enclosures, and only those mice with hematocrits of 65% or greater were used for study. For each experiment, the mice were separated into groups of five animals, and the animals

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in each group received a single i.p. injection of either 5 U of erythropoietin (Step III, 3.2 U/mg protein, Connaught Medical Research Laboratory, Willowdale, Ontario, Canada) in 0.5 ml of 0.5% bovine serum albumin (type V, Sigma Chemical Co., St. Louis, Mo.) in saline or 0.5 ml of 0.8% albumin-saline solution.

Preparation of spleen cell nuclei. Mice were killed by cervical dislocation 1, 3, 8, 12, 18, and 36 h after injection. The spleens were removed and bisected for preparation of Wright's-stained imprints before the nuclear isolation procedure. Spleen cell nuclei were isolated by a modification of the method of Blobel and Potter (7) as previously described (8). As judged by phase microscopy, over 95% of the nuclei were free of cytoplasmic contamination. The final nuclear pellet was suspended on the basis of the initial spleen weights, in 5 vol of 0.05 M Tris-HCl (pH 7.9), 25% glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA, and 2.5 mM dithiothreitol (TGMED),[1] to give a final DNA concentration of 1–2 mg/ml. DNA content was determined by the method of Burton (9), and the yield of DNA from the original homogenate was 60%.

Assay of nuclear RNA polymerase. The assay is a modification of a procedure previously described (8). At low ionic strength, in the absence of $(NH_4)_2SO_4$, the reaction mixture contained in a volume of 250 μl, Tris-HCl (pH 8.5), 25 μmol; NaF, 1.5 μmol; $MgCl_2$ 1 μmol; phosphoenolpyruvate, 1 μmol; pyruvate kinase (rabbit skeletal muscle type I, Sigma Chemical Co.) 5 μg; ATP, CTP, GTP, 0.15 μmol each; unlabeled UTP, 0.025 μmol (P-L Biochemicals, Inc., Milwaukee, Wis.); [^{3}H]UTP, 1.5 μCi (sp act 30–50 mCi/mmol, New England Nuclear, Boston, Mass.); and 100 μl of nuclei (100–200 μg DNA) suspended in TGMED. 1 μg of α-amanitin (Calbiochem, La Jolla, Calif.) was also added to the reaction mixture except where noted.

At high ionic strength, in the presence of $(NH_4)_2SO_4$, the buffer was Tris-HCl (pH 7.5), 25 μmol, and $MnCl_2$, 0.2 μmol, was substituted for $MgCl_2$. 50 μl of a saturated solution of $(NH_4)_2SO_4$, neutralized to pH 7.5 with NH_4OH, was added to give a final concentration of 0.4 M, and only 50 μl of nuclei (50–100 μg DNA) suspended in TGMED was added to the reaction mixture. Except for the omission of α-amanitin and the addition of $(NH_4)_2SO_4$, the other reagents were present in the same concentrations as in the assay at low ionic strength.

As a consequence of the TGMED diluent used for the final nuclear suspension, the following reagents were also present in the reaction mixture at low ionic strength: Tris-HCl (pH 7.9), 5 μmol; glycerol, 10%; $MgCl_2$, 0.5 μmol; EDTA, 0.01 μmol; and dithiothreitol, 0.25 μmol. At high ionic strength, the respective concentrations were Tris-HCl (pH 7.9), 2.5 μmol; glycerol, 5%; $MgCl_2$, 0.25 μmol; EDTA, 0.005 μmol, and dithiothreitol, 0.125 μmol.

The reaction mixtures were incubated in a shaking water bath at 30°C in room air for 10 min in the absence of $(NH_4)_2SO_4$ and for 30 min in its presence (8). The reaction was stopped by placing the tubes in an ice bath and adding 0.5 ml of cold 10% trichloroacetic acid (TCA) containing 0.04 M sodium pyrophosphate and 0.4 μmol of unlabeled UTP. Torula RNA, 200 μg, (Sigma Chemical Co., St. Louis, Mo.) was added as carrier. After 5 min in ice, 4 ml of 5% TCA-0.02 M sodium pyrophosphate was added and the suspensions were centrifuged at 1,500 *g* for 8 min at 4°C. The supernates were discarded, and the pellets were washed again before being collected on nitrocellulose filters (HA type, 0.45 μm, Millipore Corp., Bedford, Mass.). The filters were washed with 10 ml of 5% TCA-0.02 M sodium pyrophosphate, dried, and placed into counting vials with 10 ml of a toluene-based scintillation fluor. Counting efficiency for tritium was 30%. Nuclear RNA polymerase activity was expressed as picomoles of UMP incorporated into an acid-insoluble product per milligram of nuclear DNA. The results of each assay represent the pooled values of five separate determinations. Student's *t* test was used for analysis of the significance of differences.

Erythropoietin assay. Mice were made polycythemic as described above. 6 days after release from hypoxia, mice with hematocrits of 65% or greater were injected i.p., in groups of five, with either 5 U erythropoietin in 0.5 ml of 0.5% albumin-saline or 0.5 ml of 0.8% albumin-saline solution. 24 h later, 2 μCi of ^{59}Fe as ferrous citrate (sp act 17.6 mCi/mg, Mallinckrodt Chemical Works, St. Louis, Mo.) was injected (i.p.). 48 h later, 200 μl of orbital venous blood was obtained from each animal for determination of ^{59}Fe incorporation into circulating red cells.

RESULTS

Effect of erythropoietin on splenic erythropoiesis. Imprints from the spleens of polycythemic mice taken 5 days after release from hypoxia contained no recognizable immature erythroid cells. After single injection of erythropoietin, a wave of erythropoiesis occurred in the spleen (Table I). Within 8 h, isolated early erythroblasts appeared. By 18 h, the number of early erythroblasts had increased and they now occurred in clusters of three to four cells. There was a shift in maturation of the erythroblast population by 36 h, with the first appearance of late stage erythroblasts, and by 72 h these cells were predominant.

Quantitation of erythropoietin-stimulated erythropoiesis, using ^{59}Fe, indicated a 20-fold increase in red cell

TABLE I

Changes in Spleen Cell Erythroblast Distribution after Erythropoietin Stimulation*

Time interval after injection of erythropoietin (5 U)	Erythroblast stage in percent			Percent erythroblasts (1,000 nucleated spleen cells scored)
	Early	Intermediate	Late	
h				
0.5–3	—	—	—	—
8	100	—	—	1.0
12	100	—	—	0.9
18	100	—	—	1.8
36	15	73	12	10.9
45	3	55	42	21.3
72	1	33	66	26.3

* Imprints were taken from bisected spleens before the isolation of nuclei for the polymerase assay and stained with Wright's stain. 500 nucleated erythroid cells were counted to obtain the differential count, and the number of erythroblasts per 1,000 nucleated splenic cells was determined to obtain the relative change in size of the nucleated erythroid cell population. The data represent the average of two separate determinations.

[1] *Abbreviations used in this paper:* TCA, trichloroacetic acid; TGMED, 0.05 M Tris-HCl (pH 7.9), 25% glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA, and 2.5 mM dithiothreitol.

TABLE II

*Effect of Inhibitors of DNA-dependent RNA Synthesis on Erythropoietin-Stimulated Red Cell Radioiron Incorporation**

Material injected (i.p.)	Percent incorporation of ^{59}Fe into red blood cells at 72 h
Erythropoietin (5 U)	6.6±0.7‡
0.8% albumin-saline	0.3±0.1
Actinomycin D, 10 μg, 1 h before erythropoietin (5 U)	0.4±0.1
α-Amanitin, 8 μg, 1 h before erythropoietin (5 U)	0.8±0.6

*24 h after injection of either erythropoietin, albumin-saline, or an inhibitor followed by erythropoietin, 2 μCi of ^{59}Fe as ferrous citrate was injected (i.p.). 48 h later 200 μl of blood was obtained from each animal for determination of red cell radioiron incorporation.

‡ Mean±SEM.

production over controls, and this effect of erythropoietin was markedly reduced by either actinomycin D or α-amanitin given 1 h before administration of the hormone (Table II).

Assay of nuclear RNA polymerase activity. Nuclear RNA polymerase activity can be divided into three major forms on the basis of divalent cation and ionic strength requirements and sensitivity to inhibition by the fungal toxin α-amanitin (10). Polymerase I activity is optimal at low ionic strength in the presence of Mg^{++} and is resistant to α-amanitin. Polymerase II activity is optimal at high ionic strength in the presence of Mn^{++} and is sensitive to low concentrations of the toxin (less than 1 μg/ml). Polymerase III activity is not sensitive to variation in ionic strength, has a preference for Mn^{++}, and is inhibited only at high concentrations of α-amanitin (greater than 10 μg/ml). The sensitivity of mouse splenic nuclear RNA polymerase activity to varying concentrations of α-amanitin is shown in Fig. 1. In the absence of $(NH_4)_2SO_4$, α-amanitin in concentrations up to 20 μg/ml resulted in only 10% inhibition of [^{3}H]-UTP incorporation; in the presence of $(NH_4)_2SO_4$ there was 90% inhibition with 2 μg/ml of the toxin, and further increments did not result in greater inhibition. These data suggest that polymerase III activity could account for no more than 10% of total splenic nuclear RNA polymerase activity. In the studies to follow, [^{3}H]UTP incorporation at low ionic strength, in the presence of 1 μg/ml of α-amanitin, is designated as polymerase I activity. Incorporation of [^{3}H]UTP occurring under conditions of high ionic strength, in the absence of the inhibitor, is designated as polymerase II activity.

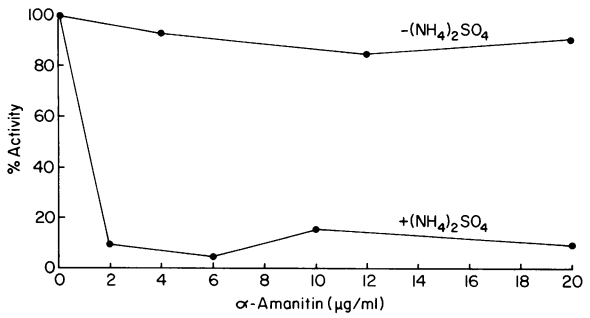

FIGURE 1 Effect of α-amanitin on splenic nuclear RNA polymerase activity in the presence and absence of $(NH_4)_2SO_4$. Nuclei were isolated from the spleens of ex-hypoxic polycythemic mice for assay of RNA polymerase activity 12 h after administration of erythropoietin.

Requirements for optimal in vitro nuclear RNA polymerase activity in the mouse spleen, including the effects of divalent cations, pH, and ionic strength have been described previously (8). Studies demonstrating that incorporation of [^{3}H]UTP into an acid-insoluble product represented the DNA-dependent enzymatic formation of a heteropolyribonucleotide both before and after administration of erythropoietin are shown in Table III. In addition, an estimation of the composition of the RNA synthesized in the presence and absence of $(NH_4)_2SO_4$ was made by comparing the ratio of [^{3}H]UTP to [^{3}H]-GTP incorporated under these conditions (11). In the absence of $(NH_4)_2SO_4$ the U/G ratio was 0.54, suggesting the formation of ribosomal RNA; in the presence of $(NH_4)_2SO_4$ the ratio was 0.82, which is typical of DNA-like RNA ($P < 0.05$) (11).

Effect of erythropoietin on splenic nuclear RNA polymerase activities. The effect of erythropoietin on splenic nuclear polymerase I activity is seen in Table IV and on polymerase II activity in Table V. Variation in the base-line activity levels was observed between different

TABLE III

Characteristics of Nuclear DNA-dependent RNA Polymerase Activity

Conditions	Polymerase I	Polymerase II
	pmol UMP/mg DNA	
Unstimulated nuclei		
No inhibitors or omissions	9.5±0.7*	52.0±6.7
Actinomycin D (50 μg/ml)	0	3.6±1.6
α-Amanitin (1 μg/ml)	8.2	3.0±2.4
RNase (100 μg/ml)	0	6.0±0.6
−ATP, CTP, GTP	3.8	0
After erythropoietin (18 h)		
No inhibitors or omissions	15.6±1.8	112.5±24.6
Actinomycin D (50 μg/ml	5.7±0.6	3.4
α-Amanitin (1 μg/ml	9.9±0.9	3.6±1.6
−ATP, CTP, GTP	4.9±1.2	19.4±3.5

* Mean±SEM.

TABLE IV

*Changes in Spleen Nuclear RNA Polymerase I Activity after Erythropoietin Stimulation**

Time interval after injection of erythropoietin (5 U) or albumin-saline solution	Nuclear RNA polymerase I activity		Percent change
	Control	Erythropoietin	
h	*pmol UMP/mg DNA*		
0.5	23.1±1.0‡	20.1±0.6	NS§
	7.8±0.4	5.9±1.5	NS
1	10.0±0.8	8.0±0.4	NS
	6.3±0.4	6.0±0.3	NS
2	8.1±0.3	7.2±0.4	NS
	13.5±1.5	10.2±1.2	NS
3	11.3±0.5	13.8±0.2	22‖
	15.1±1.0	17.9±1.2	19‖
8	17.4±0.6	30.3±0.6	74‖
	16.4±1.7	24.2±1.8	48‖
12	11.0±0.5	18.3±0.8	66‖
	13.8±0.8	28.9±0.4	109‖
18	11.1±0.4	12.2±0.4	NS
	17.5±1.7	20.7±2.2	NS
36	12.2±0.6	9.6±0.4	NS
	15.6±0.3	13.8±0.6	NS

* Data are shown for two separate assays at each time point.
‡ Mean±SEM.
§ No significant difference.
‖ $P < 0.05$.

experiments and was most marked for polymerase II (Table V, 3 and 36 h). These variations appeared to be due to differences between groups of mice studied at different times. No such variation was observed when repeated experiments were performed with mice taken from the same group.

An increase in polymerase I activity occurred 3 h after administration of erythropoietin at a time when morphologically identifiable erythroblasts were not present (Table IV). Polymerase I activity reached a peak at 12 h and declined by 18 h. In contrast, within 0.5 h after administration of the hormone, there was a significant increase in polymerase II activity (Table V). The increase was transient, subsiding by 2 h. At 12 h, a second increase in polymerase II activity was observed, which disappeared between 18 and 36 h. Peak activity of both polymerase I and II coincided at a time when the splenic erythroblast population consisted only of early forms. With the transition to intermediate and late stage erythroblasts, the activity of both polymerases was not different from that observed in control animals.

Ribonuclease activity in isolated nuclei. The observed differences in nuclear RNA polymerase activity between control and erythropoietin-treated animals could be explained by differences in nuclear ribonuclease activity. This possibility was investigated by measuring ribonuclease activity in the isolated nuclei at each of the time intervals by either the method of Neu and Heppel with yeast RNA as substrate (12) or the enzymatic hydrolysis of [^{3}H]polyuridylate. In both assays, a small amount of nuclease activity was found but was always the same for control and erythropoietin-treated animals (data not shown).

Specificity studies. Since the erythropoietin preparation used in these experiments is impure, several studies were performed to determine whether the observed effects on nuclear RNA polymerase activity were due to erythropoietin as opposed to another foreign protein. In these studies the original experimental protocol was followed, and spleens were obtained 1 and 12 h after injection of erythropoietin or albumin-saline solution. These time intervals were chosen since maximal change

TABLE V

*Changes in Spleen Nuclear RNA Polymerase II Activity after Erythropoietin Stimulation**

Time interval after injection of erythropoietin (5 U) or albumin-saline solution	Nuclear RNA polymerase II activity		Percent change
	Control	Erythropoietin	
h	*pmol UMP/mg DNA*		
0.5	70.2±2.4‡	101.3±1.3	44§
	79.7±2.1	94.3±2.8	18§
1	30.5±3.4	51.0±3.7	67§
	31.4±1.0	46.1±4.4	47§
2	108.9±6.9	80.9±6.2	NS‖
	95.7±4.5	90.7±6.8	NS
3	29.8±5.9	22.9±3.2	NS
	76.3±1.9	79.6±2.4	NS
8	56.1±3.9	58.0±4.3	NS
	53.7±3.2	53.2±4.1	NS
12	40.0±3.1	77.3±7.9	93§
	49.4±4.7	75.7±7.3	53§
18	65.3±2.0	87.8±3.9	34§
	68.5±5.2	89.7±5.4	31§
36	34.4±1.0	29.8±2.1	NS
	66.2±3.4	59.5±5.4	NS

* Data are shown for two separate assays at each time point.
‡ Mean±SEM.
§ $P < 0.05$.
‖ No significant difference.

TABLE VI

Effect of Erythropoietin on Nuclear RNA Polymerase Activity in the Normal Mouse Spleen

Time interval after injection of erythropoietin (5 U) or albumin-saline solution	Nuclear RNA polymerase activity		Percent change
	Control	Erythropoietin	
h	*pmol UMP/mg DNA*		
Polymerase I			
1	5.4±0.3*	5.3±0.2	NS‡
12	5.9±0.1	12.0±0.4	103§
Polymerase II			
1	25.1±2.5	49.8±8.4	98§
12	37.1±1.8	55.6±4.3	50§

* Mean±SEM.
‡ No significant difference.
§ $P < 0.05$.

in polymerase activity was observed at 1 and 12 h in the ex-hypoxic polycythemic mouse.

When normal mice were injected with erythropoietin, the changes in nuclear RNA polymerase activity were identical to those found in the ex-hypoxic polycythemic mouse (Table VI). In the normal mice approximately 10% of spleen nucleated cells were erythroid.

The effect of erythropoietin on polymerase activity was then examined in mice whose spleens were active

TABLE VII

*Effect of Erythropoietin on Nuclear RNA Polymerase Activity in the Ex-hypoxic Erythroid Mouse Spleen**

Time interval after injection of erythropoietin (5 U) or albumin-saline solution	Nuclear RNA polymerase activity		Percent change
	Control	Erythropoietin	
h	*pmol UMP/mg DNA*		
Polymerase I			
1	31.2±0.9‡	29.4±1.8	NS§
12	14.0±0.7	14.5±1.0	NS
Polymerase II			
1	22.6±2.0	17.7±2.3	NS
12	61.2±1.7	60.4±3.8	NS

* Splenic erythropoiesis was induced by exposing mice to reduced oxygen tension for 1 wk in dimethyl silicone membrane cages. The mean hematocrit after 1 wk of hypoxia was 71%, the mean reticulocyte count 8%, and approximately 50% of the nucleated cells from splenic imprints were erythroid precursors.
‡ Mean±SEM.
§ No significant difference.

TABLE VIII

Effect of Acid Desialation on Erythropoietin-Stimulated Nuclear RNA Polymerase Activity

Time interval after injection of desialated erythropoietin (5 U) or albumin-saline solution	Nuclear RNA polymerase activity		Percent change
	Control	Erythropoietin	
h	*pmol UMP/mg DNA*		
Polymerase I			
1	15.1±0.4*	12.7±0.9	NS‡
12	7.9±0.8	6.9±0.5	NS
Polymerase II			
1	104.1±3.6	89.2±3.1	NS
12	89.2±3.1	81.3±4.5	NS

* Mean±SEM.
‡ No significant difference.

in erythroid cell production. For these experiments mice were housed in dimethyl silicone membrane enclosures for 1 wk. 12 h after removal from the hypoxic environment, the mice were injected with either erythropoietin or albumin-saline solution. Approximately 50% of the nucleated cells obtained on imprints of these spleens were erythroid, the majority of which were intermediate stage erythroblasts. No change in nuclear RNA polymerase activity was observed in erythropoietin-treated animals as compared with controls (Table VII). Polymerase II activity was lower than expected in the first 13 h after hypoxia in both control and erythropoietin-

TABLE IX

Changes in Liver Nuclear RNA Polymerase Activity after Erythropoietin Stimulation

Time interval after injection of erythropoietin (5 U) or albumin-saline solution	Nuclear RNA polymerase activity		Percent change
	Control	Erythropoietin	
h	*pmol UMP/mg DNA*		
Polymerase I			
1	45.0±2.8*	49.4±2.1	NS‡
12	67.0±2.8	73.3±2.4	NS
18	63.8±1.7	51.8±3.7	NS
Polymerase II			
1	239.0±2.7	236.8±11.8	NS
12	290.6±8.0	250.5±14.5	NS
18	258.4±14.6	256.4±30.0	NS

* Mean±SEM.
‡ No significant difference.

treated mice. This was a consistent observation and could not be explained by differences in spleen DNA content, ribonuclease activity, or substrate concentration.

The ability of a biologically inactive erythropoietin preparation to stimulate splenic nuclear RNA polymerase activity was also examined. Erythropoietin and albumin-saline solution were heated at 80°C for 30 min in the presence of 0.03 M HCl (pH 2.0) in order to remove the sialic acid residues as described by Goldwasser et al. (13). Before injection, the pH was adjusted to 7.0. Erythropoietin treated in this fashion failed to stimulate in vivo incorporation of ^{59}Fe into red cells and also failed to stimulate splenic nuclear RNA polymerase activity in ex-hypoxic plethoric mice (Table VIII).

Finally, in order to determine whether the effect of erythropoietin on the spleen was organ specific, changes in liver nuclear RNA polymerase were also examined. As shown in Table IX no change in liver nuclear RNA polymerase activity was observed at 1, 12, or 18 h after administration of the hormone.

DISCUSSION

The results of these experiments indicate that erythropoietin stimulates RNA polymerase activity in the nuclei of splenic cells in the ex-hypoxic polycythemic mouse. Although the erythropoietin preparation used was impure, several observations suggest that we are not observing a nonspecific phagocytic or immunologic response of the mouse spleen to foreign protein. First, nuclear RNA polymerase activity was stimulated by the erythropoietin preparation but not by bovine serum albumin, a protein of similar size and from a closely related species. Second, the minimally erythroid spleen (normal or ex-hypoxic plethoric) was able to respond to erythropoietin while an actively erythroid spleen was not. Third, removal of sialic acid residues resulted in loss of biologic activity (^{59}Fe incorporation into red cells) and, simultaneously, the ability to stimulate RNA polymerase activity. Finally, the erythropoietin preparation did not stimulate nuclear RNA polymerase activity in liver, another organ containing reticuloendothelial cells.

Activation of transcription in the spleen occurred within 0.5 h of administration of the hormone when no morphologically identifiable erythroid precursors were present. This correlates well with the observation of Orlic et al. that [^{3}H]uridine could be identified in spleen cell nuclei by autoradiography within 1 h after injection of erythropoietin (1). Maximal stimulation of transcription occurred 12 h after erythropoietin, at a time when only early erythroblasts were present in the spleen. By 36 h, when the erythroid cell population consisted mainly of intermediate and late stage normoblasts, nuclear RNA polymerase activity had declined to control levels. The decrease in polymerase activity is consistent with the morphologic changes (reduction in cytoplasmic ribosomes, condensation of nuclear chromatin) which are observed in erythroblasts in the later stages of maturation (14).

Stimulation of transcription by erythropoietin involved multiple forms of nuclear RNA polymerase and activation of these was sequential. The earliest change following administration of the hormone was a transient increase in the activity of polymerase II, the enzyme that transcribes a DNA-like RNA. Neither the RNA transcribed at this time nor the cell involved have been identified. However, in vivo inhibition of erythropoietin-mediated red cell ^{59}Fe incorporation by α-amanitin, a specific inhibitor of polymerase II, suggests that the early transient increase in polymerase II activity has a physiologic role in erythropoiesis. A similar transient increase in polymerase II activity has been observed in rat uterine nuclei after treatment with 17β-estradiol, (15) and in chick oviduct nuclei polymerase II activity increased before polymerase I activity after injection of diethylstilbestrol (16). In vivo, administration of α-amanitin was found to inhibit cortisone-induced activation of transcription in rat liver (17) and induction of phosvitin production in chicks by 17β-estradiol (18). These observations suggest that stimulation of polymerase II activity is a common property of many growth-promoting hormones. In contrast, during phytohemagglutinin-stimulated lymphocyte transformation, activation of polymerase I precedes that of polymerase II by 2 h (19).

The activation of multiple nuclear RNA polymerases by erythropoietin provides an enzymatic basis for the synthesis of a wide spectrum of RNA molecules observed by Gross and Goldwasser after the addition of erythropoietin to rat bone marrow cultures (3). The more limited number of RNA species produced in vitro in response to erythropoietin by mouse fetal liver cells appears to be due to a restriction of the cell population to mainly proerythroblasts and basophilic erythroblasts (4).

Although our data provide evidence that erythropoietin activates transcription in target cells, the mechanism for this remains unresolved. An increase in nuclear RNA polymerase activity can be the result of an increase in the number of polymerase molecules, a modification of the enzyme resulting in an increase of activity, or an alteration in chromatin template capacity. In connection with the latter possibility, one of us has recently reported that an increase in spleen chromosomal protein synthesis occurred within 3 h of administration of erythropoietin (20). The relationship of this alteration of chromatin composition to polymerase activity remains to be determined.

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