BALANCED ACCUMULATION OF α- AND β-GLOBIN mRNA IN DIFFERENTIATING FETAL MOUSE ERYTHROID CELLS

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1. Introduction

Mammalian reticulocytes synthesize nearly equal amounts of α- and β-globin chains. Mouse and human reticulocytes, also, contain nearly equal amounts of α- and β-globin mRNA, as estimated by molecular hybridization to purified complementary DNA, or by electrophoresis in formamide polyacrylamide gels [1–3]. The relative accumulation of α- and β-globin mRNA during the induction of erythroid differentiation by dimethylsulfoxide in cultures of murine erythroleukemic cells has been measured [1]. They report that early in induction α-mRNA is present in excess ($\alpha/\beta = 3.7$). The α/β ratio approaches 1 at later times. We have recently confirmed these findings [4]. However, in the erythropoietic spleens of mice recovering from phenylhydrazine-induced anemia, the poly(A)-rich RNA fraction contains a 2-fold excess of β-globin mRNA [2]. In humans, unequal amounts of α- and β-globin mRNA are found in patients with thalassemia syndromes [5,6]. In all these instances, in which the amounts of α- and β-globin mRNA were measured directly, erythroid differentiation is abnormal either because of viral transformation (murine erythroleukemia cells), hematopoietic stress (phenylhydrazine-treated animals) or genetic defect (thalassemias).

In this report we have studied the accumulation of α- and β-globin mRNA in fetal mouse erythroid cells at different stages of differentiation. These cells have been used extensively for the study of erythroid differentiation. Our results indicate that the α/β mRNA ratio in erythroid precursor cells and in peripheral blood reticulocytes is close to one and that the α/β ratio remains unchanged during erythroid differentiation.

2. Materials and methods

Fetal-liver erythroid cells were prepared from white mice (CD-1) on day 13 of gestation and were purchased from Charles River Laboratories, (Wilmingon, MA). Erythroid precursor cells were isolated from total fetal-liver erythroid cells by selective immune cytolysis in the presence of complement and a rabbit antiserum prepared against adult mouse erythrocytes [7]. Cultures of erythroid precursor cells were carried out in the presence of 0.3 U/ml human urinary erythropoietin, as described [8]. For morphologic identification the cells were stained with benzidine and Wright-Giemsa stain. Total RNA from erythroid cells was extracted twice with phenol (55°C) and a third time with ice-cold phenol, as described [9]. The cDNA probes were prepared using reverse transcriptase from avian myeloblastosis virus and purified α- and β-globin mRNA.

The two mRNAs were separated by preparative polyacrylamide gel electrophoresis in formamide according to [10]. The isolated α- and β-globin mRNA, assayed in a wheat germ cell-free system, gave 98% α- and 98% β-globin, respectively. The purity of the α- and β-cDNA probes was estimated by back hybridization to their templates, as greater than 96% for α-cDNA

Table 1

Preparation	% Pro-erythroblasts	% Basophilic erythroblasts	% Hemoglobinized cells	Amount RNA added at half-hybridization with α-cDNA[a]	Amount RNA added at half-hybridization with β-cDNA[a]	α/β mRNA
Total liver-erythroid cells from day 13, fetuses						
prepn A	10	30	60	0.52 μg	0.50 μg	0.96
prepn B	8	27	65	0.40 μg	0.40 μg	1.00
Erythroid precursor cells from day 13 fetuses						
prepn A	38	61	1[b]	10.00 μg	10.00 μg	1.00
prepn B	35	64	1[b]	6.67 μg	6.67 μg	1.00
Erythroid precursor cells cultured for 23 h with erythropoietin	22	63	15	3.00 μg	3.00 μg	1.00
Peripheral blood reticulocytes from phenylhydrazine-treated mice	–	–	100	0.07 μg	0.07 μg	1.00

[a] The values indicated represent the amount of total RNA present in the hybridization mixture which gave 50% protection of the cDNA probes upon hydrolysis of single stranded cDNA with micrococcal nuclease

[b] Most of these cells are mature embryonic erythrocytes of the primitive cell lineage [15]

and greater than 93% for β-cDNA. The sensitivity of the α- and β-cDNA probes to detect differences in the relative amounts of α- and β-mRNA was tested by hybridization to artificial mixtures of α- and β-mRNA preparations. When the control mixture contained 1.5 parts α-mRNA and 1 part β-mRNA ($\alpha/\beta = 1.5$), the hybridization data gave an estimated ratio of 1.6. Conversely, when the control mixture contained an α/β ratio of 0.67, the estimated value was 0.71.

Hybridizations to total RNA were carried out in 10 μl reaction mixtures using 180 pg cDNA. The reaction mixtures were incubated for 20—24 h at 68°C before processing [9].

3. Results and discussion

Cells, prepared from the livers of day 13 fetuses, include all stages of erythroid differentiation. Table 1 shows the cell composition of the preparations used in the present studies. Using an antiserum directed against mouse erythrocytes, almost all hemoglobinized cells are lysed; the remaining cells are proerythroblasts and basophilic erythroblasts (erythroid precursor cells) [7]. These precursor cells proliferate and differentiate in vitro when cultured with erythropoietin [11]. In the preparation in which erythroid precursor cells were cultured for 23 h with erythropoietin, the proportion of hemoglobin-containing cells (cells stained with benzidine) increased from 1% at 0 time to 15%. The same preparation contained 65% hemoglobinized cells after 43 h cultures, indicating that during this period the cells were actively differentiating. Figure 1 shows the relative amounts of α- and β-globin mRNAs in total RNA from reticulocytes and mouse fetal erythroid cells as determined by molecular hybridization with globin-specific c[^{3}H]DNAs. The relative amounts of α- and β-globin mRNA present in cells at different stages of maturation are presented in table 1. In peripheral blood reticulocytes the α/β ratio is 1, in agreement with [1,2]. The antibody-isolated erythroid precursor cell population is contaminated by only 1% hemoglobinized cells. The α/β mRNA ratio in these immature precursor cells is, also, one. This ratio remains unchanged after 23 h culture, by which time there is clear evidence of differentiation as measured by cytologic criteria and

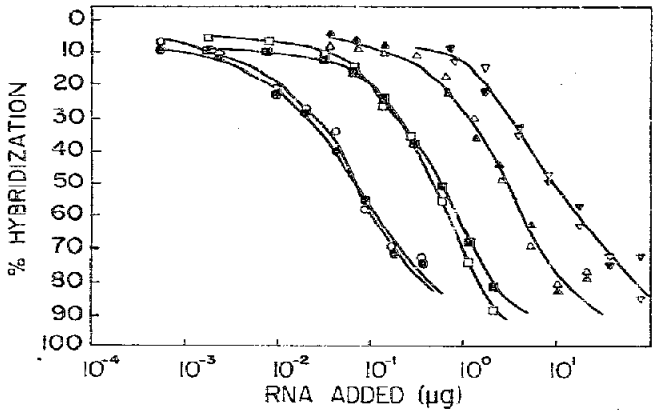

Fig.1. Hybridization of total cellular RNA to α- and β-cDNA. Hybridization to α-cDNA (●,■,▲,▼); hybridization to β-cDNA (○,□,△,▽). Reticulocyte RNA (●,○), RNA from total erythroid cells of day 13 fetuses, prepn A (▼,▽); RNA from erythroid precursor cells of day 13 fetuses, prepn A (▾,▿); RNA from erythroid precursor cells cultured for 23 h with erythropoietin (▲,△).

by the 4-fold increase in the relative amount of globin mRNA (table 1). These results indicate that in fetal mouse erythroid cells the α/β mRNA ratio remains close to unity throughout differentiation.

Evidence for differences in the affinities of α- and β-mRNA for initiation sites, at least in the rabbit, is available [12—14]. It is concluded that balanced synthesis of globin chains cannot be achieved by equal amounts of α- and β-globin mRNA but rather demands an excess of α-mRNA to compensate for the lower affinity of α-mRNA for initiation sites. Whether this also applies to mouse hemoglobin synthesis is not established. The present methodology is not sufficiently sensitive to determine whether the α/β mRNA ratio is exactly one and not slightly higher than one, as suggested [12—14].

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