CONCISE REPORT

Stability of α and β Globin Messenger RNA During Induced Differentiation of Mouse Erythroleukemia Cells

By Roberto Gambari, Richard A. Rifkind, and Paul A. Marks

Murine erythroleukemia cells (MELC) are induced to express erythroid differentiation when cultured with hexamethylene bisacetamide (HMBA). Newly synthesized α and β globin mRNA are both relatively stable, half-life (t1/2) > 50 hr, early in the course of induced differentiation. In fully induced cells there is a decrease in stability of both newly synthesized α and β globin mRNA. The decay of α mRNA is faster, (t1/2, 10–12 hr) than β globin mRNA (t1/2, 20–22 hr). Thus, differences in stability of α and β globin mRNA plays a role in determining the ratio of α to β mRNA content in differentiated erythroid cells.

MELC, derived from DBA/2 mice infected with Friend virus, can be induced to express erythroid differentiation when cultured with one of several types of inducers.1 The differentiation of MELC is characterized by the accumulation of globin mRNA and hemoglobin,3-5 as well as by other biochemical and morphological changes similar to those that occur during normal erythropoiesis.6 At a late stage of induction of MELC the content of α globin mRNA is approximately equal to that of β globin mRNA,7,8 as it is in human, rabbit, and mouse reticulocytes.9-12 During the course of induction by HMBA, accumulation of α globin mRNA precedes that of β globin mRNA by 8–12 hr and, thereafter, α mRNA accumulates at a greater rate than β globin mRNA.7,8 In Me2SO-induced MELC early in differentiation, globin mRNA is relatively stable (t1/2 > 50 hr), whereas later, mRNA decays with a t1/2 of 17 hr, indicating a marked decrease in stability during induction.13 The present experiments show that there are differences in stability of α and β globin mRNA that may be factors determining the final ratio of α and β mRNA content in fully induced cells.

MATERIALS AND METHODS

Cell Culture

MELC, Strain 745A, kindly provided by Dr. Charlotte Friend, was subcloned, and the subclone (DS19) maintained in suspension culture by diluting the cells twice weekly at 10⁶/ml in fresh medium.2 HMBA (final concentration 4 mM) was added to the cultures as indicated below. Cell number and benzidine-reactive cells were determined as previously described.2

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Preparation of Newly Synthesized RNA

Synthesis of RNA was determined by incubation of an aliquot of MELC (2 x 10^6 cells/ml) with 3H-uridine (30-45 Ci/m mole) at the times indicated for each experiment; RNA is extracted from cytoplasmic fractions as previously described. In experiments designed to evaluate the stability of α and β globin mRNA, cells were labeled with 3H-uridine for the periods indicated, then suspended in fresh media as described by Lowenhaupt and Lingrel.13

RNA: cDNA Hybridization

Purified globin mRNA was prepared from DBA/2 mouse reticulocytes. Mouse globin 3H-cDNA was prepared according to Nudel et al.8 The newly synthesized globin mRNA was assayed by oligo(dt) globin cDNA cellulose chromatography.13

Preparation of α and β Globin DNA Filters

Bacteriophage lambda recombinants, CH3AHb117 (containing mouse α globin cDNA) and CH3AHb4 (containing mouse β globin cDNA), derived from the bacteriophage lambda-cloning vector, charon 3A, were a gift of F. R. Blattner. Charon phages were grown under P2-EK2 containment conditions, and DNA was prepared from the phage as described by Blattner et al. Filters containing α or β mouse globin DNA were prepared according to the procedure of Gillespie and Spiegelman.17 DNA prepared from CH3AHb117 or CH3AHb4 was bound at 3–8 μg/filter.

Procedures for 3H-RNA: DNA Filter Hybridizations

Increasing amounts of 3H-RNA (3 x 10^6–2 x 10^6 cpm) were incubated with filters containing α or β globin DNA for 18 hr at 66°C in scintillation vials containing 0.65 M NaCl, 1 mM EDTA, 10 mM TRIS-HCl (pH 7.2), 0.5% sodium dodecylsulfate, 50 μg/ml of poly(A), and 200 μg/ml of yeast tRNA in a final volume of 0.35 ml. After incubation, each filter was washed, treated with pancreatic RNAase (50 μg/ml) (Worthington) for 30 min at 24°C, washed, and radioactivity determined in Aquasol. In preliminary studies, conditions to insure excess of α and β globin DNA relative to 3H-RNA were established. Cytoplasmic 3H-RNA, purified from induced MELC, was separated on a sucrose density gradient; only the fraction corresponding to 9S contained 3H-RNA that hybridized to α or β DNA filters. The amount of 3H-RNA retained by filters with a mixture of α and β globin DNA was approximately equal to the sum of 3H-RNA counts retained on separate α globin DNA and β globin DNA filters (Table 1). This suggests that there is little cross-hybridization of α globin DNA with β globin 3H-RNA or β globin DNA with α globin 3H-RNA. Comparing hybridization of 3H-RNA with globin DNA on filters and with the oligo(dt) globin cDNA cellulose column suggests that the cDNA-cellulose matrix retains some nonglobin RNA sequences (Table 1). Very little 3H-RNA was retained by filters bound with DNA from phage charon 3A (containing no globin DNA insert) (Table 1).

Table 1. Detection of Newly Synthesized Globin mRNA

<table>
<thead>
<tr>
<th>Technique for Hybridization*</th>
<th>Source of 3H-RNA</th>
<th>Uninduced MELC (%)</th>
<th>HMBA-Induced MELC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligo(dt)globin cDNA cellulose chromatography</td>
<td></td>
<td>0.011</td>
<td>0.620</td>
</tr>
<tr>
<td>Filter hybridization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charon-α DNA</td>
<td></td>
<td>0.011</td>
<td>0.253</td>
</tr>
<tr>
<td>Charon-β DNA</td>
<td></td>
<td>0.013</td>
<td>0.184</td>
</tr>
<tr>
<td>Charon-α DNA + charon-β DNA</td>
<td></td>
<td>0.020</td>
<td>0.450</td>
</tr>
<tr>
<td>Charon DNA (no globin DNA insert)</td>
<td></td>
<td>0.006</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*For details of techniques of hybridization see text. 3H-RNA was prepared from uninduced MELC and from HMBA (4 mM) induced MELC after 72 hr of culture.
† 3H-RNA retained on filter or column as percent of applied cytoplasmic 3H-RNA.
RESULTS

Accumulation of Newly Synthesized α and β Globin mRNA

In earlier studies, α and β globin mRNA content was measured by hybridization of cytoplasmic RNA with labeled α and β globin cDNA. In these experiments, the kinetics of accumulation of newly synthesized cytoplasmic α and β globin mRNA (labeled with \(^{3}H\)-uridine) were examined during culture of MELC with HMBA, employing more specific probes, namely, α and β globin DNA contained in cloned charon phage. Cells were cultured with 4 mM HMBA for 20, 40, and 72 hr, then exposed to \(^{3}H\)-uridine for 2 hr to label newly synthesized RNA. By 20 hr, there is an increase in rate of accumulation of newly synthesized cytoplasmic α globin mRNA, but not of β globin mRNA. By 40 and 72 hr in culture, there is an increase in rate of accumulation of both α and β globin mRNA; the rate of accumulation of newly synthesized α globin mRNA exceeds that of β globin mRNA (Table 2). In cells cultured without inducer, there is little or no change in the rate of accumulation of newly synthesized α or β globin mRNA.

Stability of Newly Synthesized α and β Globin mRNA

The observation that the rate of accumulation of newly synthesized α globin mRNA exceeds that of β globin mRNA throughout induction suggested that there may be a difference in the stability of newly synthesized α and β mRNA to account for the approximately equal amounts of α and β globin mRNA found in MELC at advanced stages of differentiation.

In MELC cultured with 4 mM HMBA, globin mRNA content, measured by hybridization of cytoplasmic RNA with \(^{3}H\)-globin cDNA (Fig. 1A), increases during the first 85–90 hr in culture, then decreases. The rate of accumulation of newly synthesized globin mRNA relative to the rate of accumulation of total newly synthesized RNA increases throughout the period of culture, up to 100 hr (Fig. 1B).

To determine the stability of newly synthesized α and β globin mRNA during induction, the following experiments were performed (Table 3). At 36 and 72 hr, aliquots of the culture were removed, incubated with \(^{3}H\)-uridine for 3 hr, and then...
transferred to fresh media and culture continued for an additional 48 hr. During this 48-hr chase, aliquots were removed at 12, 24, and 48 hr to determine the residual content of radioactive α and β globin mRNA. As expected, the accumulation of newly synthesized α globin mRNA is greater than β globin mRNA at both 36 and 72 hr of culture with HMBA. At 36 hr, newly synthesized α and β globin mRNA are stable for at least 50 hr (Table 3, Fig. 2). By 72 hr, there is a marked decrease in the stability of both α and β globin mRNA. A major component of α globin mRNA decays with a $t_1/2$ of 12–14 hr, while a major component of β globin mRNA decays with a $t_1/2$ of 20–22 hr. Precise values for the half-life of α or β globin
Fig. 2. Stability of \(\alpha\) and \(\beta\) globin mRNA in MELC cultured with HMBA for 36 or 72 hr, pulsed with \(\text{\textsuperscript{3}}\text{H}\)-uridine for 3 hr, and chased for up to 48 hr, as described in Materials and Methods; the residual levels of \(\alpha\) and \(\beta\) globin \(\text{\textsuperscript{3}}\text{H}\)-mRNA was determined by hybridizing \(\text{\textsuperscript{3}}\text{H}\)-uridine-labeled cytoplasmic RNA with \(\alpha\) or \(\beta\) charon phage DNA filters. These data are derived from those presented in Table 3.

**Table 3. Stability of Newly Synthesized \(\alpha\) and \(\beta\) Globin mRNA During Induced MELC Differentiation**

<table>
<thead>
<tr>
<th>Culture With Inducer*</th>
<th>Chase (hr)</th>
<th>Total Residual Cytoplasmic (\text{\textsuperscript{3}}\text{H})-RNA</th>
<th>(\frac{\text{\textsuperscript{3}}\text{H}}{\text{\textsuperscript{3}}\text{H}})-Globin mRNA (% of total cytoplasmic (\text{\textsuperscript{3}}\text{H})-RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>0</td>
<td>100</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>100</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>100</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>85</td>
<td>0.07</td>
</tr>
<tr>
<td>72</td>
<td>0</td>
<td>100</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>100</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>100</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>80</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* MELC were cultured with 4 mM HMBA under conditions described in the text. At 36 and 72 hr, aliquots of cells were removed for a 3-hr period of labeling with \(\text{\textsuperscript{3}}\text{H}\)-uridine, followed by chase in fresh medium for 12, 24, and 48 hr.

† Total residual cytoplasmic \(\text{\textsuperscript{3}}\text{H}\)-RNA at each time point of the chase is expressed as a percent of the total \(\text{\textsuperscript{3}}\text{H}\)-cytoplasmic RNA recovered from \(10^6\) cells at initiation of the chase.

mRNA cannot be determined from these data. At 72 hr, the decay curves for both \(\alpha\) and \(\beta\) globin mRNA suggest that each contains at least two species with respect to stability, a major component with a relatively short \(t/2\) (\(\approx\) 20 hr) and a minor component with a considerably longer \(t/2\) (\(>\) 50 hr). By 72 hr, greater than 90% of these cells have become benzidine-reactive (contain hemoglobin), indicating the extent of differentiation induced by HMBA under these conditions.

**DISCUSSION**

This study demonstrates that there is a change in the stability of both \(\alpha\) and \(\beta\) globin mRNA during induced differentiation of MELC. Early in the course of induction, both \(\alpha\) and \(\beta\) globin mRNA are relatively stable, with a \(t/2\) \(>\) 50 hr. By 72 hr, the \(t/2\) of newly synthesized globin mRNA, both \(\alpha\) and \(\beta\), has shortened considerably; the major component of \(\alpha\) globin mRNA has a \(t/2\) of 12–14 hr and that of \(\beta\) globin mRNA, approximately 20–22 hr. Our present findings are in agreement with previous reports\(^{13,15}\) that the \(t/2\) of total globin mRNA early in induction is over 50 hr and falls, later in induction, to about 17 hr.
During HMBA-induced MELC differentiation, the rate of accumulation of newly synthesized α globin mRNA exceeds that of β globin mRNA. Nevertheless, in fully differentiated MELC (as in normal mature erythroid cells), the content of α and β globin mRNA are close to equal. The present findings suggest that in late stages of induced differentiation, the more rapid decay of α than β globin mRNA is an important factor determining the ultimate content of these RNA species.

The decrease in stability of globin mRNA during MELC differentiation could reflect an alteration in mRNA structure, configuration, or an increase in activity of nucleases active in degrading mRNA. The present data are more consistent with a difference in mRNA structure and/or configuration occurring as MELC differentiate. Were there a change in nuclease activity by 72 hr, it would be predicted that a more rapid decay in residual labeled mRNA would have been detected in cells labeled at 36 hr and chased for an additional 48 hr (Fig. 2, left panel). Potential changes in structure or configuration could include changes in degree of polyadenylation at the 3' end of the mRNA, changes in proteins involved in mRNA packaging, or a change in available polyribosomal sites for newly synthesized mRNA.

In normal erythropoiesis, the ratio of the contents of α and β globin mRNA approaches unity in reticulocytes, the most mature cells that still support protein synthesis; the half-life of globin mRNA in these cells is approximately 17 hr. These observations suggest that studies in MELC may provide data relevant to understanding the control of globin mRNA metabolism during normal erythropoiesis.

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