Variable Globin Chain Synthesis in Mouse Erythroleukemia Cells

By Blanche P. Alter and Sabra C. Goff

Various mouse erythroleukemia cell lines show unique responses to chemical inducers. Lines 745 and 707, from DBA/2 mice, produced 25%-48% β-minor and 75%-52% β-major globin following culture with DMSO. Butyric acid treatment led to <40% β-minor globin, while induction with hemin resulted in over 80% β-minor synthesis. Line FSD was developed independently. DMSO induction led to less than 10% β-minor globin, while hemin and butyric acid both resulted in >40% β-minor synthesis. T3C12 and 5000 originated in DDD mice. With these lines all inducers led to only β-major and no β-minor synthesis. The inducers did not influence protein stability or initiation of globin synthesis. Translation of poly A-containing cytoplasmic RNA in a wheat germ cell-free system led to proportions of β-minor that were the same as those seen in the intact cells. Thus regulation of the type of globin chain produced in erythroleukemia cells following culture with several inducing agents occurs at either the level of gene transcription or posttranslational processing, or by growth of a selected cell population.

MOUSE ERYTHROLEUKEMIA CELLS (Friend cells, ELC) grow in vitro and show slight erythroid differentiation that is enhanced markedly with inducing agents such as dimethylsulfoxide (DMSO), butyric acid, or hemin. Uninduced Friend cells produce a small amount of mouse hemoglobin and synthesize adult mouse globin chains in amounts that increase following DMSO induction. The mice in which Friend cells were first developed, DBA/2, have approximately 80% “major” and 20% “minor” hemoglobin due to β chain heterogeneity. The two β chains differ by 6–12 amino acids, depending on the strain of mouse.

Several studies have been reported in which DMSO induction led to varying proportions of total β/α chain synthesis when different Friend cell lines were studied. In addition, Kabat et al. observed that the proportion of β-minor synthesis varied in different lines. We previously reported that the percentage β-minor globin synthesized in a single line (745) varied with different inducing agents, and we suggested that these agents acted either at the level of globin gene transcription or by selective enhancement of the growth of cells within the mass culture that were preprogrammed for synthesis of a specific β chain. The inducing agents might act at the levels of initiation of translation or posttranslational stabilization of protein. The agents might also influence transcription, processing, or stabilization of specific globin mRNA.

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We have now examined the proportion of β-minor synthesis following the induction of several different lines of Friend cells with a variety of unrelated agents. Within each related line of ELC, the amount and type of globin produced depends on the inducing agent and is characteristic of each ELC group. We examined globin chain stability, the initiation of globin mRNA translation, and the product of cell-free protein synthesis by poly A-containing cytoplasmic RNA. Our findings suggest that the induction of globin synthesis depends on the specific interaction of erythroid precursors with their external milieu (the inducing agents) and is due to either the regulation of transcription, differential posttranscriptional stabilization of mRNA, or the selective propagation and maturation of cells whose specific type of globin synthesis was predetermined.

MATERIALS AND METHODS

Cell cultures. Friend cells were grown in suspension in Dulbecco Modified Eagle Medium with 10% fetal calf serum (FCS) (Flow Laboratories, Rockville, Md.) as described previously. A single lot of serum was used for all experiments. Group A (Table 1) stocks were passed thrice weekly at 5 x 10^4 cells/ml and groups B and C at 2 x 10^4 cells/ml. Line 745-IMR was purchased from the Institute for Medical Research, Camden, N.J. 745-9M, 707, and TELC were kind gifts of Dr. Albert Deisseroth. 745-9M had been subcloned by him from 745-IMR and then grown in mass culture for more than 1 yr. TELC had been formed by the fusion of enzyme-deficient clones of 745-9M and 707. T3C12 and 5000 had originated from a single T3C12 line cultured at the NIH by Dr. Stuart Orkin. T3C12 was maintained in continuous culture by Dr. Robert Troxler, while 5000 had been recloned and then grown by Dr. Orkin. Line FSD-B8 was kindly provided by Dr. David Housman.

Induction. DMSO (Fisher Scientific, Medford, Mass.) was stored frozen and added directly to cultures; final concentration was 2% (280 mM) for group A and 1.5% (210 mM) for groups B and C. Hemin was prepared as described previously and used at a final concentration of 75 μM. Butyric acid (Aldrich Chemical, Metuchen, N.J.) was used at 1 mM final concentration. Hexamethylenebisacetamide (HMBA), a gift of Dr. Roberta Reuben, was dissolved in medium and used at 5 mM final concentration.

Globin synthesis. On the fourth or fifth day after induction, 10^7 cells were centrifuged, resuspended in medium containing 3H- or 14C-leucine (New England Nuclear, Boston, Mass.; 3H, 100 Ci/mM; 14C, 270 mCi/mM), and labeled for 4 hr (except as indicated in Results). The cells were harvested and lysed and the lysates analyzed by electrophoresis on sodium dodecyl sulfate (SDS) polyacrylamide gels in urea followed by carboxymethylcellulose (CMC) chromatography of the globin chains, as we described previously. Double-label experiments were analyzed by counting 3H with <1% spill into the 14C channel and 14C with approximately 15% spill into the 3H channel. Data were corrected for the spill ratios. The relative amount of globin synthesis compared to total protein synthesis was calculated as follows:

\[
\text{Percent globin} = \frac{\text{Counts in globin region}}{\text{Counts in } \beta-\text{major + } \beta-\text{minor + } \alpha \text{ regions}} \times \frac{\text{Total counts on SDS gel}}{\text{Total counts on CMC column}} \times 100.
\]

### Table 1. Types of Erythroleukemia Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Line Designation</th>
<th>Mouse Strain</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>745</td>
<td>DBA/2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>TELC</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>707</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>T3C12</td>
<td>DDD</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>C</td>
<td>FSD-B8</td>
<td>DBA/2</td>
<td>15, 22</td>
</tr>
</tbody>
</table>
The proportion of $\beta$-minor synthesis was determined from the CMC columns:

\[
\text{Percent } \beta\text{-minor } = \frac{\beta\text{-minor counts}}{\beta\text{-major + } \beta\text{-minor counts}} \times 100.
\]

The range of precision of this calculation was determined to be ±5% in triplicate analyses of DMSO-induced lysates and ±10% following hemin induction.

**Hemoglobin content.** Blood was obtained from mice of several strains, washed in saline, and lysed in 4 vol. 1 mM phosphate pH 7.4, and stroma were removed with CCl₄:toluene (2:1). Lysate (25 $\mu$g) was electrophoresed on 7.5% polyacrylamide gels at pH 8.9 and then stained with Amido black.²⁴

**RNA preparation.** DBA/2 mice were injected with 0.1-0.2 ml 0.8% phenylhydrazine (in 0.15% sodium acetate with the pH adjusted to 7.0) given intraperitoneally as three injections at 12-hr intervals. Mice were bled on the seventh day. The cells were washed in saline, lysed with 10 vol cold distilled water, and centrifuged at 12,000 g for 30 min to remove membranes. The supernatant was adjusted to final concentrations of 0.02 M Tris, 0.1 M NaCl, 0.001 M EDTA, and 1% SDS.

**Protein synthesis** was analyzed in a wheat germ cell-free system.²⁸ Each 50 $\mu$l assay contained 0.5 $\mu$g (50% saturation) of reticulocyte or 5 $\mu$g of ELC poly A-containing RNA, 15 $\mu$g of wheat germ S-30, and buffer and salts as described.²⁸ We found that 5 $\mu$g ELC RNA was well below saturation; materials were insufficient to determine the saturation point. Globin mRNA is less than 0.02% of total cellular RNA in induced ELC,²⁸ and poly A-containing RNA was 0.5%-2.5% of cytoplasmic RNA in our experiments. Thus 5 $\mu$g of cytoplasmic poly A-containing RNA contained much less than 0.5 $\mu$g of globin mRNA. Optimal K⁺ concentration was 80 mM and Mg²⁺ 3 mM. Each incubation contained 16 $\mu$Ci $^3$H-leucine (80 Ci/mM). Incubations were for 1 hr at 25°C. Then 10 $\mu$l of the samples was treated with 50 $\mu$l pancreatic ribonuclease A (1 mg/ml) for 20 min at 37°C. Trichloroacetic acid (TCA)-precipitable counts were determined as we described previously.¹⁷ The ribonuclease-treated samples were electrophoresed on SDS-polyacrylamide gels and the globin region recovered and analyzed on CMC columns as described.¹⁷

In one experiment, 0.5 $\mu$g of reticulocyte RNA was translated in the wheat germ system in the presence of increasing concentrations of hemin. The translation products were analyzed directly on CMC columns.

**RESULTS**

The Friend cells studied fall into three groups (Table 1). They all originated with Friend leukemia virus supplied by Dr. Charlotte Friend, but the lines were developed in different strains of mice and maintained in different parts of the world. All these strains of mice have both major and minor hemoglobins.¹⁰,²⁹ (Fig. 1).

All lines that we examined were inducible with DMSO. By day 4 or 5, 8%–20% of newly synthesized protein was globin (Table 2). In uninduced cells 0.02%–0.4% of new protein synthesis was globin at this time. Induction with
hemin was more variable, and globin production was only greater than that of uninduced cells in lines 745-9M and 745-IMR of group A and in FSD. There was also some variation in the responses to butyric acid. In group A, only 745-9M was inducible; induction was rapid and was complete within 2 days.\(^7\) Cells of groups B and C were inducible with butyric acid. Line 745-9M cells were also cultured with combinations of hemin and DMSO or HMBA, and these agents also led to globin synthesis.

Induction with the different agents also led to unique patterns of globin chain synthesis (Table 2). DMSO induction of the group A lines resulted in 25%-48% $\beta$-minor globin, similar to the 20%-25% $\beta$-minor globin seen in DBA/2 mice. Hemin led to over 80% $\beta$-minor globin synthesis in those lines induced by hemin (745-9M and 745 IMR). Although uninduced cells also produced over

| Table 2. Comparison of Globin Synthesis (%) in Intact Erythroleukemia Cells |
|-------------------|----------------|----------------|----------------|----------------|
|                  | None | DMSO | Hemin | Butyric Acid |
| Group | Line    | Globin | $\beta$-minor | Globin | $\beta$-minor | Globin | $\beta$-minor | Globin | $\beta$-minor |
| A     | 745-9M  | 0.4    | 78       | 19.8  | 25          | 3.0    | 81           | 2.4†  | 37‡         |
|       | 745-IMR | 0.2    | 76       | 9.5   | 27          | 1.2    | 87           | 0.4   | 61          |
|       | 707     | 0.2    | 74       | 8.3   | 48          | 0.5    | 84           | 0.1   | 85          |
|       | TELC    | 0.1    | 74       | 8.5   | 31          | 0.2    | 92           | 0.3   | 69          |
| B     | T3C12   | 0.1    | 0        | 10.8  | 0           | 0.4    | 0            | 2.6   | 0           |
|       | 5000    | 0.03   | 0        | 9.2   | 0           | 0.3    | 0            | 10.9  | 0           |
| C     | FSD     | 0.02   | 23       | 9.0   | 2           | 1.3    | 39           | 2.4   | 37          |

Cells were cultured with inducers for 4 or 5 days, then labeled with $^3$H-leucine for 4 hr.

* Globin as percentage of total protein synthesized; see Materials and Methods.
† Percentage $\beta$-minor/total $\beta$ chain synthesized; see Materials and Methods.
‡ Analysis on day 2.
70% β-minor globin, hemin induction led to >1% globin synthesis, while the uninduced cells produced <0.5% globin. Thus hemin led to overall globin stimulation. Induction of 745-9M with butyric acid led to 37% β-minor globin. The CMC column patterns of the studies in 745-9M mice were published previously. When 75 μM hemin and DMSO were used simultaneously on cells of 745-9M mice, β-minor synthesis was 34% when the DMSO level was 2% and 58% when the latter was 0.5%. The presence of DMSO increased the total globin synthesis from 3% with hemin alone to 8% and 12% in the combinations, but the percentage of β-minor globin synthesis depended on the hemin:DMSO ratio. Results from culture with HMBA resembled those with DMSO. There was 15% globin synthesis (29% β-minor).

Figure 2 shows the CMC patterns following induction of T3C12 with DMSO and 5000 with butyric acid. Globin synthesis did significantly increase, but it was all β-major and α chain. Culture with hemin led to only slight induction of globin, but β-minor synthesis remained undetectable.

Line FSD was inducible with all three agents (Fig. 3). However, the proportion of β-minor synthesis remained less than in group A. There was 2%-10% β-minor synthesis following DMSO induction and close to 40% with hemin or butyric acid (Table 2). Thus the general pattern of globin synthesis and type of β chain produced was different with each inducer in each group of ELC.

In all experiments in which globin synthesis was induced, total β/α synthetic ratios were approximately 1.

To determine if culture of ELC with inducing agents altered the stability of globin chains, line 745-9M cells were induced for 5 days with DMSO or hemin. 4C-leucine was used to label the cells for 4 hr, and 3H-leucine was added for the last 15 min. The results are shown in Table 3. The proportion of globin synthesis was higher with the longer incubation time, which indicates that some of the nonglobin proteins produced by ELC are more labile than globin. However, the percentage of β-minor synthesis in induced cells was similar with short and long

![Fig. 2](image.png)

**Fig. 2.** CMC chromatography of globin from group B erythroleukemia cells. Cells were cultured with inducers for 5 days and labeled for 4 hr with 3H-leucine. o, counts per minute; o, A280 (280 nm) of carrier DBA/2 globin. (A) T3C12, induced with 210 mM DMSO; (B) 5000, induced with 1 mM butyric acid.
incubation times. Thus hemin does not result in a relative excess of β-minor synthesis because of stabilization of β-minor relative to β-major globin.

The influence of hemin on globin chain translation was evaluated in the wheat germ cell-free system. When DBA/2 reticulocyte mRNA was translated, 30% of the protein synthesized cochromatographed on the CMC columns with globin chains, and 25% of the β chain was β-minor (Table 4). Addition of low concentrations of hemin led to similar results and did not stimulate overall protein synthesis. As the hemin concentration rose to ≥50 μM, total globin synthesis was actually inhibited, as was total protein synthesis (data not shown). The

<table>
<thead>
<tr>
<th>Table 3. Protein Turnover</th>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>DMSO</td>
</tr>
<tr>
<td>Hemin</td>
</tr>
</tbody>
</table>

Line 745-9M cells were cultured with inducers for 5 days and then labeled for 4 hr with 14C-leucine; 3H-leucine was added for the last 15 min. Globin and β-minor percentages were determined as described in Materials and Methods.

* Total counts were very low.
Cells were cultured with inducers for 4 days. Numbers represent percentage of $\beta$-minor synthesis as described in Materials and Methods.

Table 4. Effect of Hemin on Globin Synthesis in Wheat Germ Cell-free System

<table>
<thead>
<tr>
<th>Addition</th>
<th>$\alpha$-Globin (%)</th>
<th>$\beta$-Minor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Buffer</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Hemin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 $\mu$M</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td>30 $\mu$M</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>50 $\mu$M</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

DBA/2 mouse reticulocyte mRNA was used in all assays.
* See Materials and Methods.
† $\beta$-Minor/total $\beta$ radioactivity x 100.

percentage of $\beta$-minor synthesis began to decrease. Thus addition in vitro of hemin did not lead to increased initiation of mRNA for $\beta$-minor globin.

The cell-free translation system was used to examine the type of translatable globin mRNA present in the cytoplasm of ELC following induction. As shown in Table 5, 25% $\beta$-minor globin was synthesized in the cell-free system in which DBA/2 reticulocyte mRNA was used at half saturation, and 25% $\beta$-minor globin was synthesized by intact DBA/2 reticulocytes. In the intact cells of line 745, the percentage of $\beta$-minor globin produced was 84% in the control, 28% with DMSO, 90% with hemin, and 60% with butyric acid. Translation of poly A-containing RNA in the wheat germ cell-free system led to 82%, 31%, 83%, and 43% $\beta$-minor synthesis from the control-, DMSO-, hemin-, and butyric acid-induced cultures, respectively. When line FSD was used for these studies, the percentage of $\beta$-minor globin synthesized was again almost identical in intact cells and in the products of cell-free translation of isolated poly A-RNA. The production of $\beta$-minor globin that occurred in intact cells was thus reflected in the translation products of the poly A-containing RNA from these cells.

DISCUSSION

The Friend murine erythroleukemia cell system has been used extensively as a model in vitro for erythroid differentiation. The studies described in this manuscript suggest that these cells may provide a model in vitro for hemoglobin regulation as well as cellular differentiation. The different ELC lines are hetero-

Table 5. Comparison of $\beta$-Minor Synthesis (%) in Intact Cells and in Cell-free Translation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Inducer</th>
<th>None</th>
<th>DMSO</th>
<th>Hemin</th>
<th>Butyric Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBA/2 mouse</td>
<td>Intact cell</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cell-free</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Line 745-IMR</td>
<td>Intact cell</td>
<td>84</td>
<td>28</td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Cell-free</td>
<td>82</td>
<td>31</td>
<td>83</td>
<td>43</td>
</tr>
<tr>
<td>Line FSD</td>
<td>Intact cell</td>
<td>47</td>
<td>8</td>
<td>27</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Cell-free</td>
<td>36</td>
<td>12</td>
<td>27</td>
<td>48</td>
</tr>
</tbody>
</table>

Cells were cultured with inducers for 4 days. Numbers represent percentage $\beta$-minor synthesis as described in Materials and Methods.
geneous in their origins, chromosome numbers \(^{19,30}\) (also Alter BP: unpublished observations), and maturation with inducing agents. We show here that they are also heterogeneous in the type of globin chain synthesized after culture with inducing agents. This variation in percentage \(\beta\)-minor synthesis may be a model for the human \(\gamma\)-\(\beta\) switch that occurs during ontogeny and that is abnormal in various disorders. \(^{31}\) Data recently published by Whitney \(^{32}\) provide further support for this suggestion: in mouse ontogeny, the relative synthesis of \(\beta\)-minor/ \(\beta\)-major globins declines. Thus shifts in the relative proportions of \(\beta\)-minor and \(\beta\)-major globins may provide a model for the human \(\gamma\)-\(\beta\) switch.

Several studies have now confirmed that ELC of lines 745 or 707, i.e., what we have termed group A, produce approximately 25\%–30\% \(\beta\)-minor synthesis when induced with DMSO \(^{16-18}\) (Table 2), 40\% with butyric acid, \(^{17,18}\) and over 75\% with hemin \(^{17,33}\) or in the small amount of globin made in uninduced cells. \(^{17,34}\) In contrast, FSD produced less \(\beta\)-minor globin than cells in group A with any agent, although the relative trends are similar—less than 10\% with DMSO \(^{16}\) (Table 2) and 25\%–40\% with butyric acid or hemin—as well as in uninduced cells (Table 2). Our lines of group B, T3C12 and 5000, did not produce \(\beta\)-minor globin with any agent examined, although they did produce \(\beta\)-major globin when induced with DMSO or butyric acid. The line of T3C12 that Ross found to induce with hemin \(^8\) also produced only \(\beta\)-major globin (data not shown). One previous report also indicated that T3 cells made only one hemoglobin. \(^{29}\)

The catalogue of ELC response to inducers shown in Table 2 may be instructive with regard to the mechanism of \(\beta\) chain regulation. In the lines that produce some \(\beta\)-minor globin, DMSO induction led to the smallest proportion, butyric acid to more, and hemin to the most. However, since overall induction of globin compared to total protein was less with the latter two agents, the total amount of \(\beta\)-minor relative to protein was similar with all three inducers. \(^{17}\) This observation led us to suggest previously \(^{17}\) that the inducers might act by promotion of the growth of cells destined for the synthesis of a specific globin chain. For example, DMSO would stimulate growth of \(\beta\)-minor cells (25\%) as well as \(\beta\)-major cells (75\%), while hemin would stimulate growth of only \(\beta\)-minor cells. Thus \(\beta\)-major globin predominates when globin synthesis comprises a high proportion of total protein production. Further support for the cell selection model is provided by our analysis of group B cells. These lines came from DDD mice, which produce two hemoglobins. At some time during the cloning of these lines, the single cell that was propagated happened to be a "\(\beta\)-major" cell. The lines of T3 that we examined were induced poorly with hemin, which by our model should induce only \(\beta\)-minor cells, which are lacking in these lines.

Although cells of line 745 have been cloned in the past, our work was done from mass cultures. The terms "\(\beta\)-major" and "\(\beta\)-minor" cells refer to cells whose phenotypic expression is unique. They do not refer to true clones, since they are not genetic mutants. In man, erythroid cells contain the genetic material for the synthesis of both fetal and adult hemoglobin, but they may not express both simultaneously. During ontogeny, erythrocytes contain both fetal and adult hemoglobins. Whether they synthesize both simultaneously or sequentially remains to be clarified. The cells of line 745 with which we worked may
still have the potential for synthesis of both \( \beta \)-major and \( \beta \)-minor globins, although environmental influences could determine which choice was relevant in a specific cell and its progeny. In fact, this determination could be made on a stochastic basis, as was proposed for erythroid commitment by Gusella et al.\(^3\) The probability of the progeny of a cell producing \( \beta \)-minor or \( \beta \)-major globin would be determined by the genetics (the line) and the environment (the inducer).

Our data suggest that inducing agents do not affect the relative stability of the two \( \beta \) chains. Although hemin has a role in globin synthesis,\(^3\) perhaps related to initiation of translation of mRNA,\(^3\),\(^3\) this regulatory role may not be critical in the ELC hemoglobin phenotype.

The data shown in Table 5 indicate that the effect of inducing agents on globin synthesis in intact cells is due to alterations in the cytoplasmic mRNA. The poly A-containing RNA that is translatable in the cell-free system reliably reflects the globin synthesis that occurs in intact cells.

Our data fail to distinguish regulation at the level of transcription or of RNA processing from selective cell growth. Both our studies and those of Nudel et al.\(^8\) involved large number of cells and demonstrate the results in a population of cells but do not describe what occurs at the level of the single cell. Further studies must be focused on this level of investigation in order to explain the precise mechanism for the regulation of hemoglobin synthesis in this model system.

**ACKNOWLEDGMENT**

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