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5-Aminolevulinate Synthase Expression and Hemoglobin Synthesis in a Human Myelogenous Leukemia Cell Line[1]

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We examined the effect of hemin, TGF-β1 and cytosine arabinoside (Ara-C) on the levels of mRNAs for the erythroid-specific 5-aminolevulinate synthase (ALAS-E) and γ-globin in various human myelogenous leukemia cell lines. Detailed analyses were also made using one of them, YN-1, which was isolated and established in culture from a patient with chronic myelogenous leukemia. Our results demonstrate that γ-globin protein level and the percentage of benzidine-positive cells in the cell line increased markedly (10- to 30-fold) upon treatment with hemin, TGF-β1, or Ara-C. In contrast, γ-globin mRNA was already markedly expressed prior to treatment in 4 out of 9 cell lines examined, including YN-1, and the level increased only marginally after treatment with hemin. ALAS-E mRNA levels were increased in YN-1 cells after treatment with TGF-β1 and Ara-C, while hemin treatment had little effect. These results indicate that heme supply is insufficient in YN-1 cells and suggest that hemin increases hemoglobin synthesis principally at the post-transcriptional level, whereas TGF-β1 and Ara-C stimulate hemoglobin synthesis by activating efficient endogenous heme supply in the cells.

Key words: 5-aminolevulinate synthase, globin mRNA, hemin, hemoglobin synthesis, human leukemia cells.

The first and the rate-limiting step of heme biosynthesis in animal cells is catalyzed by 5-aminolevulinate synthase (ALAS) [EC 2.3.1.37] (*1*). There are two tissue-specific isozymes of ALAS: one is exclusively expressed in erythroid cells (the erythroid-specific ALAS, ALAS-E, or ALAS2), and the other is expressed ubiquitously in all cells including erythroid cells (the nonspecific ALAS, ALAS-N, or ALAS1) (*2-8*). In murine Friend-virus transformed erythroleukemia (MEL) cells, the level of ALAS-E mRNA was found to increase markedly, while that of ALAS-N mRNA was down-regulated when these cells were induced to undergo erythroid differentiation by treatment with various chemicals including dimethylsulfoxide (Me_2SO) (*9*). This finding suggests that ALAS-E expression may be critically involved in the erythroid differentiation process that requires a large quantity of heme for hemoglobin synthesis. Consistent with this conclusion are the findings that ALAS activity in bone marrow cells of patients with X-linked sideroblastic anemia is markedly decreased (*10-13*), and that a point mutation in the ALAS-E gene has recently been identified in such patients (*12, 13*).

Erythroid differentiation can also be induced in a number of human cell lines established from patients with erythroleukemia or chronic myelogenous leukemia (CML) by treatment with various chemicals, cytokines or growth factors (*14-26*). The spectrum of the inducers of erythroid differentiation in human leukemia cell lines is, however, markedly different from that for MEL cells (*14, 27, 28*). For example, hemin is a potent inducer of erythroid differentiation of human cell lines (*14, 15*), while Me_2SO is not (*15*). In contrast, Me_2SO is a potent inducer of erythroid differentiation of MEL cells, whereas hemin is a relatively weak inducer (*9, 29-31*).

Thus, using human leukemic cell lines, we examined in this study the effect of hemin, and two chemical inducers of erythroid differentiation, *i.e.*, TGF-β1 and Ara-C (*21, 32*), on the levels of benzidine (BZ)-positive cells and mRNAs for ALAS-E and γ-globin. Our results demonstrate that untreated cells of at least 4 human leukemic cell lines expressed substantial amounts of γ-globin mRNA, but the mRNA was not efficiently translated into the γ-globin protein. In one of the cell lines, YN-1, which was studied in detail, it was shown that hemin treatment increased hemoglobin synthesis at the translational level. In contrast, treatment of these cells with Ara-C or TGF-β1 induced ALAS-E expression and increased hemoglobin synthesis.

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Abbreviations: AIA, allylisopropylacetamide; ALA, 5-aminolevulinic acid; ALAS, 5-aminolevulinate synthase; ALAS-E, erythroid-specific 5-aminolevulinate synthase; ALAS-N, non-specific 5-aminolevulinate synthase; Ara-C, cytosine arabinoside; BZ, benzidine; Me_2SO, dimethylsulfoxide: MEL, murine erythroleukemia.

These results indicate that YN-1 cells lack sufficient heme and can be induced to undergo erythroid differentiation either by treatment with hemin or by treatment with agents that can activate ALAS-E expression.

MATERIALS AND METHODS

Cell Culture—YN-1 (*33*), Y-1K (*33*), KYO-1 (*34*), KU812 (*35, 36*), KCL-22 (*37*), NALM-1 (*38*), and K562 (*39*) were Philadelphia (Ph^1) chromosome-positive cell lines established from peripheral blood cells of patients with CML in blastic crisis. Cytochemical and surface marker analyses demonstrated that YN-1 cells were exclusively committed to the erythroid lineage. Hemoglobin analyses showed that HbF, Hb Bart's, and Hb Portland were synthesized in YN-1 cells, the major species being HbF (*33*). Y-1K cells were less differentiated than YN-1 cells in that Y-1K cells did not show any BZ(+) cells before or after chemical treatment (*33*). KYO-1 cells had a unique capacity to differentiate spontaneously along the erythroid and monocytoid lineage when they were initially isolated from a patient with blastic crisis of CML, but after several months of passages this property was significantly reduced (*34*). KU812 cells were leukemic basophil precursors (*35, 36*). KCL-22 cells were positive for acid phosphatase and PAS, and expressed Fc receptors, but they were negative for BZ staining or glycophorin A, the two major erythroid markers (*37*). NALM-1 cells had a specific antigen of acute lymphoblastic leukemia, but expressed no cell-surface or cytoplasmic immunoglobulins (*38*). K562 cells were able to synthesize embryonic hemoglobins such as Hb Gower 1, Hb Portland, and Hb Bart's (*14*).

HL60 was a myelomonocytic cell line (*40*) which was isolated from a patient with acute myelogenous leukemia (M2). KG-1a was an undifferentiated blast cell line that was isolated as a variant subclone of an acute myelogenous leukemia cell line, KG-1 (*41*). These two cell lines were used as non-erythroid control cell lines in this study.

YN-1 and Y-1K cells were cultured in Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum (FBS) and 50 units/ml of penicillin and 50 μg/ml of streptomycin at 37°C in 5% CO_2 (*33*). K562, KYO-1, KCL-22, KU812, NALM-1, KG-1a, and HL60 cells were cultured in RPMI1640 supplemented with 10% FBS and the antibiotics as above.

Induction of Hemoglobin Synthesis in YN-1 Cells—Optimal concentrations of hemin, and Ara-C for hemoglobin induction in YN-1 cells have previously been reported (*33*). An optimal concentration of TGF-β1 for hemoglobin synthesis was determined in this study to be 1 ng/ml. These chemicals were added to culture media which contained 1×10^5 cells/ml, and cells were harvested after various periods of incubation as indicated in the legends to the figures. BZ(+) staining cells were counted at each time point, as described previously (*16*).

RNA Blot Hybridization Analysis—The expression of mRNAs encoding γ-globin, ALAS-E, or ALAS-N was examined by RNA blot hybridization analysis. Total RNA was isolated from 1×10^7 cells by the procedure described previously (*42*). Twenty micrograms of total RNA was electrophoresed in a formaldehyde/1% agarose gel, and electrophoretically transferred to a nitrocellulose filter (Hybond C-extra, Amersham Japan, Tokyo). The JW151 γ-globin cDNA clone and the HG126 clone of the ribosomal RNA gene were obtained from the Japanese Cancer Research Resources Bank (JCRB), Tokyo. The plasmid JW151 was digested with *Nco*I and *Eco*RI, and the resulting 359 bp fragment was isolated, and used as a γ-globin probe.

cDNAs encoding human ALAS-E and ALAS-N were isolated independently in our laboratory. The cDNA fragments which match nucleotides 216 to 1,160, and 750 to 1,338 of the reported human ALAS-E and ALAS-N cDNA sequences, respectively (*43, 44*), were used as probes in this study. The cDNA fragments were labeled by the random primer extension method (*45*) and used as probes. Hybridization and washing of nitrocellulose filters were performed using standard procedures.

Immunoblot Analysis—For immunoblot analysis, 1×10^7 YN-1 cells were homogenized in 100 μl of a solution containing 0.25 M sucrose, 20 mM Tris-HCl (pH 7.4), 0.2 mM dithiothreitol, 0.1 mM pyridoxal 5'-phosphate, and 0.5 mM EDTA. In addition, 10 μg/ml each of leupeptin, chymostatin, antipain, and pepstatin (Peptide Institute, Osaka) were added as protease inhibitors. The homogenate was centrifuged at $600\times g$ for 7 min at 4°C. The supernatant fraction was centrifuged again at $10{,}000\times g$, for 7 min. The resulting precipitate was dissolved directly in 100 mM Tris-HCl (pH 7.4) containing 3.2% SDS, 10% [w/v] glycerol, and 8% [v/v] 2-mercaptoethanol (SDS sample buffer). Mitochondrial fraction from anemic rat blood cells was prepared as described previously (*7*). Positive controls for ALAS-N were obtained by injecting allylisopropylacetamide (AIA) into rats to induce liver expression of ALAS-N (*46*).

For the immunoblot assay of hemoglobin, 1×10^7 cells were homogenized in SDS sample buffer or urea/SDS sample buffer [1% SDS, 8 M urea, 1% 2-mercaptoethanol, and 10 mM sodium phosphate buffer (pH 6.8)], and then centrifuged at $600\times g$ for 7 min. The supernatant was used directly for the immunoblot analysis of hemoglobin. Protein concentration was determined by using a Protein Assay Kit (BioRad). Five micrograms of protein was loaded onto either an 18% Laemmli gel system (*47*) or an urea/SDS gel electrophoresis system (*48*), and electrophoretically separated. Proteins were then transferred to a sheet of polyvinylidene difluoride (PVDF) membrane (Immobilon-P, Millipore, Bedford, MA). After blocking with Tris-buffered saline (TBS) containing 3% skimmed milk at 4°C overnight, the membrane was reacted with the primary antibodies in TBS containing 1% bovine serum albumin (BSA), and subsequently reacted with goat anti-rabbit IgG conjugated with horseradish peroxidase. The immune complex on the membrane was visualized by the enhanced chemiluminescence (ECL) method (Amersham). Antibodies used in this study were rabbit anti-rat ALAS-N antibody (*7, 49*), and anti-human HbF antibody (Calbiochem, La Jolla, CA). The former antibody has been shown to cross-react with rat ALAS-E protein (*7*), and human ALAS-N and ALAS-E proteins (unpublished observation). The latter antibody had been adsorbed against human HbA prior to use.

Immunofluorescent Staining with Anti-γ-Globin Chain Antibody—Smears of YN-1 cells, with or without hemin treatment, were prepared on glass slides using Cytospin 3 (Shandon, Cheshire, UK). Cells were fixed in methanol for 30 min at room temperature and incubated with the HbF

antibody at 1:200 dilution. Then cells were incubated with fluorescein isothianate (FITC)-conjugated goat anti-rabbit-IgG [F(ab')2] for 30 min at room temperature, and examined using a fluorescence microscope (Olympus, Tokyo).

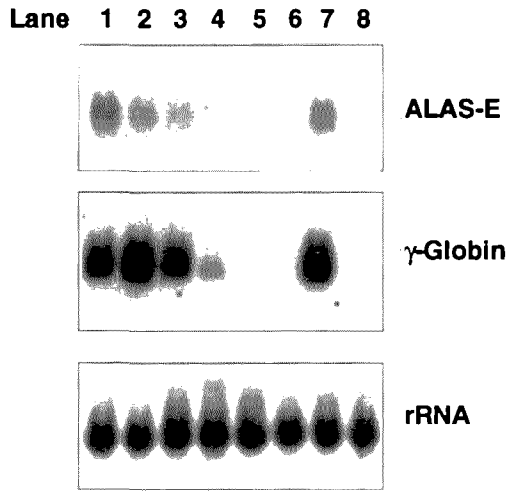


Fig. 1. **RNA blot analysis of γ-globin and ALAS-E expressions in human myelogenous leukemia-derived cell lines.** Human myelogenous leukemia cell lines with erythroid properties express γ-globin mRNA abundantly. K562 (lane 1), YN-1 (lane 2), KU812 (lane 3), Y-1K (lane 4), and KYO-1 (lane 7) are cell lines committed to the erythroid lineage. KG-1a (lane 6) and KCL-22 (lane 7) cell lines are both myeloid cell lines and NALM-1 (lane 8) is a lymphoid cell line; these three cell lines do not show any erythroid marker expression. ALAS-E and γ-globin mRNA expression was analyzed by RNA blotting. The filter was first hybridized to ALAS-E (48 h exposure to imaging plate) and then to γ-globin probe (2 h exposure). It was washed in water, and rehybridized to a ribosomal RNA probe to confirm the amount of RNA loaded. 18S rRNA bands are shown.

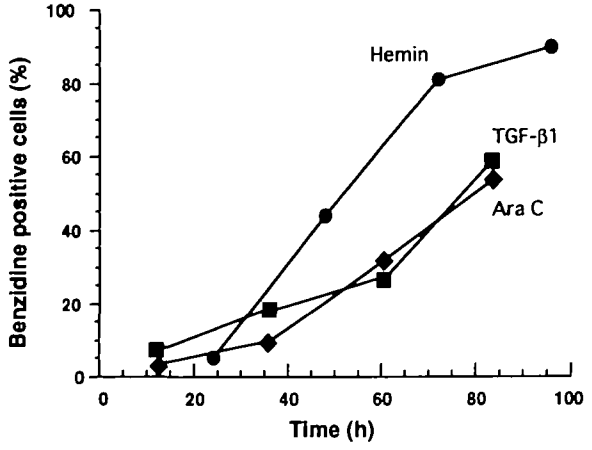


Fig. 2. **Induction of hemoglobin synthesis in YN-1 cells after treatment with hemin, TGF-β1, or Ara-C.** YN-1 cells were incubated with hemin (100 μM), TGF-β1 (1 ng/ml), or Ara-C (100 nM). Cells were harvested at various time points as indicated in the figure. Viable cells and BZ(+) cells were counted at each time point using a method as described previously (*16*). These experiments were repeated three times and averages of the percentage values are shown.

RESULTS

γ-Globin mRNA Is Abundantly Expressed in Several Human Leukemia Cell Lines—Nine human leukemia cell lines were examined in this study, and seven of them were positive for the Ph[1] chromosome. These cell lines were found to express little or no hemoglobin as judged from the small number of BZ-positive cells (less than 10%). In contrast to the result of BZ staining, we found substantial levels of γ-globin mRNA expression in 4 out of 7 Ph[1] chromosome-positive cell lines (YN-1, KYO-1, KU812, and K562) in an RNA blot hybridization analysis (Fig. 1). A similar finding has been reported in other human leukemic cell lines (*e.g.*, *50*). Three other cell lines had either a lesser but detectable level (Y-1K) or an undetectable level (KCL-22 and NALM-1) of γ-globin mRNA expression. We also examined two Ph[1] chromosome-negative leukemia cell lines, KG-1a and HL60, but these cell lines showed no detectable γ-globin mRNA expression (Fig. 1 and data not shown). These findings in several human leukemia cell lines are in contrast to that in MEL cells, which show only a small amount of globin mRNAs expression before Me_2SO treatment (*9, 51*), and suggest that globin mRNAs in human leukemia cell lines may not be efficiently translated into globin proteins.

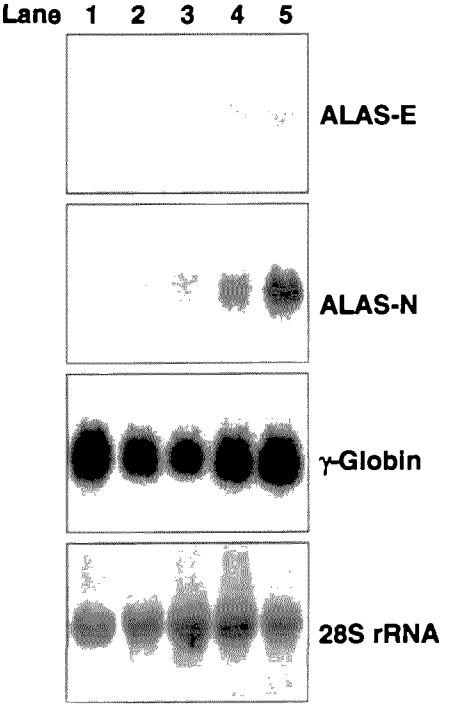


Fig. 3. **RNA blot analysis of ALAS-E, ALAS-N, and γ-globin mRNAs in YN-1 cells treated with Ara-C.** YN-1 cells were incubated with Ara-C (100 nM), and RNA was prepared at 12 (lane 2), 36 (lane 3), 60 (lane 4), and 84 h (lane 5) after the addition of Ara-C. RNA was also prepared at the 12 h time point from Ara-C-untreated cells which were replated at the same time as the Ara-C-treated cells, and used as a control (lane 1). Total RNA from each time point was hybridized to ALAS-E, ALAS-N, or γ-globin probes. Exposure times of the filter to the photoimaging plate were 12 h, 24 h, and 1 h for ALAS-E, ALAS-N, and γ-globin probes, respectively. A probe detecting ribosomal RNA was used as an internal control.

In untreated YN-1 cells, globin mRNA was markedly expressed and only a brief exposure period was required for detection in RNA blotting analysis, whereas the ALAS-E mRNA level in these cells was low and required an extended exposure period for detection, even using a BAS-2000 image analyzer plate (Fig. 1).

The level of ALAS-E mRNA among other untreated leukemia cell lines was highest in K562 cells, while it was very low in Y-1K cells. The percentage of BZ(+) cells was 7-10%, 0%, and 1-5% for untreated K562, Y-1K, and YN-1 cells, respectively. Thus, there is an approximate correlation between the level of ALAS-E mRNA and the percentage of BZ(+) cells.

To gain an insight into the nature of the discrepancy between γ-globin mRNA levels and hemoglobin contents, we examined the time course of induction of hemoglobin synthesis after treatment of YN-1 cells with hemin. A similar study was made with Ara-C, or TGF-β1, either of which is known to induce erythroid differentiation in other cell lines (*21, 43*) (Fig. 2). The percentage of BZ(+) cells increased with time, from 1-5% in untreated YN-1 cells to >50% at 84 h or thereafter, upon treatment of cells with hemin, Ara-C or TGF-β1. Results with hemin and Ara-C were similar to those which have been reported in two other cell lines (*21, 43*). Hemin was the strongest inducer of hemoglobin synthesis, since the percentage of BZ(+) cells reached more than 90% at 96 h after hemin treatment, while Ara-C, or TGF-β1, could induce BZ(+) cells to the extent of approximately 60% at 84 h.

The induction of BZ(+) cells with TGF-β1 in the YN-1 cell system indicates that TGF-β1 is a significant inducer of erythroid differentiation of human leukemia cell lines.

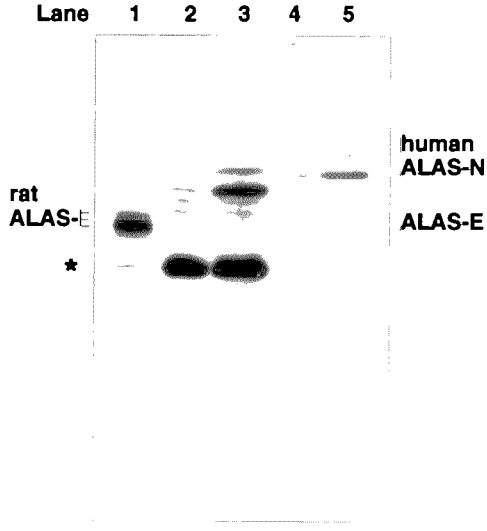


Fig. 4. **Immunoblot analysis of ALAS-E and ALAS-N in YN-1 cells.** Mitochondrial fractions from anemic rat blood cells (lane 1), from the livers of rats either untreated (lane 2) or treated (lane 3) with AIA, and from YN-1 cells untreated (lane 4) or treated (lane 5) with Ara-C were prepared, separated electrophoretically and transferred to a PVDF membrane. The membrane was incubated with rabbit anti-rat ALAS-N antibody which cross-reacts with ALAS-E. The band in lane 1 representing rat ALAS-E and bands in lane 5 representing human ALAS-N (both precursor and mature forms) and ALAS-E (mature form) are indicated. Intense bands in lanes 2 and 3 (shown by asterisk) are non-specific, and these bands were not induced by AIA.

Under the conditions of these experiments, cellular growth curves for hemin-treated and TGF-β1-treated cells were only marginally affected as compared with that for untreated cells, whereas Ara-C-treated cells showed significant retardation of cell growth (data not shown).

Ara-C Increased Both ALAS-E and ALAS-N mRNA Levels in YN-1 Cells—Changes in the levels of mRNAs encoding γ-globin, ALAS-E and ALAS-N were examined by RNA blot hybridization analysis in YN-1 cells after treatment with Ara-C. Treatment of YN-1 cells with 5×10^{-6} M Ara-C resulted in a time-dependent increase in both ALAS-E and ALAS-N mRNAs (Fig. 3). At 84 h, ALAS-E and ALAS-N mRNAs increased 30- and 7-fold, respectively, over the untreated control level. In contrast, the level of γ-globin mRNA appeared not to be increased substantially (Fig. 3). These experiments were repeated three times, and the marked induction of both ALAS-E and ALAS-N mRNAs was confirmed in each experiment, as was the relatively small change in γ-globin mRNA levels. The results suggest that induction of hemoglobin synthesis by Ara-C reflects increases in the ALA synthases, rather than a transcriptional activation of the γ-globin gene.

To examine whether the increase in mRNAs resulted in the increase in the enzyme proteins, immunoblot analysis of ALA synthases was performed using a rabbit antibody directed against rat ALAS-N (*7, 49*). This antibody recognized both precursor form (the largest band) and mature

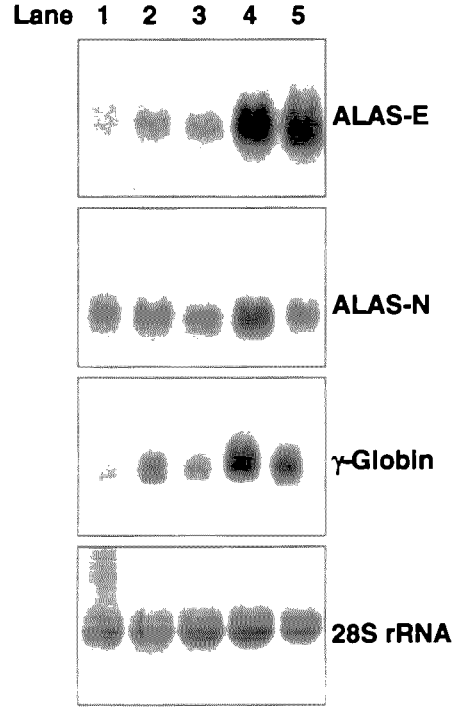


Fig. 5. **RNA blot analysis of ALAS-E, ALAS-N, and γ-globin mRNAs in YN-1 cells treated with TGF-β1.** YN-1 cells were incubated with 1 ng/ml of TGF-β1 and RNA was prepared at 12 (lane 2), 36 (lane 3), 60 (lane 4), and 84 h (lane 5) after the addition of TGF-β1. RNA was also prepared at the 84 h time point from untreated cells which were replated at the same time as the TGF-β1-treated cells, and used as a control (lane 1). Exposure times of the filters to the photoimaging plate were 48 h for ALAS-E and ALAS-N probes, and 0.5 h for the γ-globin probe. A ribosomal RNA probe was used as an internal control.

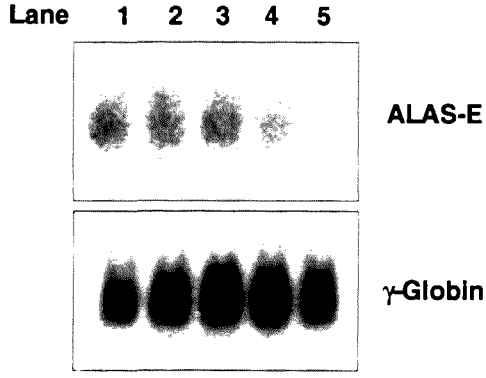


Fig. 6. **RNA blot analysis of ALAS-E and γ-globin mRNAs in YN-1 cells treated with hemin.** YN-1 cells were incubated with 100 μM hemin, and RNA was prepared at 24 (lane 2), 48 (lane 3), 72 (lane 4), and 96 h (lane 5) after the addition of hemin. RNA was also prepared at the 24 h time point from untreated cells which were replated at the same time as the hemin-treated cells, and used as a control (lane 1). RNA blot analysis was performed using ALAS-E and γ-globin probes. Exposure times of the filters to the photoimaging plate were 19 h and 1 h for ALAS-E and γ-globin probes, respectively.

form (the second largest band) of the ALAS-N in the liver of rats treated with AIA (Fig. 4, lane 3). In addition, this antibody cross-reacted with ALAS-E, as shown by the formation of a specific band (approximately 56 kDa, Ref. 7) with the mitochondrial fraction from rat reticulocytes (lane 1). The specificity of this antibody to ALAS-E has also been shown previously using a purified rat ALAS-E protein (7).

Using the mitochondrial fraction of YN-1 cells, a single band of 65 kDa was detected in untreated control cells (lane 4), whereas the intensification of the 65 kDa band and appearance of 75 kDa band were observed in Ara-C-induced cells (lane 5). The 65 kDa band corresponds to human mature ALAS-N, whereas the 75 kDa band corresponds to human ALAS-N precursor. Thus, the sizes of human ALAS-N and its precursor were found to be larger than those of rat ALAS-N and its precursor. The increase of ALAS-N and its precursor in the Ara-C-treated YN-1 cells is an intriguing finding in this experiment. The accumulation of the precursor protein seems to be unique for ALAS-N among mitochondrial matrix proteins encoded by the nuclear genome. In this regard, we have previously shown the association of the ALAS-N precursor with mitochondria in the rat liver after the induction of ALAS-N

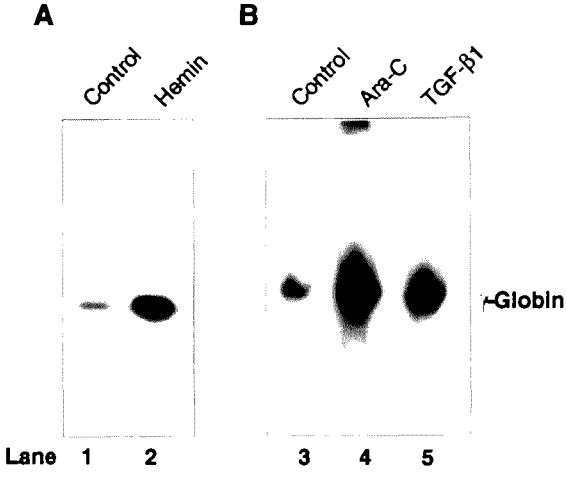
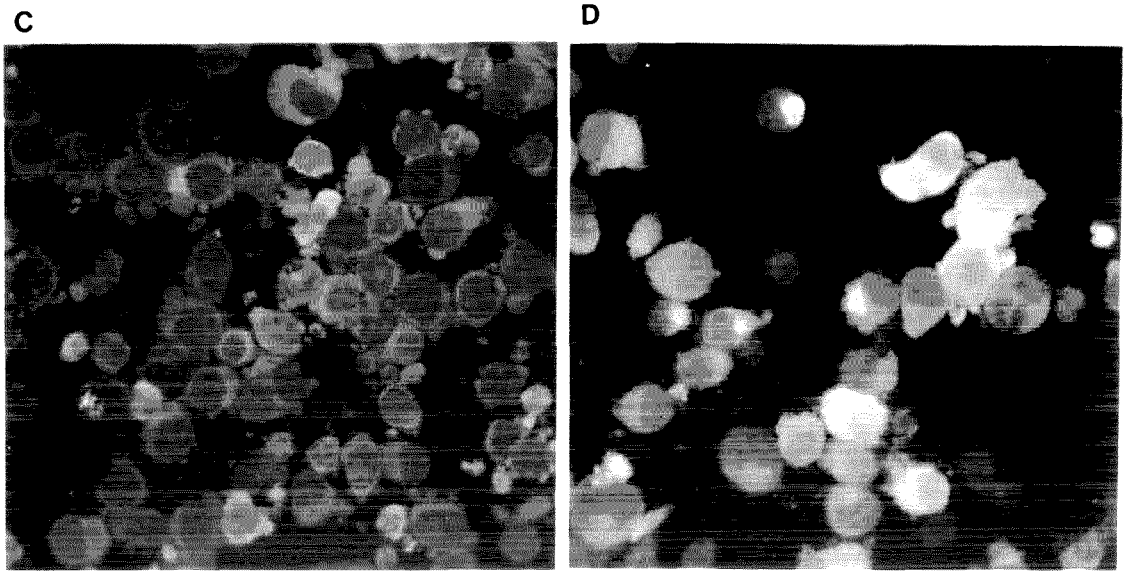


Fig. 7. **Immunoblot and immunofluorescent staining analysis of γ-globin in YN-1 cells treated with hemin, Ara-C, or TGF-β1 with anti-γ-globin chain antibody.** A and B, cell lysates were prepared from YN-1 cells treated for 72 h with 100 μM hemin (lane 2), 100 nM Ara-C (lane 4), or 1 ng/ml TGF-β1 (lane 5), or from YN-1 cells untreated with inducers (lanes 1 and 3). Five micrograms of protein were separated electrophoretically by 18% Laemmli gel (lanes 1 and 2), or urea/SDS gel (lanes 3-5), and transferred to PVDF membranes. The membranes were incubated with rabbit anti-human HbF antibody. C and D, smears of YN-1 cells, with or without treatment by hemin, were fixed and incubated with the HbF antibody. Cells were incubated with fluorescein isothianate (FITC)-conjugated goat anti-rabbit-IgG and examined using a fluorescence microscope.



by treatment of animals with AIA (*52*).

It should also be noted that a band, which is similar in size to that of ALAS-E in rat reticulocytes, is induced markedly and becomes detectable in YN-1 cells after the Ara-C treatment (lane 5). This band is most likely to represent the human mature form ALAS-E. These findings indicate that both ALAS-E and ALAS-N proteins are increased by the Ara-C treatment of YN-1 cells.

TGF-β1 Increased ALAS-E mRNA Levels in YN-1 Cells—We also examined the effects of TGF-β1 on the levels of mRNAs encoding γ-globin, ALAS-E, and ALAS-N in YN-1 cells. Treatment of cells with TGF-β1 (1 ng/ml) markedly increased ALAS-E mRNA levels (Fig. 5). This result indicates that TGF-β1 is a potent inducer of erythroid differentiation in YN-1 cells, as is the case for HEL cells (*53*). In addition to ALAS-E, γ-globin mRNA and ALAS-N mRNA levels were also increased by the TGF-β1 treatment (Fig. 5). The increase in BZ(+) cells was greater than 7-fold at 60 h after treatment with TGF-β1 (Fig. 2), which is similar to the increase in ALAS-E mRNA.

Hemin Induced Neither ALAS-E nor ALAS-N mRNA in YN-1 Cells—The percentage of BZ(+) cells in YN-1 cells increased more than 30-fold 96 h after treatment with hemin (1×10^{-4} M) (Fig. 2). This finding confirms our previous observation that hemin is the most potent inducer of hemoglobin synthesis in YN-1 cells (*33*). The level of γ-globin mRNA was, however, increased less than 2-fold by hemin treatment (Fig. 6), which was not sufficient to account for the marked increase in BZ(+) cells (Fig. 2). Both ALAS-E (Fig. 6) and ALAS-N mRNA levels (data not shown) decreased after hemin treatment. These results indicate that exogenously added hemin stimulated hemoglobin synthesis at a post-transcriptional step, rather than activating transcription of the γ-globin and ALAS-E genes.

Translation of γ-Globin mRNA Is Stimulated by Hemin, Ara-C, and TGF-β1—In addition to the BZ staining, changes in the γ-globin protein levels were examined using a rabbit anti-human HbF antibody, by immunoblot and by immunofluorescence analyses. In contrast to the small change in γ-globin mRNA levels, γ-globin protein synthesis increased markedly after treatment with hemin (Fig. 7A), Ara-C (Fig. 7B), or TGF-β1 (Fig. 7B). This was also shown at the cellular level by immunofluorescence studies which were carried out using the anti-HbF antibody. While uninduced cultures showed few fluorescent cells (Fig. 7C), hemin-treated cultures showed as much as 80% fluorescent cells (Fig. 7D). Thus, these observations suggest that hemin treatment of YN-1 cells stimulates γ-globin synthesis principally by enhancing the translation of γ-globin mRNA.

DISCUSSION

The present study demonstrates that γ-globin mRNA is abundantly expressed in several untreated human leukemia cell lines, while hemoglobin production is significantly suppressed in these cells. This situation was observed in at least 4 out of 7 Ph^1 chromosome-positive leukemia cell lines examined in this study. Expression of different globin mRNAs to variable degrees was also reported in some other human leukemia cells lines (*17, 18, 21, 22, 50, 54*), though they were not always examined with respect to the balance between heme and globin synthesis.

In comparison to γ-globin mRNA expression, ALAS-E mRNA in YN-1 cells was present at much lower levels as judged from RNA blotting analysis. Immunoblot analysis utilizing anti-rat ALAS antibody showed that ALAS-E protein was undetectable in untreated YN-1 cells (*44*). These findings suggest that the discrepancy between γ-globin mRNA expression and its protein product is in part due to heme deficiency. In fact, hemin treatment of these cells led to the formation of hemoglobin, without activating the ALAS-E gene. Stimulation of hemoglobin synthesis was also reported in other human leukemia cell lines after treatment with hemin or ALA (*18, 20-22, 24, 25, 32*).

While some authors have reported the transcriptional effect of heme on hemoglobin formation (*50*), there are many other reports which have proposed the post-transcriptional control (*55-57*). For example, heme deficiency leads to the formation of a heme-controlled translational inhibitor, which inhibits protein synthesis at the translational level by enhancing phosphorylation of the α-subunit of the eukaryotic initiation factor 2 (*58*), and it is thus tempting to speculate that such an inhibitor may also play a role in these cells. In contrast to hemin, treatment of YN-1 cells with Ara-C or TGF-β1 showed a similar increase in ALAS-E mRNA levels and BZ(+) cells, and the change in γ-globin mRNA level was more substantial than in the case with hemin. These findings suggest that Ara-C and TGF-β1 activate the ALAS-E gene, which in turn raises heme concentrations to permit hemoglobin synthesis.

The lack of ALAS-E expression and hemoglobin synthesis has also been described in other cell lines of various origin which express globin mRNA. For example, mouse DR-1 cells, a subclone of DS19 MEL cells, express β-globin mRNA, while they fail to show hemoglobin synthesis upon Me_2SO treatment. The level of β-globin mRNA declines, rather than increases, in response to Me_2SO. Notably, these cells lack ALAS-E mRNA expression (*9*). The defect in DR-1 cells in hemoglobin synthesis can be partially corrected by the addition of exogenous hemin, suggesting a critical role of heme in their hemoglobin synthesis (*61*). Another cell line, HD-6, a temperature sensitive (ts)-avian erythroblastosis virus (AEV)-transformed chicken erythroid cell line, is known to fail to undergo erythroid differentiation (*59*), and also lacks the expression of ALAS-E mRNA and its protein product (unpublished observation). A subclone of K562 cells, termed K562-L, expresses comparable levels of γ-globin mRNA to those in K562 cells (*60*), while they completely lack the expression of ALAS-E mRNA (unpublished observation), and show no detectable BZ(+) cells (*61*). These findings thus support the hypothesis that the expression of ALAS-E is an essential event in erythroid differentiation, and that the abrogation of its expression and resultant lack of hemoglobin synthesis may be a frequent event in human leukemia cell lines. The fact that ALA and hemin are generally effective in stimulating hemoglobin synthesis in human leukemia cell lines (*18, 20-22, 24*) also supports this conclusion.

The expression of fetal or embryonic globin genes in leukemic cell lines may be either a reflection of a fetal or embryonic potential in normal definitive hematopoietic progenitors (*20*) or defective heme synthesis due to the lack of ALAS-E expression in these cells. In either case, heme deficiency may interfere with normal development of

erythroid cells, and thereby make such cells vulnerable to oncogenic transformation by various stimuli. In support of this hypothesis is the fact that v-ErbA, an oncogene in AEV, suppresses the expression of erythroid-specific genes such as ALAS-E, band 3 and carbonic anhydrase, which then enhances the transforming activity of the v-ErbB oncogene *via* the blockade of the erythroid development of the infected cells (*62*). Thus, the blockade of heme biosynthesis, including that which occurs at the level of ALAS-E expression, may have significant influence on the expression of the transformed phenotype of leukemia cells.

Another line of evidence implicating heme deficiency in cellular transformation involves refractory anemia with ringed sideroblasts (or primary acquired sideroblastic anemia) (*63, 64*). Some patients with this disorder are known to develop leukemic transformation (*65, 66*), and many patients with this condition have decreased ALA synthase expression (*63, 64*), suggesting that there may be an association between these events.

Our findings in this study demonstrated that Ara-C and TGF-β1 can promote erythroid development of certain human leukemia cell lines. We also obtained similar findings in K562 cells following treatment with activin A (data not shown). Hemoglobin synthesis in these cells was largely dependent on the heme supply, which was determined by the level of ALAS-E expression (*9*). These findings suggest that it may be possible to treat patients having certain chronic myelogenous leukemias with significant globin mRNA expression by using hemin, which may facilitate hemoglobin synthesis, and thus cell differentiation. Hemin has been used to treat patients with porphyrias (*67*), and sideroblastic anemias (*68*), or myelodysplastic syndromes (*69, 70*) with a variable degree of success.

Recently genomic DNA clones encoding human ALAS-E gene have been isolated and analyzed (*43*). We have also isolated genomic DNA clones for human ALAS-E independently, and analyzed the regulatory region of the gene (unpublished observation). A cluster of *cis*-acting elements which are known to be important in determining erythroid-specific transcriptional activation, *e.g.*, GATA-1, AP-1/NF-E2, CACCC, and CCAAT factor binding sequences (*71-73*), was found in the promoter region of the ALAS-E gene. The region(s) which is important for the TGF-β1, Ara-C, and activin A-mediated activation of the ALAS-E gene is currently being investigated in our laboratory by functional analysis of its regulatory region.

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REFERENCES

1. Kappas, A., Sassa, S., Galbraith, R.A., and Nordmann, Y. (1995) The Porphyrias in *The Metabolic Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valle, D., eds.) 7th ed., pp. 2103-2159, McGraw-Hill, New York
2. Brooker, J.D., Srivastava, G., Borthwick, I.A., May, B.K., and Elliott, W.H.(1983) Evidence that 2-allyl-2-isopropylacetamide, phenobarbital and 3,5-diethoxycarbonyl-1,4-dihydrocollidine induce the same cytochrome P450 mRNA in chick embryo liver. *Eur. J Biochem.* **136**, 327-332
3. Yamamoto, M., Yew, N.S., Federspiel, M., Dodgson, J.B., Hayashi, N., and Engel, J.D. (1985) Isolation of recombinant cDNAs encoding chicken erythroid δ-aminolevulinate synthase. *Proc. Natl. Acad. Sci. USA* **82**, 3702-3706
4. Riddle, R.D., Yamamoto, M., and Engel, J.D. (1989) Expression of δ-aminolevulinate synthase in avian cells: Separate genes encode erythroid-specific and nonspecific isozymes. *Proc. Natl. Acad. Sci. USA* **86**, 792-796
5. Bishop, D.F. (1990) Two different genes encode δ-aminolevulinate synthase in humans: nucleotide sequences of cDNAs for the housekeeping and erythroid genes. *Nucleic Acids Res.* **18**, 7187-7188
6. Cox, T.C., Bawden, M.J., Abraham, N.G., Bottomley, S.S., May, B.K., Baker, E., Chen, L.Z., and Sutherland, G.R. (1990) Erythroid 5-aminolevulinate synthase is located on the X-chromosome. *Am. J. Hum. Genet.* **46**, 107-111
7. Munakata, H., Yamagami, T., Nagai, T., Yamamoto, M., and Hayashi, N. (1993) Purification and structure of rat erythroid-specific δ-aminolevulinate synthase. *J. Biochem.* **114**, 103-111
8. Yamamoto, M., Kim, L.-C., Nagai, T., Furuyama, K., and Engel, J.D. (1994) Structure and regulation of vertebrate δ-aminolevulinate synthase in *Regulation of Heme Protein Synthesis* (Fujita, H., ed.) pp. 11-25, AlphaMed Press, Dayton, Ohio
9. Fujita, H., Yamamoto, M., Yamagami, T., Hayashi, N., and Sassa, S. (1991) Erythroleukemia differentiation: Distinctive responses of the erythroid-specific and the nonspecific δ-aminolevulinate synthase mRNA. *J. Biol. Chem.* **266**, 17494-17502
10. Aoki, Y., Muranaka, S., Nakabayashi, K., and Ueda, Y. (1979) δ-Aminolevulinic acid synthetase in erythroblasts of patients with pyridoxine-responsive anemia. Hypercatabolism caused by the increased susceptibility to the controlling protease. *J. Clin. Invest.* **64**, 1196-1203
11. Buchanan, G.R., Bottomley, S.S., and Nitschke, R. (1980) Bone marrow δ-aminolaevulinate synthase deficiency in a female with congenital sideroblastic anemia. *Blood* **55**, 109-115
12. Cotter, P.D., Baumann, M., and Bishop, D.F. (1992) Enzymatic defect in "X-linked" sideroblastic anemia: Molecular evidence for erythroid δ-aminolevulinate synthase deficiency. *Proc. Natl. Acad. Sci. USA* **89**, 4028-4032
13. Cox, T.C., Bottomley, S.S., Wiley, J.S., Bawden, M.J., Matthews, C.S., and May, B.K. (1994) X-linked pyridoxine-responsive sideroblastic anemia due to a THR388-to-SER substitution in erythroid 5-aminolevulinate synthase. *N. Engl. J. Med.* **330**, 675-679
14. Rutherford, T.R., Clegg, J.B., and Weatherall, D.J. (1979) K562 human leukaemic cells synthesize embryonic haemoglobin in response to haemin. *Nature* **280**, 164-165
15. Hoffman, R., Ibrahim, N., Murnane, M.J., Diamond, A., Forget, B.G., and Levere, R.D. (1980) Hemin control of heme biosynthesis and catabolism in a human leukemia cell line. *Blood* **56**, 567-570
16. Rowley, P.T., Ohlsson Wilhelm, B.M., Farley, B.A., and LaBella, S. (1981) Inducers of erythroid differentiation in K562 human leukemia cells. *Exp. Hematol.* **9**, 32-37
17. Martin, P. and Papayannopoulou, T. (1982) HEL cells: a new human erythroleukemia cell line with spontaneous and induced globin expression. *Science* **216**, 1233-1235
18. Papayannopoulou, T., Nakamoto, B., Kurachi, S., and Nelson, R. (1987) Analysis of the erythroid phenotype of HEL cells: clonal variation and the effect of inducers. *Blood* **70**, 1764-1772
19. Takihara, Y., Rutherford, T., Shiokawa, S., Fukumaki, Y., Endo, T., and Okano, H. (1987) Differential induction of adult and fetal globin gene expression in the human erythremia cell line KMOE. *Leukemia* **1**, 673-676
20. Enver, T., Zhang, J.W., Anagnou, N.P., Stamatoyannopoulos, G., and Papayannopoulou, T. (1988) Developmental programs of human erythroleukemia cells: globin gene expression and methylation. *Mol. Cell. Biol.* **8**, 4917-4926
21. Papayannopoulou, T., Nakamoto, B., Kurachi, S., Tweeddale, M., and Messner, H. (1988) Surface antigenic profile and globin phenotype of two new human erythroleukemia lines: characteri-

zation and interpretations. *Blood* **72**, 1029-1038
22. Kitamura, T., Tange, T., Terasawa, T., Chiba, S., Kuwaki, T., Miyagawa, K., Piao, Y.F., Miyazono, K., Urabe, A., and Takaku, F. (1989) Establishment and characterization of a unique human cell line that proliferates dependently on GM-CSF, IL-3, or erythropoietin. *J. Cell Physiol.* **140**, 323-334
23. Okuno, Y., Suzuki, A., Ichiba, S., Takahashi, T., Nakamura, K., Hitomi, K., Sasaki, R., Tada, K., and Imura, H. (1990) Establishment of an erythroid cell line (JK-1) that spontaneously differentiates to red cells. *Cancer* **66**, 1544-1551
24. Chiba, S., Takaku, F., Tange, T., Shibuya, K., Misawa, C., Sasaki, K., Miyagawa, K., Yazaki, Y., and Hirai, H. (1991) Establishment and erythroid differentiation of a cytokine-dependent human leukemic cell line F-36: a parental line requiring granulocyte-macrophage colony-stimulating factor or interleukin-3, and a subline requiring erythropoietin. *Blood* **78**, 2261-2268
25. Endo, T., Ishibashi, Y., Shiokawa, S., Fukumaki, Y., and Okano, H. (1994) Differential induction of adult and fetal globin gene expression in the human CML cell subline KU-812F/33. *J. Biochem.* **115**, 540-544
26. Busfield, S.J. and Klinken, S.P. (1992) Erythropoietin-induced stimulation of differentiation and proliferation in J2E cells is not mimicked by chemical induction. *Blood* **80**, 412-419
27. Rutherford, T.R. and Weatherall, D.J. (1979) Deficient heme synthesis as the cause of noninducibility of hemoglobin synthesis in Friend erythroleukemia cell line. *Cell* **16**, 415-423
28. Reuben, R.C., Rifkind, R.A., and Marks, P.A. (1980) Chemically induced murine erythroleukemic differentiation. *Biochim. Biophys. Acta* **605**, 325-346
29. Ross, J. and Sautner, D. (1976) Induction of globin mRNA accumulation by hemin in cultured erythroleukemic cells. *Cell* **8**, 513-520
30. Dabney, B.J. and Beaudet, A.L. (1977) Increase in globin chains and globin mRNA in erythroleukemia cells in response to hemin. *Arch. Biochem. Biophys.* **179**, 106-112
31. Granick, J.L. and Sassa, S. (1978) Hemin control of heme biosynthesis in mouse Friend virus-transformed erythroleukemia cells in culture. *J. Biol. Chem.* **253**, 5402-5406
32. Hirata, J., Sato, H., Takahira, H., Shiokawa, S., Endo, T., Nishimura, J., Katsuno,M., Masuda, S., Sasaki, R., Fukumaki, Y., Nawata, H., and Okano, H. (1990) A novel CD10-positive erythroid cell line, RM10, established from a patient with chronic myelogenous leukemia. *Leukemia* **4**, 365-372
33. Endo, K., Harigae, H., Nagai, T., Fujie, H., Meguro, K., Watanabe, N., Furuyama, K., Kameoka, J., Okuda, M., Hayashi, N., Yamamoto, M., and Abe, K. (1994) Two chronic myelogenous leukaemia cell lines which represent different stages of erythoid differentiation. *Br. J. Haematol.* **85**, 653-662
34. Ohkubo, T., Kamamoto, T., Kita, K., Hiraoka, A., Yoshida, Y., and Uchino, H. (1985) A novel Ph^1 chromosome positive cell line established from a patient with chronic myelogenous leukemia in blastic crisis. *Leukemia Res.* **9**, 921-926
35. Kishi, K. (1985) A new leukemia cell line with philadelphia chromosome characterized as basophil precursors. *Leukemia Res.* **9**, 381-390
36. Nakazawa, M., Mitjavila, M.T., Debili, N., Casadevall, N., Mayeux, P., Rouyer Fessard, P., Dubart, A., Romeo, P.H., Beuzard, Y., and Kishi, K. (1989) KU812: a pluripotent human cell line with spontaneous erythroid terminal maturation. *Blood* **73**, 2003-2013
37. Kubonishi, I. and Miyoshi, I. (1983) Establishment of a Ph^1 chromosome-positive cell line from chronic myelogenous leukemia in blast crisis. *Int. J. Cell Cloning* **1**, 105-117
38. Minowada, J., Tsubota, T., Greaves, M.F., and Walters, T.R. (1977) A non-T, non-B human leukemia cell line (NALM-1): Establishment of the cell line and presence of leukemia-associated antigens. *J. Natl. Cancer Inst.* **59**, 83-87
39. Lozzio, C.B. and Lozzio, B.B. (1975) Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. *Blood* **45**, 321-334
40. Collins, S.J., Gallo, R.C., and Gallagher, R.E. (1977) Continuous growth and differentiation of human leukemia cells in suspension culture. *Nature* **270**, 347-349
41. Koeffler, H.P., Billing, R., Lusis, A.J., Sparkes, R., and Golde, D.W. (1980) An undifferentiated variant derived from the human acute myelogenous leukemia cell line (KG-1). *Blood* **56**, 265-273
42. Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidium thionate-phenol-chloroform extraction *Anal. Biochem.* **162**, 156-159
43. Conboy, J.G., Cox, T.C., Bottomley, S.S., Bawden, M.J., and May, B.K. (1992) Human erythroid 5-aminolevulinate synthase: gene structure and species-specific differences in alternative RNA splicing. *J. Biol. Chem.* **267**, 18753-18758
44. Bawden, M.J., Borthwick, I.A., Healy, H.M., Morris, C.P., May, B.K., and Elliott, W.H. (1987) Sequence of human 5-aminolevulinate synthase cDNA. *Nucleic Acids Res.* **15**, 8563
45. Feinberg, A.P. and Vogelstein, B. (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**, 6-13
46. Yomogida, K., Yamamoto, M., Yamagami, T., Fujita, H., and Hayashi, N. (1993) Structure and expression of the gene encoding rat nonspecific form δ-aminolevulinate synthase. *J. Biochem.* **113**, 364-371
47. Laemmli, U.K. (1970) Cleavage of structural proteins during assembly of the head of the bacteriophage T4. *Nature* **227**, 680-685
48. Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (1990) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York
49. Yamamoto, M., Fujita, H., Watanabe, N., Hayashi, N., and Kikuchi, G. (1986) An immunochemical study of δ-aminolevulinate synthase and δ-aminolevulinate dehydratase in liver and erythroid cells of rat. *Arch. Biochem. Biophys.* **245**, 76-83
50. Charnay, P. and Maniatis, T. (1983) Transcriptional regulation of globin gene expression in the human erythroid cell line K562. *Science* **220**, 1281-1283
51. Fukuda, Y., Fujita, H., Garbaczewski, L., and Sassa, S. (1994) Regulation of β-globin mRNA accumulation by heme in dimethyl sulfoxide (DMSO)-sensitive and DMSO-resistant murine erythroleukemia cells. *Blood* **83**, 1662-1667
52. Hayashi, N., Watanabe, N., and Kikuchi, G. (1983) Inhibition by hemin of in vitro translocation of chicken liver δ-aminolevulinate synthase into mitochondria. *Biochem. Biophys. Res. Commun.* **115**, 700-706
53. Hooper, W.C., Jackson, D., Pruckler, J., and Evatt, B.L. (1991) Cellular kinetics of transforming growth factor-beta induced hemoglobin accumulation in the HEL erythroleukemia cell line. *Leukemia Res.* **15**, 745-751
54. Seigneurin, D., Champelovier, P., Mouchiroud, G., Berthier, R., Leroux, D., Prenant, M., McGregor, J., Starck, J., Morle, F., and Micouin, C. (1987) Human chronic myeloid leukemic cell line with positive Philadelphia chromosome exhibits megakaryocytic and erythroid characteristics. *Exp. Hematol.* **15**, 822-832
55. Rabinovitz, M., Freedman, M.L., Fisher, J.M., and Maxwell, C.R. (1969) Translational control in hemoglobin synthesis. *Cold Spring Harbor Symp. Quant. Biol.* **34**, 567-578
56. London, I.M., Clemens, M.J., Ranu, R.S., Levin, D.H., Cherbas, L.F., and Ernst,V. (1976) The role of hemin in the regulation of protein synthesis in erythroid cells. *Fed. Proc.* **35**, 2218-2222
57. Rovera, G., Vartikar, J., Connolly, G.R., Magarian, C., and Dolby, T.W. (1978) Hemin controls the expression of the β-minor globin gene in Friend erythroleukemic cells at the pretranslational level. *J. Biol. Chem.* **253**, 7588-7590
58. Pal, J.K., Chen, J.J., and London, I.M. (1991) Tissue distribution and immunoreactivity of heme-regulated eIF-2α kinase determined by monoclonal antibodies. *Biochemistry* **30**, 2555-2562
59. Beug, H., Doederlein, G., Freudenstein, C., and Graf, T. (1982) Erythroblast cell lines transformed by a ts mutant of avian erythroblastosis virus: a model system to study erythroid differentiation in vitro. *J. Cell Physiol. Suppl.* **1**, 195-207
60. Sasaki, D., Kosunago, S., Hirano, J., Matsuda, K., Hata, Y.,

Komiyama, Y., Mikami, T., Matsumoto, T., and Suzuki, M. (1993) Cloning and characterization of K562 cells on hemoglobin synthetic activity. *Biol. Pharm. Bull.* **16**, 548-551
61. Sasaki, D., Mikami, K., Matsumoto, T., and Suzuki, M. (1992) Proliferation and differentiation of human leukemia cell lines with different levels of hemoglobin. *Seikagaku* **64**, 898 (abstract)
62. Zenke, M., Kahn, P., Disela, C., Vennstrom, B., Leutz, A., Keegan, K., Hayman, J.M., Choi, H.-R., Yew, N., Engel, J.D., and Beug, H. (1988) v-erbA specifically suppresses transcription of the avian erythrocyte anion transporter (band 3) gene. *Cell* **52**, 107-119
63. Bottomley, S.S., Tanaka, M., and Self, J. (1973) δ-Aminolevulinic acid synthetase activity in normal human bone marrow and in patients with idiopathic sideroblastic anemia. *Enzyme* **16**, 138-145
64. Aoki, Y., Urata, G., Wada, O., and Takaku, F. (1974) Measurement of δ-aminolevulinic acid synthase activity in human erythroblasts. *J. Clin Invest.* **53**, 1326-1334
65. Hast, R. (1983) Hereditary ring sideroblastic anaemia and Christmas disease in a Swedish family. *Scand. J. Haematol.* **30**, 444
66. Yoshida, Y., Oguma, S., Uchino, H., Maekawa, T., and Nomura, T. (1990) Reappraisal of sideroblastic erythropoiesis in refractory myelodysplastic anemias. *Acta Haematol. Jpn.* **53**, 860-865
67. Pierach, C.A., Bossenmaier, I., Cardinal, R., Weimer, M., and Watson, C.J. (1980) Hematin therapy in porphyric attacks. *Klin. Wochenschr.* **58**, 829-832
68. Volin, L. (1989) Haem arginate treatment for hereditary sideroblastic anaemia. *Eur. J. Hematol.* **42**, 60-66
69. Volin, L., Ruutu, T., Knuutila, S., and Tenhunen, R. (1988) Heme arginate treatment for myelodysplastic syndromes. *Leukemia Res.* **12**, 423-431
70. Ruutu, T., Volin, L., and Tenhunen, R. (1987) Haem arginate as a treatment for myelodysplastic syndromes. *Br. J. Haematol.* **65**, 425-428
71. Orkin, S.H. (1992) GATA-binding transcription factors in hematopoietic cells. *Blood* **80**, 575-581
72. Engel, J.D., George, K.M., Ko, L.J., Kornhauser, J.M., Leonard, M.W., Ting, P., and Yamamoto, M. (1991) Transcription factor regulation of hematopoietic cells. *Semin. Hematol.* **28**, 158-169
73. Evans, T., Felsenfeld, G., and Reitman, M. (1990) Control of globin gene transcription. *Annu. Rev. Cell Biol.* **6**, 95-124