Megakaryocytic Differentiation Induced in 416B Myeloid Cells by GATA-2 and GATA-3 Transgenes or 5-Azacytidine Is Tightly Coupled to GATA-1 Expression

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The GATA 'zinc-finger' transcription factors are thought to have important roles in the control of hematopoiesis. GATA-1 and GATA-2 are found in the erythroid, mast cell, and megakaryocytic lineages, and GATA-3 in T lymphocytes. GATA-1 is required for erythroid development and has recently been shown by gene transfer to direct megakaryocytic differentiation of the primitive myeloid cell line 416B. Here we show that enforced expression in 416B cells of either the GATA-2 or GATA-3 gene also induces megakaryocytic differentiation, as assessed by cellular morphology, acetylcholinesterase activity, polyploid DNA content, and loss of Mac-1 expression. No erythroid or mast cell differentiation was found. Unexpectedly, the level of endogenous GATA-1 mRNA had increased 20- to 30-fold among the transfectants, whereas that of GATA-2 mRNA was unaltered and endogenous GATA-3 transcripts remained undetectable. This finding suggests that GATA-2 and GATA-3 lie upstream of GATA-1 in a regulatory hierarchy and that, in 416B cells, GATA-1 may mediate the phenotypic changes induced by GATA-2 or GATA-3. Furthermore, 416B cells treated with the DNA demethylating agent 5-azacytidine underwent megakaryocytic differentiation accompanied by a marked increase in the level of GATA-1 mRNA but not that of GATA-2 or GATA-3. These results strongly implicate GATA factors in megakaryocytic differentiation and suggest that, at least for 416B cells, GATA-1 is a dominant regulator of maturation along this lineage.

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mine whether GATA-2 or GATA-3 also had this capacity. Here we report that introduction of a GATA-2 or GATA-3 expression vector into 416B cells also elicited megakaryocytic development. However, the dramatic increase in endogenous GATA-1 expression found in these transfecants suggests that the GATA-1 gene can be activated by either GATA-2 or GATA-3 and that the phenotypic changes may be mediated by GATA-1. By analogy with the myogenic system, the extremely low GATA-I expression in untransfected 416B cells might reflect methylation of the gene. Therefore, we tested the effect of 5-azaC and found that megakaryocyte production was indeed induced, in concert with markedly elevated expression of GATA-1. Taken together, these findings strongly implicate GATA factors in megakaryocytic maturation. Moreover, the tight coupling of GATA-1 expression with megakaryocytic differentiation in 416B cells suggests that it may be a key regulator of that pathway.

**MATERIALS AND METHODS**

**Plasmids.** The expression vector pEF-MC1neo has been described previously. A 2.2-kb human GATA-2 cDNA fragment derived from the hGATA-2/pXM expression vector12 and a 2.2-kb human GATA-3 cDNA fragment derived from hGATA-3/pCDNA1neo3 were inserted into a blunt Xhol site in the CDMS polylinker 3' of the pEF-α promoter10 of pEF-MC1neo. Clones in the sense orientation were selected. The vector MC1neoPA provided a control.

**Cell lines, electroporation conditions, and treatment with 5-aza-cytidine.** The 416B line15 and transfecants derived from this line were passaged in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal calf serum (FCS). Cell lines other than those generated in this work have been cited previously.18,22 For electroporation, 1 × 10^7 416B cells in 0.5-mL HEPEs-buffered RPMI medium containing 20 µg of SalI-linearized GATA-2 or NdeI-linearized GATA-3 expression vector were left at room temperature for 5 minutes before pulsing at 270 V, 960 µF using a Bio-Rad Gene Pulser (Bio-Rad, Hercules, CA). After 5 minutes on ice, the cells were diluted into 50 mL of warm DMEM, 10% FCS. Twenty-four hours later, the cells were plated in 24-well plates at 5 × 10^4 cells/mL in medium containing 1.5 mg/mL G418 (Gentec, Sigma, St Louis, MO). The electroporation frequency was estimated to be 1 in 2 × 10^6 cells. Clonal lines were generated by limiting dilution in 96-well plates.

For treatment with 5-azaC, 416B cells at 5 × 10^4 or 10^6 cells/mL in 24-well plates were exposed to 0.5 to 5.0 µmol/L drug (Sigma) for 48 hours. Cells from 3 to 4 wells at the same concentration of 5-azaC were pooled, washed three times and replated in fresh DMEM/10% FCS. Megakaryocytes were observed in all wells after 5 to 6 days. Clonal lines were derived by limiting dilution in 96-well plates.

**DNA and RNA analysis.** Nucleic acids were isolated and fractionated as described previously.23 The probes used for Southern or Northern analysis included the following: mouse GATA-1, a 1.8-kb Xhol fragment24; 5 mouse GATA-1, a 0.35-kb PvuII-Xhol fragment from the 3' untranslated region; human GATA-2, a 2.2-kb Eco RI fragment12; human GATA-3, a 2.2-kb NotI fragment27; mouse SCL, a 1.9-kb EcoRI fragment18; a 0.6-kb fragment from the mouse AChE gene8; a mouse platelet factor 4 fragment (nucleotides 1 to 306); and a 0.7-kb XbaI-EcoRI fragment from pEF-BOS,8 spanning the human granulocyte-colony stimulating factor (G-CSF) polyadenylation region. Mouse cDNA probes were hybridized in standard buffer containing 50% formamide whereas human cDNA probes were hybridized under reduced stringency (30% formamide) and also washed at lower stringency, at 65°C in 2 × sodium chloride/sodium citrate (SSC)/0.1% sodium dodecyl sulfate (SDS) instead of 0.2 × SSC/0.1% SDS. A rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe36 was used to monitor RNA loading and transfer efficiency. Probes were labeled with α-32P-dATP by priming with random hexamers (Bresatec, Adelaide, South Australia).

**Flow cytometric analysis and measurement of DNA content.** The monoclonal antibodies (MoAbs) Ter-11927 and M1/70 (eMac-1)28 were used in flow cytometric analyses as described. Ter-119 was directly conjugated to fluorescein isothiocyanate (FITC) while eMac-1 was biotinylated and used in conjunction with phycoerythrin (PE)-streptavidin (Caltag, San Francisco, CA). All staining was performed in the presence of MoAb 2.4G2 (anti-Fcy receptor) to block nonspecific binding of antibodies.

DNA content29 was estimated as follows: 1 × 10^6 cells in 1 mL of propidium iodide solution (50 µg/mL) containing 0.1% sodium citrate, 0.1% Triton X-100 (BDH Chemicals, Victoria), were lysed for 10 minutes at room temperature, then filtered through a 44-µm nylon membrane before flow cytometric analysis. Fluorescence above 570 µmol/L was measured and the percent cells with a DNA content greater than 4 N calculated.

**Cytotoxic staining.** Cytocentrifuge preparations were stained for acetylcholinesterase as described. Standard cytochemical stains for cell morphology included modified Wrights (Diff-Quik, Lab-Aids, New South Wales) and May-Grunwald–Giemsa. Benzidine–HCl was used to detect the pseudoperoxidase activity of hemoglobin in cell suspensions.

**RESULTS**

**GATA-1 and GATA-2 have a similar pattern of mRNA expression in the hematopoietic system.** As expression of the GATA-2 gene has been examined only in a limited number of avian and human lines,24 we compared the distribution of its mRNA with that of GATA-1 in 35 murine hematopoietic cell lines, 23 of which are shown in Fig 1. Consistent with previous reports, a high level of two GATA-I and GATA-2 RNA species (2.9 and 3.5 kb) was observed (Fig 1) in four erythroid lines, a megakaryocytic line, and both mast cell lines examined. GATA-2 transcripts were also abundant in the multipotential lines 32D and B6SuTA1, in the early myeloid lines 416B and FDC-P1, and in the LyH7 and BaF3 (not shown) lines, which have both pro-B and promyelocytic features. No GATA-2 expression was detectable in 17 B- or 1-lymphoid lines, 7 of which are shown. This expression pattern resembles that of GATA-1 (Fig 1), but the relative levels of their expression differed considerably among cell lines representative of a particular lineage. For example, only a very low level of GATA-1 transcripts was found in the myeloid line FDC-P1 and the mastocytoma line P815 (visible on longer autoradiographic exposure) whereas GATA-2 mRNA is abundant in these lines (Fig 1). Conversely, GATA-1 mRNA was much more abundant than GATA-2 mRNA in the erythroid line FL-F4N. Hence, expression of neither of these GATA genes is tightly coupled to that of the other. As expected, GATA-3 transcripts were observed in seven murine T-cell lines but, among the other lines, a low level was also apparent in two macrophage....
lines (P388D1 and WR19) and the HC.3 mastocytoma line (data not shown).

Generation of 416B clones with high GATA-2 or GATA-3 expression. The human GATA-2 and GATA-3 cDNAs were introduced into the vector pEF-MCI neo, which had previously yielded a high level of expression of GATA-1 and SCL.

In this vector, the powerful promoter from the translational elongation factor-1α (EF-1α) gene directs expression of the gene of interest and a separate neomycin resistance cassette serves as a selectable marker. The linearized expression vectors were electroporated into 416B cells; G418-resistant transfectants were selected and clonal lines were generated by limiting dilution. Southern blot analysis showed that most transfectants harbored 2 to 20 copies of the intact EF-1α expression cassette and confirmed that the lines were of independent origin (data not shown).

Both GATA transgenes were expressed abundantly in the transfectants. The level of transcripts expressed from the GATA-2 transgene (2.9-kb transcript in Fig 2A) was generally fivefold higher than the endogenous level in 416B cells and comparable with that in several erythroid, megakaryocytic, and mast cell lines, as exemplified by the HC.3 line. The transgene-derived GATA-2 transcripts could be distinguished by hybridization with a fragment from the pEF-MCI neo vector that provides the polyadenylation region (Fig 2B). Similarly, the level of GATA-3 mRNA expressed from the EF-1α promoter was at least 20-fold higher than that of the endogenous (3.5-kb) transcript in three murine T-cell lines, illustrated by Tikaut in Fig 3A. The two transgene products in Fig 3A appear to result from alternative sites of polyadenylation. The 2.9-kb mRNA reflects use of the exogenous polyadenylation sequence contributed by the expression vector, as indicated by its hybridization to the relevant vector probe (3′ transgene; Fig 3B), whereas the 2.2-kb transcript in Fig 3A presumably terminates after the polyadenylation signal in the introduced GATA-3 cDNA.

GATA-2 and GATA-3 provoked megakaryocytic differentiation of 416B cells. After 6 days of selection in G418, large megakaryocytes appeared in every pool of 416B cells electroporated with the GATA-2 expression vector (48 wells) or the GATA-3 vector (24 wells), whereas none appeared with the control vector MCINeo (72 wells). The pools of transfectants did not contain hemoglobinized (benzidine+) erythroid cells, nor were any mast cells evident on staining with May-Grunwald-Giemsa.

The morphologic changes induced by enforced GATA-2 or GATA-3 expression in clonal derivatives are depicted in Fig 4, where the top panel shows cell suspensions and the lower panel shows stained cytocentrifuge derivatives. The control 416neo line, which was indistinguishable from the parental line, consisted of small blast cells with a large nuclear to cytoplasmic ratio. In contrast, GATA-2 and GATA-3–expressing transfectant lines comprised not only 416B-like blast cells and intermediate-sized cells, but also a distinctive subpopulation (approximately 4% to 8%) of mature megakaryocytes, characterized by their large size, multi-lobed nuclei, and granular cytoplasm (Fig 4).

An important megakaryocytic marker is acetylcholinesterase (AChE) activity. Cytochemical staining for AChE activity showed very high levels within the cytoplasm of the mature megakaryocytes and intermediate levels within the smaller cells (data not shown). In three independent GATA-2–expressing lines, from 50% to 90% of cells exhibited moderate or intense staining and another 7% to 25% had a lower level. Similarly, in two GATA-3 transfectants, 79% to 85% of cells exhibited at least moderate staining and 11% to 18% had a lower level, whereas neither the parental line nor the control 416neo cells exhibited any detectable AChE activity. Thus, a large proportion of the cells expressing either GATA-2 or GATA-3 appeared to be committed to the megakaryocytic lineage. Consistent with these cytochemical data, Northern blot analysis (Figs 2D, E; Figs 3D, E) showed that the level of mRNAs for both the AChE gene and another megakaryocyte-specific gene, platelet factor 4 (PF4), was 3- to 10-fold higher in lines showing megakaryocytic differentiation than in the control lines.
Mature megakaryocytes are polyploid and both the GATA-2 and GATA-3 transfectants contained a subpopulation of such cells. Flow cytometric analysis of DNA content showed that 3.7% of cells in the GATA-2 transfectant C6 (Fig 5A) and 1.9% in the I2 line (not shown) contained nuclei with greater than 4 N DNA content. Similarly, for the GATA-3 transfectants F7 (Fig 5A) and C5 (not shown), 5.6% and 1.9% of cells were polyploid whereas only 0.2% of cells in the control 416neo contained nuclei with greater than 4 N DNA content.

As noted previously for GATA-1 transfectants, the myeloid lineage marker Mac-1 was substantially downregulated in the lines expressing the GATA-2 or GATA-3 transgene (Fig 5B). Whereas only approximately 3% of 416B cells lacked this surface marker, 29% of cells in the megakaryocytic line GATA-2.C6 and 89% in the GATA-3.F7 line were negative. Similarly, in three other 416GATA lines (GATA-2.I2, GATA-3.C5, GATA-3.A3), from 14% to 89% of cells lacked Mac-1. The bimodal distribution, particularly evi-
GATA FACTORS IN MEGAKARYOCYTIC DEVELOPMENT

Fig 4. Morphology of 416B transfectants. The upper panel depicts cell suspensions of (A) 416B cells electroporated with the neo<sup>+</sup> gene alone (416neo), (B) a GATA-2 transfectant, and (C) a GATA-3-expressing clonal line. Original magnification X 250. The lower panel shows cytocentrifuge preparations stained with modified Wright’s (Diff-Quick) of (A) 416neo control cells, (B) and (C) GATA-2 and GATA-3 expressing lines, respectively. Original magnification X 500.

Fig 5. Analysis of DNA content and cell-surface marker expression in 416neo cells and GATA-gene transfectants. (A) Relative DNA content of 416B cells transfected with MC1-neoPA (neo), a GATA-2 transfectant, and a GATA-3 transfectant. DNA content was estimated by flow cytometry of propidium-iodide-stained DNA complexes. The relative positions of 2N and 4N are shown. Both axes represent linear scales. (B) Flow cytometric analysis of Mac-1 and Ter-119 surface antigens on control 416neo cells (neo), the GATA-2 expressing clone GATA-2.C6 and the GATA-3 transfectant GATA-3.F7. Unstained cells gave fluorescent intensities within the lower left quadrant.

dent in the GATA-2.C6 line, correlates with the presence in the cultures of undifferentiated cells as well as those at different stages of differentiation along the megakaryocytic lineage.

GATA-1 mRNA is markedly elevated in GATA-2 and GATA-3 transfectants. In the parental 416B cells, GATA-2 transcripts (2.9 and 3.5 kb) were moderately abundant, but the level of GATA-1 mRNA (1.9 kb) was very low (Fig 1) and no GATA-3 mRNA (3.5 kb) was detectable (not shown). Among megakaryocytic lines generated by any of the three GATA transgenes, the amount of endogenous GATA-2 transcripts remained unaltered and no endoge-
A Probe
3' GATA-1
GATA-1 clones
GATA-2

B
GATA-1
GATA-2 clones

C
GATA-3 clones

D
5-azaC clones

Fig 6. Northern analysis of 416B cells transfected with GATA genes or treated with 5-azaC. Filters containing poly(A)⁺ RNA samples (3 μg) were hybridized sequentially with mouse GATA-1 (high stringency) and human GATA-2 probes (moderate stringency). The 3' GATA-1 probe used in (A) corresponds to a 3' untranslated sequence excluded from the expression vector construct and is therefore specific for endogenous GATA-1 mRNA. The control GAPDH hybridizations for the GATA-2 and GATA-3 clones are displayed in Figs 2 and 3, respectively, whereas that for these GATA-1 expressing lines has been shown previously. In (D), four clonal megakaryocytic lines isolated after treatment of 416B cells with 5-azaC are shown. Equivalent amounts of intact RNA were loaded on the basis of ethidium bromide staining of the gel.

DISCUSSION

Although the immature myeloid line 416B originally could differentiate in vivo along the megakaryocytic and granulocytic lineages, no differentiation now occurs spontaneously, nor can any be induced by interleukin-6 (IL-6) or erythropoietin (Epo), growth factors that normally promote megakaryocytic maturation. We recently showed that introduction of a GATA-1 expression vector into this line provoked megakaryocytic differentiation and report here that enforced expression of the GATA-2 or GATA-3 gene induced the same phenotypic change (Fig 4). Like GATA-1 transfectants, the lines bearing a GATA-2 or GATA-3 transgene comprised a heterogeneous population that included undifferentiated 416B-like cells in addition to cells at

ous GATA-3 transcripts were found (Figs 6A and 6C and data not shown). However, unexpectedly the level of GATA-1 transcripts increased 20- to 30-fold in every GATA-2- or GATA-3-expressing megakaryocytic line (Figs 6B and C). These findings raise the possibility that both the GATA-2 and the GATA-3 factors can activate transcription of the endogenous GATA-1 gene (see Discussion). In striking contrast, the GATA-1 transgene did not provoke a significant increase in endogenous GATA-1 mRNA in the megakaryocytic GATA-1 transfectants. Hybridization with a probe specific for endogenous GATA-1 transcripts (Fig 6A) showed at most a twofold increase, observed for certain clones but not their siblings. Thus, the GATA-1 gene appears to be regulated by other GATA members but no autoregulation was observed.

The mRNA for the presumptive transcription factor SCL increased threefold to fivefold in every transfected expressing GATA-2 (Fig 2C) or GATA-3 (Fig 3C), as it did in all GATA-1 transfectants. Hence, one or more of the GATA factors may stimulate transcription of the SCL gene (see Discussion).

5-azaC induces megakaryocytic differentiation of 416B cells. No phenotypic change in the 416B line was provoked by treatment with chemicals such as phorbol ester, butyrate, or dimethyl sulfoxide (DMSO). However, in four independent experiments, treatment with the demethylating agent 5-azaC at 0.5 to 5 μmol/L induced the appearance of megakaryocytes. After 6 to 7 days these cells were evident in every cell pool. Limiting dilution indicated that 21% to 50% of cells in each pool were committed to the megakaryocytic lineage. Clonal lines consisted of mature megakaryocytes (4% to 10%) together with cells of an intermediate or small size. These lines were indistinguishable from those generated by enforced expression of the GATA genes and a similar proportion of cells exhibited intense staining for AChE activity.

Significantly, northern blot analysis showed that GATA-1 expression in clonal lines derived from 5-azaC-treated cells was 20- to 30-fold higher than that in the parental line (Fig 6D). In contrast, GATA-2 mRNA levels remained unchanged (Fig 6D) and GATA-3 was not expressed (data not shown). These data implicate GATA-1 as the key factor in regulating megakaryocytic differentiation in this early myeloid line.
various stages of megakaryocytic maturation. A subpopulation of 4% to 8% mature megakaryocytes was evident morphologically and by polyploid DNA content (Fig 5A). The majority of cells in the cultures were apparently committed to the megakaryocytic lineage, as 50% to 90% exhibited strong AChE staining. As well as AChE, the megakaryocytic lines contained increased levels of mRNA for the megakaryocytic differentiation potential of many myeloid progenitors to the megakaryocytic pathway.

Although enforced expression of the SCL (or TAL) gene, which has a hematopoietic expression pattern remarkably similar to that of GATA-1 and GATA-2,18,19 has been shown to provoke maturation in an erythroid lineage20 it did not provoke megakaryocytic (or erythroid) maturation of 416B cells. Nevertheless, SCL may well cooperate with other factors in regulating megakaryocytic maturation, because SCL mRNA levels increased several fold in all the GATA-2 and GATA-3 transfectants (Figs 2C and 3C) as well as in those expressing the GATA-1 transgene.16 Whereas this increase might be an indirect consequence of megakaryocytic maturation, one (or more) of the GATA factors may instead act directly on the SCL promoter, which contains a GATA binding site that can mediate transcriptional activation by GATA-1.20

Members of a gene family often have overlapping functions, presumably to protect critical cellular pathways. Despite the distinctive expression patterns of the GATA factors, transient transfection assays and DNA-binding experiments have not delineated specific roles for these factors.1 Disruption of the GATA-1 gene has established that this factor is essential for erythroid development,2 but functional redundancy could hold for the megakaryocytic and mast cell lineages as development along these pathways may still be operative in GATA-1 null embryos.

Although all three GATA genes could induce megakaryocytic differentiation in 416B cells, GATA-1 appeared to be the most important for implementing the megakaryocytic program. Because GATA-1 mRNA levels were elevated 20- to 30-fold in all the lines expressing the GATA-2 or GATA-3 transgene (Figs 6B and C), we suggest that enforced expression of GATA-2 or GATA-3 stimulated GATA-1 expression, which then directed megakaryocytic differentiation (Fig 7). GATA-3 is unlikely to play a role in normal megakaryocytic differentiation as it appears to be largely restricted to T-lymphoid cells. However, because GATA-2 is expressed within the megakaryocytic lineage as well as in multipotential myeloid lines, it is also plausible that GATA-2 elicited megakaryocytic maturation directly in the GATA-2 transfectants (Fig 7). If normal megakaryocytic differentiation can be directed by either GATA-2 or GATA-1, that pathway would be maintained in GATA-1 deficient embryos. Thus, our findings emphasizing the importance of GATA-1 in megakaryocytic differentiation are not in conflict with the phenotype elicited by GATA-1 gene disruption.

Our findings (summarized in Table 1) suggest that the GATA-2 and GATA-3 genes may lie upstream of GATA-1 in a regulatory hierarchy. Consistent with this view, in human hematopoiesis expression of GATA-2 and perhaps even GATA-3 appears to precede that of GATA-1.36 Each factor may act via the double GATA motif present in the upstream region of the GATA-1 promoter and known to be required for its full activity.37,38 However, other factors must participate in GATA-1 gene regulation, because the gene is expressed very poorly in certain hematopoietic cell lines containing abundant GATA-2 mRNA (Fig 1). Interestingly, in 416B cells the GATA-1 transgene did not provoke

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**Table 1. Megakaryocytic Differentiation in 416B Cells**

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<tr>
<th>Agent</th>
<th>Expression of Endogenous Gene*</th>
<th>Megakaryocytic Differentiation</th>
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<tr>
<td>None</td>
<td>±</td>
<td>yes</td>
</tr>
<tr>
<td>GATA-1 transgene</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>GATA-2 transgene</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>GATA-3 transgene</td>
<td>++++</td>
<td>+</td>
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<tr>
<td>SCL transgene</td>
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<tr>
<td>5-azaC</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Abbreviation: ND, not done.</td>
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* refers to low levels of transcripts, detectable only on longer autoradiographic exposure.
expression of its endogenous counterpart (Fig 6A). Evidence from cotransfection experiments has indicated that GATA-1 can drive its own transcription, via the GATA sites in its promoter,\(^{37}\) but it has not been established whether other GATA factors also regulate this promoter. Our data favors control of GATA-1 gene expression by GATA-2 and GATA-3, but do not exclude the proposed autoregulation of GATA-1 expression during erythroid maturation.\(^{37,38}\)

Further support for a dominant role of GATA-1 in the differentiation of 416B cells arose from the experiments with 5-azaC. A substantial proportion (21% to 50%) of the treated cells became committed to megakaryocytic differentiation. By morphologic appearance and AChE staining, representative clonal lines were indistinguishable from those generated by GATA-factor transgenes. Moreover, their megakaryocytic differentiation was accompanied by a dramatic increase in the level of GATA-1 mRNA but not, significantly, that of GATA-2 or GATA-3 (Fig 6D and Table 1). This increase may reflect direct activation of GATA-1 expression by demethylation. The tight coupling in 416B cells of GATA-1 expression with the megakaryocytic differentiation elicited by all three transgenes as well as by the demethylating agent (Table 1) leads us to suggest that GATA-1 is a primary effector of megakaryocytic maturation.

Our results with the 416B cell line are reminiscent of those with the 10T1/2 myogenic progenitor line.\(^{13}\) The frequency (21% to 50%) with which megakaryocytes arose from 416B cells treated with 5-azaC is comparable with that of myogenic conversion (25% to 50%) of 10T1/2 cells exposed to this agent. The high frequency observed with the 10T1/2 cell line suggested that a single gene might suffice to elicit myogenesis in these cells, as subsequently proved to be the case. The establishment of permanent cell lines and their prolonged culture presumably select against expression of endogenous genes that promote differentiation, such as MyoD in 10T1/2 cells or GATA-1 in 416B cells, and de novo methylation of such genes may contribute to the selection process.\(^{14}\)

As GATA-1 and GATA-2 are coexpressed in three hematopoietic lineages, it is presumed that each of these factors contributes primarily to the control of maturation along these lineages rather than commitment to any one of them. A crucial role for GATA-1 in erythroid maturation was shown by gene disruption,\(^{2}\) and a role in megakaryocytic maturation can be inferred from our observations. However, GATA-1 cannot be the sole determinant of differentiation in any of these lineages because erythroid markers known to be regulated by GATA-1 are not present in megakaryocytes or mast cells, despite their abundant GATA-1 expression. Lineage commitment in a presumptive progenitor cell for the erythroid, megakaryocytic, and mast cell lineages is likely to require interplay between the relevant GATA factors and other cell-restricted transcription factors.

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Megakaryocytic differentiation induced in 416B myeloid cells by GATA-2 and GATA-3 transgenes or 5-azacytidine is tightly coupled to GATA-1 expression

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