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Efficient cell proliferation and predominant accumulation of ε-globin mRNA in human leukemic K562 cells which produce mostly Hb Gower 1

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Summary. Long-term cultures of K562(S) cells in 50–75 μM hemin allow the selection of 'hemin-resistant' K562 cells together with cells which proliferate efficiently while fully induced to express the human embryonic globin genes, as the hemoglobin Gower 1 ($\zeta_2\varepsilon_2$) is the predominant hemoglobin produced. Our experiments demonstrate that these K562 cells accumulate mostly ε-globin mRNA (ε-globin mRNA/γ-globin mRNA = 2.9) suggesting that the control of hemoglobin expression is at a pretranslational level.
Key words. K562; ε-globin mRNA; Hb Gower 1.

The human leukemic K562 cell line was isolated from a patient with chronic myelogenous leukemia in blast crisis[2]. These cells, when suitably cultured with a variety of chemical inducers, including hemin[3], butyric acid[4] and 5-azacytidine[5], undergo erythroid differentiation and accumulate large amounts of embryonic and fetal hemoglobins[3–7].

In a previous work we demonstrated that hemin-induced K562(S) cells retain a limited proliferative capacity but can be indefinitely subcultured in the presence of 50 μM hemin, allowing the selection of 'hemin-resistant' cell lines together with K562 cell populations which retain an efficient rate of cell proliferation while fully induced to express hemoglobin genes[5,8].

These latter cell populations accumulate predominantly Hb Gower 1[8].

The aim of this study was to determine whether this feature is correlated with a differential extent of accumulation of ε-globin and γ-globin mRNAs.

Our experiments show that in hemin-induced K562(S) cells the ratio ε-globin mRNA/γ-globin mRNA is 1.25. By sharp contrast in cell lines which proliferate while fully expressing the hemoglobin genes (Hb Gower 1 = 78%) the ratio ε-globin mRNA/γ-globin mRNA was found to be 2.9.

These results suggest the existence of a pre-translational control mechanism for ε-globin gene expression in human leukemic K562 cell lines which accumulate mostly Hb Gower 1.

Materials and methods. Cell lines and culture conditions. K562(S) cells were obtained from Dr Livia Cioè[4] (Institute of Virology, Rome University, Italy). The K562(h) cell line was developed in our laboratory by culturing K562(S) cells in 50 μM hemin. This cell line can be indefinitely subcultured in the presence of the inducer and it is composed of two major cell populations, one 'resistant' to hemin-mediated induction to hemoglobin expression and the other one proliferating and fully induced to express hemoglobin genes (these latter cells accumulate predominantly Hb Gower 1).

Cells were maintained at 37 °C in an humidified atmosphere in α-medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Flow Laboratories) as described[9].

Semi-solid cell cultures were performed in α-medium, 0.33% agar (DIFCO), 10% FCS. Hemin was prepared as described[10]. Cell growth was monitored with a ZF Coulter Counter[10]. K562 cells containing heme or hemoglobin were detected by specific reaction with a benzidine/hydrogen peroxide solution as reported elsewhere[10].

Hemoglobin determination. In order to analyze K562 hemoglobins total fresh post-mitochondrial cell lysates were electrophoresed on cellulose acetate strips (Polyphor) in Tris-EDTA-borate buffer, pH 8.7[5]. After electrophoresis the gels were stained with benzidine, photographed and the relative proportion of separated hemoglobins was quantitated from the areas of the densitometry peaks[8].

Cytoplasmic dot hybridization. Dot hybridization analysis of cellular cytoplasmic preparations was performed as described by White and Bancroft[11]. 50 μl of post-mitochondrial cell lysates were added to 30 μl of 20 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M trisodium citrate) and 20 μl of 37% (w/w) formaldehyde (Fisher). This mixture was incubated at 60 °C for 15 min and stored at −70 °C.

For analysis 20 μl of each sample were suitably diluted in 15 × SSC and 100 μl of each dilution were applied on a nitrocellulose sheet as described[12].

The nitrocellulose sheet was baked at 80 °C for 90 min and pre-hybridized at 42 °C in 50% formamide, 5 × SSC, 5× Denhardt's solution[12], 0.1% sodium dodecyl sulphate (SDS), 20 mM

Globin gene expression in K562(S) and K562(hC) cell lines cultured in the presence of 50 μM hemin for 7 days

K562 cell line	Hemoglobins (%)*					Globins (%)*			
	Gower 1 ($\zeta_2\varepsilon_2$)	X ($\varepsilon_2\gamma_2$)	F ($\alpha_2\gamma_2$)	Portland ($\zeta_2\gamma_2$)	Bart's (γ_4)	ε	γ	ε-globin* / γ-globin	ε-globin mRNA** / γ-globin mRNA
K562(S)	35.2	16	7.5	37	3.9	27.6	34.2	0.81	1.25
K562(hC)	78.2	11.8	2	7.8	–	45	10.8	4.16	2.9

*Data obtained from the experiments shown in figure 1. **Data obtained from the experiments shown in figures 2 and 3.

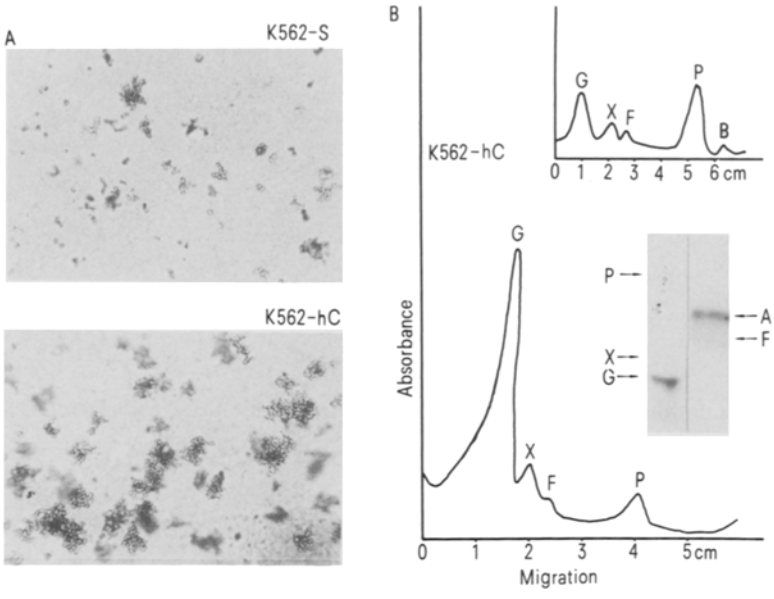

Figure 1. *A* Differential rate of cell proliferation of erythroid induced K562(S) and K562(hC) cells. Cells were cultured in semi-solid medium, 50 μM hemin, for 7 days. *B* Analysis of hemoglobin production by cellulose acetate gel electrophoresis of post-mitochondrial cell lysates from K562(hC) cells. The electrophoretic mobility of the standard hemoglobins HbA and HbF are also shown. The pattern of hemoglobin production in K562(S) cells induced to erythroid differentiation by hemin is shown in the insert. G = Hb Gower 1 ($\zeta_2\varepsilon_2$); X = HbX ($\varepsilon_2\gamma_2$); F = fetal Hb ($\alpha_2\gamma_2$); A = adult Hb ($\alpha_2\beta_2$); P = Hb Portland ($\zeta_2\gamma_2$); B = Hb Bart's (γ_4).

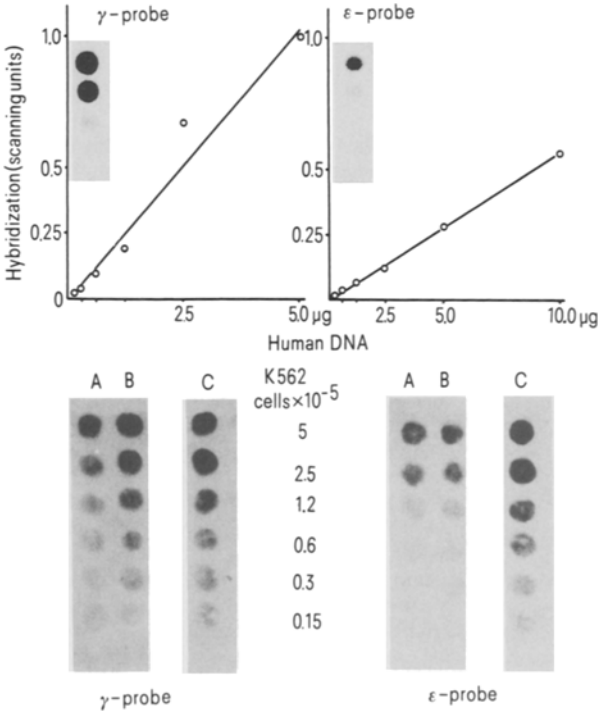

Figure 2. Upper panel: hybridization between ε-globin (pε0.7) or γ-globin (JW151) (^{32}P) DNA-probes with human DNA. As two γ-globin genes and one ε-globin gene are present in the human haploid genome, half amount of human DNA has been spotted onto the filter hybridized with γ-globin (^{32}P) DNA sequences. Lower panel: hybridization between formaldehyde-treated, dot spotted cytoplasms from uninduced K562(S) cells (A), hemin-induced K562(S) cells (B) or K562(hC) cells (C) and the ε-globin and γ-globin probes.

sodium phosphate pH 6.5, 100 μg/ml sonicated and denatured salmon sperm DNA[12].

Hybridization with nick-translated DNA plasmid JW151[13] and pε0.7[14], carrying respectively the sequences specific for human γ-globin and ε-globin mRNAs was performed for 16 h as described[12].

After the hybridization the filters were extensively washed three times with 250 ml portions of 2 × SSC, 0.1% SDS (5 min) and twice with 250 ml of 0.1 × SSC, 0.1% SDS at 50 °C for 30 min. Autoradiography and quantitation by scanning through a spectrophotometer were performed as described elsewhere[5].

Results. K562(h) cells are a mixed K562 cell population which is continously cultured in the presence of 50 μM hemin and is composed by 'hemin-resistant' cells (which proliferate but do not accumulate hemoglobins) together with cells which proliferate and at the same time accumulate large amounts of hemoglobins. Only 5–15% of K562(h) cells are therefore positive to the benzidine stain.

The K562(hC) cell line was obtained by cloning K562(h) cells by serial dilutions in 50 μM hemin[8]. After 20 days culture K562(h) clones – denominated K562(hA), K562(hB), K562(hC) ... – were stained with benzidine in order to identify erythroid induced, proliferating K562(h) cells.

91% of the K562(hC) cell population was found to be positive to the benzidine stain. This cell population was therefore compared with hemin-induced K562(S) cells and analyzed with respect to a) cell proliferation in semi-solid cell cultures; b) hemoglobin expression; c) accumulation of ε-globin and γ-globin mRNAs. Figure 1A shows that unlike hemin-induced K562(S) cells, the K562(hC) cell line retain an efficient rate of cell proliferation, which can be considered similar to that of uninduced K562(S) cells.

According with previous observations from our laboratory[5,8] this cell population accumulates predominantly Hb Gower 1 ($\zeta_2\varepsilon_2$) (78%) (fig. 1B, table). By contrast K562(S) cells accumulate both Hb Gower 1 and Hb Portland ($\zeta_2\gamma_2$) to the same extent after hemin-mediated erythroid induction (fig. 1B).

This finding might indicate a positive association between the rate of cell proliferation and the expression of Hb Gower 1, a hemoglobin which is only found in early human embryos[15].

In order to determine whether the pattern of hemoglobin expression was correlated with a differential accumulation of ε-globin and γ-globin mRNAs, cytoplasmic dot hybridizations were performed on formaldehyde-treated post-mitochondrial cell lysates[11] from hemin-induced K562(S) cells and K562(hC) cells. The (^{32}P)-nick translated probes used in these experiments were plasmid DNA JW151[13] containing DNA sequences specific for human γ-globin mRNA) and pε0.7[14] (containing ε-globin specific DNA sequences). The experiments reported in the upper panel of figure 2 suggest that the γ-globin probe is 3.1-fold more efficient that the ε-globin probe in detecting the same amounts of specific DNA sequences.

The most likely hypothesis which explains this behavior is that since the sizes of the JW151 and pε0.7 globin-specific DNA inserts are considerably different[13,14], the ε-specific probe does

hybridize only with the first and the second exons of the ε-globin mRNA[14], while the γ-specific probe contains sequences complementary to the γ-globin mRNA from preceding the initiator codon to sequences of the 3′ untranslated region following the terminator codon[13].

The results of the cytoplasmic dot hybridization performed (fig. 2) suggest that a) hemin-induced K562(S) cells accumulate 1.25 more ε-globin mRNA and 2.5 more γ-globin mRNA in comparison with uninduced K562(S) cells and b) the ε-globin mRNA/γ-globin mRNA ratio is significantly higher in K562(hC) cells in comparison with hemin-induced K562(S) cells.

In figure 3 are reported the values corrected for the differential efficiency of the hybridization performed with the ε-globin and γ-globin probes. In addition in figure 3 are shown also the cytoplasmic dot hybridization obtained using the ribosomal (^{32}P)-pXCR7 DNA probe (containing sequences for the 18S RNA and the 28S rRNA). These data suggest that K562(hC) cells accumulate 2.3-fold more ε-globin and γ-globin mRNAs with respect to hemin-induced K562(S) cells.

In addition, the ratios ε-globin mRNA/γ-globin mRNA in uninduced K562(S) cells is 1.3, in hemin-induced K562(S) cells is 1.25, while in K562(hC) cells is clearly different being 2.9 (fig. 3). These results, summarized in the table, are in good agreement with the pattern of hemoglobin expression shown in figure 1.

Discussion. In this paper we show that in highly proliferating, erythroid induced cell lines isolated from K562(h) cells the predominant accumulation of Hb Gower 1 ($\zeta_2\varepsilon_2$) is controlled at a pre-translational level, being the ε-globin mRNA preferentially expressed with respect to γ-globin mRNA (ε-globin mRNA/γ-globin mRNA = 2.9) (table, fig. 3).

This is, in our knowledge, the first report which suggest a positive correlation between efficient cell proliferation and predominant accumulation of embryonic-type globin sequences in erythroid induced K562 cells.

This is of interest with respect to at least three points of view: a) as the K562 cell population is heterogeneous with respect to cell proliferation and the type of hemoglobins produced, our results should encourage experiments aimed to determine whether a differential pattern of globin gene expression following administration of different chemical inducers[3,7,16] is due to clonal selection or to a true intracytoplasmic 'switch' in gene expression.

In addition b) as the predominant expression of ε-globin genes is found only at the very early stages of erythropoiesis of the human embryo[15], the K562(h) cell line can be used in order to produce cell clones which might be a unique model system to identify molecular mechanism(s) modulating the expression of ε-globin genes (DNA methylation, chromatin structure, differential stability and/or translation of specific mRNAs). On the other hand (c) the concqmitant expression of both differentiated functions and efficient cell proliferation (fig. 1) suggests that other tumor-associated markers, including transferrin receptors[17,18] or some sequences homologous to human cellular oncgenes (such as c-myc, c-erb, c-abl, c-rasH) (for a review see Yunis[19] and Bishop[20]) might also be highly expressed in K562 cells which retain biological features typical of the primitive embryonic erythroblasts.

Recent unpublished results from our laboratory indicate that at least the expression of two onc-gene sequences (c-abl and c-erb) appears to decrease together with the decrease of cell proliferation in hemin-induced K562(S) cells. By contrast K562(hC) cells, which proliferate while fully committed to Hb Gower 1 expression, accumulate c-abl related sequences to an extent similar to that of uninduced K562(S) cells (Gambari et al., in prep.).

This kind of experiments are of great interest especially when related to recent findings which suggest that the product of at least two onc-genes (c-erb and c-sis) are similar to the membrane receptor of the epidermal growth factor and the platelet-derived growth factor[21-23].

Cell lines similar to K562(hC) cells might therefore turn out to be of great help in studies focused on the understanding of molecular and cellular mechanisms regulating differentiation, cell proliferation and neoplastic state.

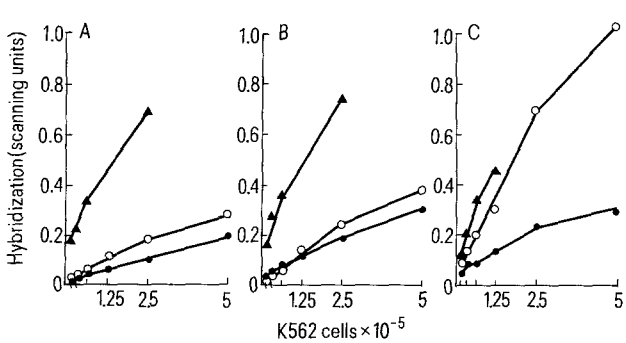

Figure 3. Densitometric analysis of the dot hybridizations performed between formaldehyde-treated cytoplasms from uninduced K562(S) cells (A), hemin-induced K562(S) cells (B) or K562(hC) cells (C) and the ε-globin (^{32}P) DNA (○), the γ-globin (^{32}P) DNA (●) or the ribosomal (^{32}P)-pXCR7 DNA probe (▲). The values of the ε-specific hybridizations have been corrected (×3.1) for the differential hybridization efficiency of the pε0.7 and JW151 probes (fig. 2, upper panel).

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