RAPID COMMUNICATION

Dynamics of GATA Transcription Factor Expression During Erythroid Differentiation

By Mark Leonard, Martha Brice, James Douglas Engel, and Thalia Papayannopoulou

Although the formation of terminally differentiated erythroid cells has been shown to require the presence of a functional GATA-1 gene in vivo, the role of this transcription factor and other members of the GATA family at earlier stages of erythroid differentiation is unclear. In this report, the expression of GATA-1, GATA-2, and GATA-3 has been examined in enriched peripheral blood progenitors before and after culture in a well-characterized liquid culture system. In addition primary leukemic cells as well as several erythroleukemic and nonerythroid cell lines were analyzed for GATA factor expression. The results show that the profile of GATA factor expression in erythroid cells is distinct from that of myeloid or lymphoid lineages. Erythroleukemic cell lines express little or no GATA-3, but high levels of GATA-1 and GATA-2. When they are induced to display the terminal erythroid phenotype, little change in the level of GATA-1 is detected but a significant decline in the levels of GATA-2 is observed commensurate with the degree of maturation achieved by the cells. Enrichment of erythroid progenitors from peripheral blood leads to selection of cells that express both GATA-1 and GATA-2. As the enriched populations are cultured in suspension in the presence of multiple cytokines, the levels of both GATA-1 and GATA-2 initially increase. However, in cultures containing only erythropoietin, which show exclusive erythroid differentiation, the levels of GATA-1 continue to increase, whereas GATA-2 expression declines as erythroid maturation progresses. In contrast, cultures lacking Epo (ie, with interleukin-3 and kit ligand) display limited progression towards both the myeloid and erythroid pathways, and high levels of expression of both GATA-1 and GATA-2 are maintained. Despite the initial upregulation of GATA-1 expression in the latter cultures, terminal erythroid differentiation does not occur in the absence of erythropoietin. These results indicate that GATA-1 upregulation is associated with both the initiation and the maintenance of the erythroid program, but that these two processes appear to be under separate regulatory control. Thus, the dynamic changes in the levels of different GATA factors that occur during primary erythroid differentiation suggest that the levels of these factors may influence the progression to specific hematopoietic pathways.

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TRANSCRIPTION FACTORS recognizing the GATA DNA consensus motif have now been described in a large number of vertebrate species and appear to exhibit a conserved pattern of expression during development and, in particular, in hematopoiesis. GATA-1, the first characterized factor in this multigene family, is abundantly expressed in erythroid-lineage cells and has proven to be a pivotal regulator of terminal erythroid differentiation in vivo. Furthermore, its presence has been recently documented in proliferating, factor-dependent cell lines and in primary hematopoietic cells with progenitor cell characteristics. However, GATA-1 is also expressed in nonerythroid lineages (megakaryocytic and mast cells and in testes) and additional members of the GATA family are expressed in erythroid cells. Thus, the precise role of GATA-1 in defining erythroid specificity and the stage of erythroid differentiation at which GATA-1 exerts its specific effects are unclear. Furthermore, parameters that govern the initiation and the maintenance of the erythroid phenotype have not been clearly defined. The contribution of other transcription factors expressed in erythroid cells (ie, SCL, NFE-2, Myb, Rb, or other GATA members) or the effects of growth factor receptor- (ie, erythropoietin receptor [Epo-R]) mediated signals potentially modulating the stabilization and/or induction of GATA-1 during terminal erythroid differentiation have not been explored.

We have investigated the patterns of expression of GATA-1, GATA-2, and GATA-3 in enriched populations of progenitors from normal peripheral blood and have observed their changes during differentiation under the influence of hematopoietic cytokines. In addition to primary cells, we have also examined the relative changes in abundance of GATA factors in erythroleukemia cell lines before and after induction of erythroid differentiation. We find that both GATA-1 and GATA-2 are expressed in enriched progenitor populations and both are upregulated further in the progeny of CD34(+) cells under the influence of hematopoietic cytokines, especially interleukin-3 (IL-3) and kit ligand (KL). However, in contrast to GATA-1, which is further induced in Epo-containing cultures in parallel with terminal erythroid differentiation, a significant decline in GATA-2 is observed in the same cultures. A similar phenomenon is also observed upon induction of erythroleukemia cell lines. It appears that the initial upregulation of GATA-1 expression occurs in the absence of Epo and is accompanied by the initiation of globin synthesis. However, this increased level of GATA-1 is not by itself sufficient for the maintenance or stabilization of the erythroid phenotype and for the survival of these cells. Thus, although it is possi-
ble that increased GATA-1 expression is required for the initiation of erythroid phenotype and globin expression, additional interactive regulatory events are needed for its maintenance. GATA-3 does not seem to have a clear role in human erythromyeloid differentiation.

MATERIALS AND METHODS

Cells

Peripheral blood cells from normal individuals were used as a source of normal cells and blood from leukemia (acute myeloid leukemia [AML] or chronic myeloid leukemia [CML]) patients as a source of primary leukemic blasts. Fetal liver samples were obtained, with informed maternal consent, from spontaneous or therapeutic abortions through the Central Laboratory for Human Embryology, University of Washington, Seattle. All procedures were performed according to institutionally approved protocols. To enrich for hematopoietic progenitors, peripheral blood mononuclear cells (PBMC), recovered after centrifugation of platelet apheresis collections, were subjected to a direct, positive immunoadherence to Petri dishes precoated with anti-CD34 or anti-c-kit monoclonal antibody (SR-1) according to previously described procedures. Adherent cells were recovered from the plates after flushing with warm Iscove's modified Dulbecco's medium/fetal calf serum (IMDM/FCS) and designated as PBMC-P. If enough cells were available after the first direct panning, a second round of direct adherence was performed to further increase the specificity of adherence or the progenitor enrichment.17

Cell Lines

Eight human erythroleukemia lines were examined: KU812,23 JK-1,24 MB02,20 HEL,21 OCI1 and OCI1M2,22 CMK,23 and F-36.24 In addition, several nonerythroid lines (HL-60 and EM-2), as well as Epstein-Barr virus (EBV)-transformed lymphocytes and other cell lines (IM9, U266, CEM, HSB2, HuT78, MOLT-3, and HEla cells)25 were studied.

Cell Cultures

Liquid suspension cultures. Enriched populations of progenitors were used (1×10^6 cells) in 1- or 2-mL aliquots for 6 to 14 days in stationary suspension cultures. Eighty percent to 90% of the medium and additives were replaced every 48 hours. The basic suspension medium was serum-containing (IMDM + 20% FCS + 10% human AB serum) with 1% bovine serum albumin (BSA), 10^-4 mol/L 2-mercaptoethanol. To these media, the various recombinant hematopoietic growth factors were added in micro-liter quantities: IL-3 (Genetics Institute, Cambridge, MA) was used at 50 U/mL; KL (stem cell factor [SCF]; Amgen, Thousand Oaks, CA) at 50 ng/mL; Epo (Genetics Institute) at 2 U/mL; and PIXY 321 (a granulocyte-macrophage colony-stimulating factor [GM-CSF]/IL-3 fusion protein; Immunex, Seattle, WA) at 10 μg/mL.

Clonal cultures. Peripheral blood cells before and after their purification were cultured in clonal methylcellulose cultures to assess the frequency of clonogenic hematopoietic progenitors and the degree of enrichment. Methylcellulose culture media were prepared in our laboratory,26 were used at optimal concentrations in phosphate-buffered saline (PBS)/BSA. In addition to immunofluorescence, globin mRNA was evaluated by a slot blot hybridization assay as previously described.27 Probes used and the procedure were described previously in detail.

Globin Analysis

Presence of globin was assessed by immunofluorescence on fixed centrifuge-prepared smears. Antibodies specific for β- or γ-globin, prepared in our laboratory,26 were used at optimal concentrations in phosphate-buffered saline (PBS)/BSA. In addition to immunofluorescence, globin mRNA was evaluated by a slot blot hybridization assay as previously described.27 Probes used and the procedure were described previously in detail.27

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Quantitative RT-PCR analysis was performed essentially as described.28 Total RNA was isolated from the cells indicated using standard protocols.26 Approximately 1 μg of total RNA was denatured (65°C for 5 minutes) before use as a template in a 20 μL cDNA synthesis reaction. To reduce variations in cDNA synthesis, all RNA samples were made into cDNA at the same time using a single master-mix containing (per sample) 1× RT-PCR buffer (50 mmol/L KCl, 20 mmol/L Tris-HCl [pH 8.4 at 23°C], 2.5 mmol/L MgCl2, 100 μg/mL BSA, 2.5 mmol/L diethanolamine [DTL], 1 mmol/L each dNTP), 17.5 U RNasin ribonuclease inhibitor (Promega, Madison, WI), 100 pmol random dN, primers (Pharmacia, Piscataway, NJ), and 2.5 U Taq DNA polymerase (Promega). The reaction was incubated for 10 minutes at 22°C followed by 90 minutes at 42°C. Aliquots of the cDNA reactions were analyzed for GATA factor expression in 10 μL PCR reactions. Again, to reduce sample to sample variability all cDNAs to be analyzed on a given gel were amplified at the same time using a master-mix containing (per sample) 1× Taq DNA polymerase buffer (Promega), 0.2 mmol/L each dNTP, 25 pmol of each primer (see below), 0.2 μL [α-32P]dCTP (3,000 Ci/μmol; ICN, Irvine, CA), and 2.5 U Taq DNA polymerase (Promega). PCR conditions were 94°C for 2.5 minutes, followed by 21 to 26 cycles (depending on the samples under analysis) of 94°C for 1 minute, 64°C for 1 minute, and 72°C for 1 minute. Control reactions were performed to ensure that the conditions used were within the linear range of PCR amplification (control lanes of figures, and data not shown). All reactions contained, as an internal control, primers to coamplify the S14 ribosomal protein gene product present in similar abundance to the GATA factors (and thus having a similar amplification profile).

Samples were analyzed on 6% denaturing polyacrylamide gels (loaded for equivalent internal control) and exposed to autoradiography before quantitation on a Molecular Dynamics PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The results shown throughout represent typical data from analysis on at least two separate occasions using at least two independent cDNA preparations. It should be noted that this methodology permits quantitative comparison of the level of a given GATA factor mRNA in different samples. However, due to differences in primer annealing/extension characteristics and amplicon length/sequence, etc, it is not possible to compare the absolute abundance of different mRNAs (GATAs or S14) in these experiments.

The following primers were used for PCR: human small ribosomal protein 14 (S14) sense (5') GCCGAGCCGAGATGATCATCA (3') and antisense (5') CAGGTTCAGGGGTCTTGCCT (3'), nucleotides 187 to 208 and 331 to 311, respectively, of the S14 sequence;26 hGATA-1 sense (5') CAAAGCGGCAATGCATACCTTAT (3') and antisense (5') CTTGCTTGTGTTGATCC (3'), nucleotides 116 to 136 and 298 to 276, respectively, of the
RESULTS

Expression of GATA Transcription Factors in Human Cell Lines and Primary Leukemic Cells

A number of studies have used Northern blot analysis to determine the tissue distribution of GATA factor mRNAs. Recently, such studies have been confirmed and extended using more sensitive RT-PCR techniques. To confirm the validity of this methodology as used here, total RNA was isolated from a variety of hematopoietic cell lines and a limited number of primary leukemic cells. hGATA-1, hGATA-2, and hGATA-3 expression was determined by RT-PCR. The results (Figs 1 through 3) show clearly distinct patterns of expression for hGATA-1 and hGATA-2 on the one hand and hGATA-3 on the other. Concordant with previous Northern blot analysis, hGATA-1 expression was detected in all eight of the erythroid cell lines examined and (at greatly reduced levels) in certain myeloid samples, but no expression was observed in either B- or T-lymphoid cells. Like hGATA-1, hGATA-2 is expressed at variable levels in all of the erythroid lines analyzed, as well as certain myeloid samples (Fig 2), but is not detected in T or B lymphocytes. In contrast, hGATA-3 is abundantly expressed only in T-lymphoid cells, although this mRNA is also present at very much reduced levels in EBV-transformed B cells. (The hGATA-3 expression detected in the peripheral blood AML sample [Fig 2] likely reflects the presence of 20% mature lymphoid cells, as assessed by immunophenotyping.)

GATA mRNA abundance was measured in four of the erythroleukemia lines before and after induction of differentiation to determine whether the absolute or relative levels of hGATA-1 and hGATA-2 expression are altered during erythroid differentiation in vitro. All of the lines tested became hemoglobinized, as judged by visual inspection for the acquisition of red pigmentation after induction, and the proportion of cells positive for human globin gene expression by immunofluorescence (Table 1). Although many cells showed morphologic signs of maturation such as decreasing cell size, accumulation of hemoglobin in the cytoplasm, and pyknotic nucleus, significant numbers of undifferentiated blast-like cells remained at the end of induction. When these cells were analyzed for GATA expression, little change in the level of hGATA-1 mRNA was detected (Fig 3). However, a significant reduction in the level of hGATA-2 was observed, particularly in the lines that showed better hemoglobinization and maturation, such as MB02 (Fig 3). Similarly, in the two independent primary erythroid (red) colonies assayed, higher levels of GATA-1 expression coincided with relatively lower levels of GATA-2 (Fig 3). These observations suggest that terminal erythroid differentiation is associated with a reduction in the levels of the transcription factor hGATA-2. Although the various leukemic myeloid cells tested express little or no hGATA-1, this mRNA is, surprisingly, essentially as abundant in primary GM + M myeloid colonies as in primary BFUe-derived colonies. This may reflect an active expression of hGATA-1 in proliferating myeloid precursors, before its inactivation as maturation progresses, as previously reported, or may be due to development of cells of eosinophilic lineage in these colonies.

GATA Factor Expression in Enriched Primary Human Progenitor Cells

The observation that more than one hGATA factor is expressed in a number of transformed erythroid cell lines led us to ask whether these GATA factors are similarly expressed in primary erythroid cells and, if so, whether there are hierarchical differences in the expression of these factors.
during erythroid lineage differentiation. PBMCs are composed mostly of T- and B-lymphoid cells with a variable proportion of monocytes (and often a small proportion of contaminating granulocytes). RNA from isolated total PBMCs was analyzed for hGATA factor expression. As expected, abundant hGATA-3 expression was observed, but little or no hGATA-1 or hGATA-2 could be detected (Fig 4). The PBMC samples were enriched for progenitors by immunoadherence to anti-CD34 or to anti-c-kit antibody (SR-1)-coated plates. Through this process, greater than 100-fold enrichment of hematopoietic progenitors was achieved (up to 30% of cells in the enriched fraction are progenitor cells, compared with less than 0.1% in the starting population). Progenitor-enriched cells consistently showed significantly reduced hGATA-3 mRNA levels and increased hGATA-1 and hGATA-2 (Fig 4). Moreover, hGATA-1 and hGATA-2 appear to be enriched with similar profiles during the purification process (Fig 4).

Table 1. Proportion of Globin (γ, β, ζ, or ε) and Benzidine-Positive Cells in Four Erythroleukemic Lines Before (−) and After Their Induction (+)

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>γ (%)</th>
<th>β (%)</th>
<th>ζ (%)</th>
<th>ε (%)</th>
<th>Benz (+) Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCIM2</td>
<td>15</td>
<td>55</td>
<td>&lt;1</td>
<td>10</td>
<td>16 64 0 8</td>
</tr>
<tr>
<td>JK-1</td>
<td>20</td>
<td>30</td>
<td>5 17</td>
<td>25</td>
<td>45 5 9 &lt;5 49</td>
</tr>
<tr>
<td>MBO2</td>
<td>9</td>
<td>100</td>
<td>3 36</td>
<td>0 0</td>
<td>0 10 &lt;1 97</td>
</tr>
<tr>
<td>F-36</td>
<td>18</td>
<td>72</td>
<td>&lt;1</td>
<td>1 3</td>
<td>5 0 0</td>
</tr>
</tbody>
</table>

* Pellets from all induced cells had red color.
In the peripheral blood samples, progenitors were also enriched from fetal liver (Fig 5). Progenitor-enriched samples (FL-P1 and FL-P2, Fig 5) were free of contaminating erythroblasts and the predominant progenitor cell was the BFUe. Interestingly, in these samples, hGATA-1 mRNA was abundant, whereas very low levels of hGATA-3 and GATA-2 expression were detected (Fig 5).

**hGATA-1 and hGATA-2 Are Abundantly Expressed in Proliferating Progenitors, But Their Expression Diverges in Terminally Differentiated Erythroid Cells**

To further analyze the pattern of expression of hGATA factors during erythroid maturation, and to test whether terminal erythroid differentiation of normal cells is associated with reduced GATA-2 expression, as suggested by data from erythroleukemic cell lines, samples of enriched progenitor cells were cultured in suspension either in the presence of early acting cytokines IL-3, KL, or PIXY-321, or in the presence of Epo. Under the influence of IL-3 and KL, there is significant cell proliferation and progenitor amplification during the first week in culture. Concurrently, cells with lineage-specific differentiative features begin to emerge. An interesting and unexpected feature was the fact that cells with both myeloid (ie, granule formation) and erythroid features were detected in these cultures. The presence of cells at later stages of erythroid differentiation (CFUe/proerythroblasts) was suspected on the basis of morphologic
Fig 6. Morphologic appearance of erythroid cells present in Epo-only cultures at 7 days. Note the characteristic proerythroblast-like or CFUe-like appearance of these blasts.

criteria (Fig 6) and confirmed by slot-blot hybridization assays for globin RNA (Fig 7) and by antiglobin immunofluorescence (data not shown). These results were obtained not only in serum-containing cultures but in serum-free cultures and under conditions in which anti-Epo antibody was present. Furthermore, no endogenous Epo mRNA was detected in these cultures by RT-PCR (data not shown). However, in the absence of Epo, erythroid cells are significantly diminished at later times in culture (12 to 14 days) and the population is virtually all granulomonocytic in nature. On the other hand, if Epo is present as a single factor in these cultures, a healthy basophilic blast population (resembling proerythroblasts, Fig 6) is present in significant numbers by the first week in culture, and 50% to 70% of these contain β-globin. At later times (days 12 to 13), there is increased β-globin mRNA expression (virtually all the cells present are β-globin-positive) and accumulation of smaller, more mature, strongly benzidine-staining cells. Thus, during the second week in culture there is a major difference between the Epo-containing cultures and those with only IL-3 and KL; the frequency of globin-positive cells decreases in IL-3 and KL cultures without any evidence of further maturation, whereas globin-positive cells in Epo-containing cultures increase to more than 90% by day 12 or 13.

The levels of hGATA-1 and GATA-2 were similar at day 0 in enriched populations of progenitors and they were found to increase further in culture (Figs 4 and 5). In experiments in which cells were studied under the stimulation of only Epo, GATA-1 expression remained high during the observation period (1 to 12 days), whereas GATA-2 expression declined (see Fig 5, Epo, days 4 and 7; and Fig 4, PBMC-P, day 12). Thus, abundant hGATA-2 expression seems to be associated with cell and progenitor proliferation (with IL-3 and KL), but terminal erythroid differentiation (in the presence of Epo) is associated with a drastic reduction in the levels of this transcription factor.

DISCUSSION

GATA-1

Enrichment of hematopoietic progenitors from peripheral blood selects for cells expressing GATA-1. Committed progenitors for all lineages are concurrently purified in these samples, raising the possibility that GATA-1 is expressed in multipotential hematopoietic lineage progenitors. Consistent with this, GATA-1 expression is detected in a multipotential bone marrow-derived cell line. However, whether heterogeneity exists in expression of GATA-1 among different types of progenitors cannot be determined from the present data.

When progenitors are cultured in suspension in the presence of various cytokines there is a significant upregulation of GATA-1 expression, in agreement with a previous report. In addition to cell proliferation and progenitor (BFUe and CFU-GM) amplification, there is a progressive decrease in CD34+ cells and a concomitant significant in-
crease in cells that have acquired lineage-specific differentiative features. For example, in cultures without Epo, apart from granulomonocytic differentiation, we have noted the generation of late erythroid progenitors (CFUe) and of globin-positive cells (in greater numbers than CFUe or BFUe) within the first week in culture.17 Thus, in contrast to previous reports,1,11 there is not exclusively myeloid differentiation in the absence of Epo in these cultures. Our findings raise the possibility that the upregulation of GATA-1 may not be due just to the proliferation of CD34+ progeny,11 but rather associated mainly with cell progeny displaying globin transcription. Thus, low levels of GATA-1 may be found in all types of progenitors, especially proliferating ones, but upregulation occurs only in erythroid and not in non-erythroid progenitors. Although further studies with separated progenitors are needed to confirm these points, indirect lines of evidence support the view that myeloid leukemia cell lines do not express significant levels of GATA-1; this factor has been found only in a small number of AML, especially in those of the undifferentiated type36 or in those from CML patients (Fig 2), in categories in which a mixed-lineage leukemia including erythroid cells is very likely.

Despite the initial upregulation of GATA-1 and the expression of globin, globin-positive cells generated in the absence of Epo are unable to mature further without this cytokine and die prematurely.17 These cells are greatly diminished after 2 weeks in culture and GATA-1 expression is also greatly reduced (data not shown). In contrast, in cultures containing Epo (Fig 1, PBMC-P, day 12), high levels of GATA-1 are maintained during the second week. One interpretation of these results is to implicate GATA-1 in both the initiation and maintenance of the erythroid program. However, these two events appear to be under separate regulatory control. Initiation is an Epo-independent event, whereas, for maintenance, additional events, triggered by Epo-R/ligand–dependent interactions, are needed and these are critical for the survival and further maturation of these erythroid cells. It is possible that, although GATA-1 may upregulate Epo-R, only subthreshold levels of Epo-R may be reached in the absence of Epo-R/ligand interactions.37 It is also possible that, in addition to Epo, other downstream-activated genes (such as SCL) are needed to sustain high levels of GATA-1 and maintain the erythroid program.

**GATA-2**

Detailed studies of GATA-2 expression during development in frogs and chickens have shown that GATA-1 and GATA-2 are expressed concurrently in development from the very onset of hematopoiesis. Similar studies of GATA-2 expression in developing human hematopoietic cells are not available. In the present studies, we show that progenitor enrichment leads to selection of cells expressing GATA-2 in addition to GATA-1. Likewise, culture of progenitors leads to upregulation of GATA-2 in addition to GATA-1, indicating that GATA-1 and GATA-2 are modulated in tandem at early stages of hematopoietic differentiation. It remains to be seen whether GATA-2 precedes GATA-1 expression in the hematopoietic hierarchy (ie, is present in progenitors with the CD34+/CD38- phenotype), as recently suggested,12 or whether there is heterogeneity in the levels of GATA-2 expression in committed progenitors. The profile of GATA-1 and GATA-2 expression during progenitor enrichment suggests that it is very likely that the same target cells express both of these factors. The presence of both GATA-1 and GATA-2 in all erythroleukemia lines tested would tend to support this conclusion (although erythroleukemia cells have more than one potential). Preparations of fetal liver that are highly enriched for erythroid progenitors express GATA-1 at much higher levels than GATA-2 (Fig 5). Furthermore, upregulation of GATA-2 is reduced or minimal in Epo-only containing cultures (in which exclusively erythroid differentiation is observed, Figs 4 and 5) compared with non-Epo-containing cultures in which a more mixed population is present. Thus, it would seem that, at a certain stage in erythroid differentiation, the expression of GATA-1 and GATA-2 diverge; earlier stages display GATA-2 expression, but with more differentiation and maturation the GATA-2 expression is dramatically reduced. The fact that GATA-2 is diminished as erythroid cells differentiate is supported by several additional pieces of evidence; small bursts (from myelocellulose cultures) with mostly differentiated mature erythroid precursors display much less GATA-2 expression than GATA-1 (Fig 3). Furthermore, upon differentiation, erythroleukemia lines display a downregulation of GATA-2 (eg, MB02, Fig 3) proportional to the degree of terminal maturation achieved; the more advanced the differentiation, the more conspicuous the GATA-2 downregulation. Furthermore, modest overexpression of GATA-2 in committed chick erythroid progenitors blocks terminal differentiation.38

**GATA-3**

GATA-3 expression in human hematopoietic cells does not appear to follow the expression pattern seen in chickens and frogs. From our studies in primary hematopoietic cells and cell lines, it is clear that the GATA-3 expression is confined only with the T-cell lineage. In contrast to findings in chickens, there is absence of GATA-3 expression in the terminal phase of human erythroid differentiation.

In summary, our data suggest that differentially regulated expression of the GATA family of transcription factors is associated with lineage selection during hematopoietic differentiation. We propose that initiation of the terminal erythroid program of gene expression and globin transcription is accompanied by an upregulation of GATA-1 and GATA-2, and does not require the presence of Epo. However, for maintenance of the erythroid program, additional regulatory events are required. For example, the second phase of GATA-1 upregulation may be dependent on Epo-R/ligand interactions or other downstream erythroid-specific regulatory genes; the downregulation of other factors such as GATA-2, or c-myb, may possibly also be critical. GATA-3 appears to be confined to lymphoid populations and not to be involved in human erythromyeloid cytodifferentiation. Nevertheless, a host of basic questions relevant to regulation...
of early stages of hematopoietic differentiation by GATA transcription factors remain currently unanswered. At what stage of differentiation is the expression of GATA-2 abrogated in erythroid versus myeloid cells? Are there quantitative differences in the expression of GATA-1 between megakaryocytic and erythroid cells at equivalent stages of differentiation? What are the necessary interactions, positive or negative, for sustaining and further upregulating GATA-1 in erythroid cells? As more sophisticated approaches become available for separation of progenitor subsets and testing them for transcription factor expression, several of these questions may be resolved.

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