The Transcription Factor GATA-1 Regulates the Promoter Activity of the Platelet Glycoprotein IIb Gene*

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Glycoprotein IIb (GPIIb) is an early and specific marker of the megakaryocytic lineage. We have previously shown that a fragment extending 643 base pairs upstream the transcription start site of the human GPIIb promoter was able to control the tissue-specific expression of the CAT gene in transfection experiments. Four potential GATA-binding sites, located at positions −483, −376, −243, and −54 are present within this fragment. Gel shift analysis revealed that nuclear extracts from the erythroleukemic cell line HEL contain a DNA-binding protein that recognizes these GATA sites. Using an antisera raised to an hydrophilic region of the transcription factor GATA-1, the HEL GATA-binding protein was found to be GATA-1. Point mutations of the different GATA sites indicated that they did not equally contribute to GPIIb promoter activity. The −463 GATA motif located in an enhancer region is essential for full transcription activity and was found to be dominant upon the other GATA motifs. When this site is mutated, the −54 GATA site appears to be essential for the remaining CAT activity. These results indicate that the transcription factor GATA-1 plays an important role in the regulation of the transcription of the megakaryocyte specific GPIIb gene.

The commitment of a totipotent stem cell into the different hematopoietic compartment is a key event in the production of the needed number of the blood cells of each type. A number of factors (cytokines, interleukines, and colony stimulating factors) and their receptors are essential for the proliferation, the differentiation and the self-renewal of the cells (1). In response to these external stimulations, some genes are turned on or off, establishing progressively a specific pattern of gene expression, representative of a specific lineage and of a differentiation stage. This pattern of gene expression is controlled by a network of transcription factors. Some of these factors are lineage specific, but can also form transcriptionally active complexes with other molecules of broader specificity. In the hematopoietic system, megakaryocytes and erythrocytes are closely related and probably share common transcription factors. One of the factors that may be implicated in both lineages is GATA-1. It is a major regulator of erythroid genes and recognizes the DNA motif WGATAR (2) within the regulatory region of all vertebrates (3). GATA-1 is a member of a family of DNA-binding proteins including GATA-2 and GATA-3, which are related by two highly conserved zinc finger DNA recognition domains (4–6). These GATA factors have a different cellular distribution although coexpression in several cell types has also been observed. GATA-1 has been found in erythroid (7), mast (8), and megakaryocytic (8, 9) cells, suggesting that this factor may be a regulator common to these lineages. To verify this hypothesis, we have used a megakaryocyte-specific marker gene to analyze the role of GATA-1 in the transcriptional activity of a megakaryocytic promoter. We have previously cloned the human GPIIb gene (10), an early and specific marker of megakaryocytopenia (11). We have also shown that a −643 to +33 fragment of the GPIIb promoter was able to drive the cell-specific expression of a reporter gene in megakaryocytic cell lines (12) and contained an enhancer active in both erythroid and megakaryocytic cell lines (13). In the present study, we have dissected the transcriptional activity of four GATA sites present in the promoter region of this gene. Using an anti-GATA-1 antisera, we demonstrate that all the GATA sites bind GATA-1 in vitro but do not equally contribute to the promoter activity.

MATERIALS AND METHODS

DNA Probes—The synthetic oligonucleotides used in mobility shift assays were prepared with an Applied Biosystems DNA synthesizer. The first strand was 5′-end-labeled and annealed with an excess of the non-labeled second strand at a one to four ratio to optimize annealing. The following oligonucleotides were prepared: 5′-CTGATGGGCCCCATATGCTTTTACCACT-3′ from the erythroid promoter of human porphobilinogen deaminase gene (PBGD) was used as a GATA-1 standard binding site (14) and 5′-GGGCTGCTATCTCCGGCCTGC-3′ from the promoter of preproendothelin-1 gene (PPET-1) used as a GATA-2 standard binding site (15); the HNF-1-binding site from the fibrinogen α promoter, 5′-GAACCAATAGGACATGGTA-3′ (12); the wild type and the mutated GATA-binding sites of human GPIIb promoter were, respectively, 5′-CCAGTTTTGATAAGAAAGAC-3′ and 5′-CCAGTTTGACGGAGAAAC-3′ for positions −61 to −42 (−54 GPIIb probe), 5′-AGACATCATTGATAAGGGCAGAACC-3′ and 5′-AGACATCATTGTCTGCAAGAACC-3′ for positions −225 to −233 (−243 GPIIb probe), 5′-GGAGGAGATTAGACTGCT-3′ and 5′-GGAGGAGATTAGACTGCT-3′ from the promoter of HNF-1 binding site from the fibronogen β promoter was 5′-GGAGGAGATTAGACTGCT-3′ (16), the CCAAT-binding site from GPIIb promoter was 5′-TAGCTCAGTGTCAATTATACATCAAGGGAG-3′ (16), and the -643 GATA site was 5′-TAGCTCAAACGTGCTCAAATATACATCAAAGGGAG-3′ (16).

Cells and Nuclear Extracts—The nuclear extracts were prepared from HEL (erythroleukemia) and Hela (epithelial carcinoma) cells as described elsewhere (12), according to the method of Dignam et al. (17).

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The human endothelial cells (HUVEC) were collected from umbilical veins as described by Jaffe (18) and were grown on purified fibronectin-coated plates in medium 199 supplemented with 2% endothelial cells growth substance (ULTROSER IBF) and 20% fetal calf serum. A rapid nuclear protein preparation was performed by the method of Schreiber et al. (19).

**Gel Retardation Assays**—The gel retardation assays were performed by a combination of the procedures of Halligan and Desiderio (20) and of Singh et al. (21).

For the binding reaction, 0.2-0.5 ng of radiolabeled DNA fragment (20,000 counts/min) were mixed with 5 μg of nuclear extracts in a final volume of 30 μl containing 10 mM Tris-HCl, pH 7.5, 25 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 15% glycerol, and 2 μg of poly(dI)-dC (dI-C) used as non-specific competitor. Specific competitors and antisera were added to each reaction as described in individual experiments. Samples were incubated for 20 min at room temperature and analyzed on 6% polyacrylamide gels in 0.5× TBE buffer (TBE, 0.089 M Tris-HCl, 0.089 M boric acid, 0.002 M EDTA).

**Plasmid Construction and DNA Transfection**—The basic CAT plasmid used was pBLCAT3 (22). The mutated GPIIb fragments were obtained by using the “oligonucleotide-directed in vitro mutagenesis system, version 2” (Amersham Corp.). A fragment of GPIIb promoter extending from -777 to +33 was subcloned at restricted sites SstI and BamHI of M13mp18 vector. Oligonucleotides used for mutagenesis are described above. Wild type and mutated promoter fragments were inserted between XhoI and BamHI sites of pBLCAT3 vector. The Ib441 construct containing a promoter fragment extending from -414 to +33 was obtained as already described (13). The pBLCAT3 plasmid was used as internal standard expressing firefly luciferase under the control of the Rous sarcoma virus promoter (23). HEL cells were transfected by electroporation as already described (13).

**Luciferase and CAT Assays**—Cells were harvested 48 h after transfection. CAT assays were performed after normalization in function of luciferase activity as previously described (13).

**Antiserum Preparation**—Antiserum against GATA-1 (aG1) was prepared by immunizing rabbits (25) with the synthetic peptide TREDSPPQAVEDLDGKGSTS corresponding to a hydrophilic region of GPIIb and a specific anti-GATA-1 antibody was produced (aG1). The antibody was raised to a potential antigenic site present on GATA-1 having no homology with any sequence of other GATA proteins. The following experiments were designed to establish the specificity of this antiserum.

In ELISA, aG1 reacted with the GATA-1 peptide but did not react with an irrelevant peptide sequence (Fig. 1). The antiserum was then tested in band shift assays using a PBGD promoter fragment (−83 to −56) containing a GATA-1 binding site (lanes 1–5) or with a GPIIb promoter fragment (−243 to −219) containing a CCAAT motif (lanes 11–13). HUEC nuclear extracts were incubated with a PPET-1 promoter fragment (−144 to −123) containing a GATA-2-binding site (lanes 6–10). The binding reactions were performed without competitor and without antiserum, lanes 1, 6, and 11; with a 100-fold excess of the unlabeled competitors: PPET-1 promoter fragment homologous to the probe, lane 7; heterologous promoter fragment carrying the HNF-1-binding site, lane 8. Prior to polyacrylamide gel electrophoresis, some of the mixtures were incubated for 15 min on ice with 1 μl of undiluted aG1 antiserum, lanes 2, 9, and 12; with 1 μl of undiluted aG1 antiserum, lanes 3–5, 8, and 11. The protein-DNA complex specific of the PBGD GATA probe is indicated by the closed arrow 1, and the protein-DNA complex specific of the PPET-1 GATA probe is indicated by the closed arrow 2. The change in mobility of the complex detected with the PBGD probe is indicated with an open arrow. This supershifted complex was inhibited when GATA-1 immunogenic peptide was added (1 μl of a 10× M solution), lane 4; it was still detected in presence of the same amount of an irrelevant peptide, lane 5.

**RESULTS**

**Production and Specificity of Anti-GATA-1 Antibody**—To identify factors that interact with the GATA protein putative binding sites of the GPIIb promoter, a specific anti-GATA-1 polyclonal antibody was produced (aG1). The antibody was raised to a potential antigenic site present on GATA-1 having no homology with any sequence of other GATA proteins. The following experiments were designed to establish the specificity of this antiserum.

In ELISA, aG1 reacted with the GATA-1 peptide but did not react with an irrelevant peptide sequence (Fig. 1). The antiserum was then tested in band shift assays using a PBGD GATA-1 probe. After incubation with HEL nuclear extracts, a major DNA-protein complex was detected (Fig. 2, lane 1). The formation of this complex was not affected by the addition of preimmune antiserum to the mixture (Fig. 2, lane 2). In the presence of aG1, the intensity of the retarded band was considerably reduced, and a supershifted complex was formed.

1 The abbreviations used are: HUVEC, human endothelial cells; bp, base pair(s); ELISA, enzyme-linked immunosorbant assay; CAT, chloramphenicol acetyltransferase.
FIG. 3. Localization and sequence of GATA motifs on human GPIIb promoter. The GATA-binding sites are represented by hatched boxes. The arrows indicate the orientation of the consensus sequences.

FIG. 4. Gel shift analysis of GPIIb promoter probes containing the GATA motif. HeLa or HEL cell nuclear extracts were incubated with four different GPIIb GATA probes that are described under "Materials and Methods." Each panel (A–D) corresponds to an individual experiment performed with one of the different endlabeled probes: −54, −243, −376, and −463 GATA probes, respectively. The binding reactions were performed without competitor, lanes 1; with a 100-fold molar excess of the following competitors: unlabeled GPIIb promoter fragment homologous to the probe, lanes 2; a PBGD promoter fragment (−83 to −56) containing the GATA motif, lanes 3; unlabeled GPIIb promoter fragment homologous to the probe and the mutated GATA site, lanes 4; heterologous promoter fragment carrying the HNF-1-binding site, lanes 5. The position of specific DNA-protein complex is indicated with an arrow.
To explore more precisely the DNA-protein interactions, a series of mobility shift assays using oligonucleotides corresponding to each of the GPIIb GATA sequence was performed. Results of these experiments are summarized in Fig. 4. In each case, a similar DNA-protein complex was formed when nuclear extracts of HEL cells were used (Fig. 4, panels A–D, lanes 1), but this complex was not detected with extracts from HeLa cells. The formation of this complex was inhibited in the presence of an excess of the unlabeled homologous oligonucleotide (lanes 2), and in the presence of the unlabeled PBGD GATA-1 oligonucleotide as well (lanes 3). In contrast, the formation of this complex was not affected by unlabeled mutated GPIIb GATA oligonucleotides (lanes 4) or by the unlabeled heterologous oligonucleotide (lanes 5), used as competitors. Several additional bands were detected with the −54 and −243 GATA probes (Fig. 4, panels A and B). With the −54 probe, the major additional band was also detected with HeLa extracts and could not correspond to a HEL-specific GATA-binding protein. With the −243 probe, all the additional bands were also present in HeLa nuclear extracts, and the formation of these complexes was not inhibited by competition with the PBGD GATA oligonucleotide, demonstrating that they could not correspond to a GATA protein.

From these results we conclude that the different putative GATA sites of the GPIIb promoter may interact with a DNA binding factor, present in HEL but not in HeLa cells, and that these interactions are specifically inhibited by the GATA motif. We suggest that this factor could be GATA-1.

To verify this hypothesis, we used αG1 in mobility shift experiments (Fig. 5). Nuclear extracts from HEL cells were incubated with the different GPIIb GATA probes (Fig. 5, panels A–D, lanes 1). An excess of the HNF-1 oligonucleotide used as heterologous competitor was added to reduce the formation of nonspecific complexes observed with the −54 and the −243 GATA probes. Formation of the specific DNA-protein complexes was not affected by the preimmune antiserum (lanes 2). In contrast when αG1 was used, formation of this complex was either inhibited or markedly reduced with the appearance of a weak supershifted band (lanes 3).

Thus, these results strongly suggest that GATA-1 is the transcription factor that binds to the GPIIb promoter GATA sites.

**Functional Analysis of the Different GATA Sites on the GPIIb Promoter Activity**—We have checked by mobility shift assays that the different probes corresponding to the GPIIb GATA sites failed to interact with nuclear proteins when mutated at the GA positions (not shown). Thus, each site was mutated within the promoter context, and the functional consequences of these mutations were examined in CAT assays after transfection in HEL cells. In these experiments, the wild type IIb777 construct corresponded to a transcriptional activity of 100% (Fig. 6).

Mutation at the −463 GATA site in the IIb777 construct resulted in a drop of the activity down to 39 ± 9%, indicating that this site is critical for full promoter activity.

Mutation of the −376 GATA site resulted in a weak reduction of the relative CAT activity, from 100% to 85 ± 11%, suggesting that the binding of GATA-1 to this site has a minor contribution to the promoter activity in vitro.

Mutation of the −243 GATA site resulted in an increased activity from 100% to 143 ± 46%, suggesting a negative effect of this site on the promoter activity. However, the standard deviation observed on an average of four independent CAT assays was significantly high as compared to the dispersity of the values obtained with the other constructs.

![Figure 5](https://example.com/fig5.png)

**FIG. 5. Antiserum to human GATA-1 reacts with the protein that binds GATA motifs of GPIIb promoter.** Each panel (A–D) corresponds to an individual experiment performed with one of the different end-labeled probes: −54, −243, −376, and −463 GATA probes, respectively. The binding reactions were performed with a 100-fold molar excess of a cold heterologous promoter fragment carrying the HNF-1-binding site (panels A and B). Prior to polyacrylamide gel electrophoresis, some of the mixtures were incubated 15 min on ice without antiserum, lane 1; with 1 µl of undiluted control antiserum, lane 2; with 1 µl of undiluted antiserum raised to a human GATA-1-specific peptide, lane 3. The position of major protein-DNA complex is indicated with a closed arrow. The change in mobility of the complex associated with antibody was sometimes difficult to observe and can be seen on 24-h autoradiography. It is indicated with an open arrow.

Mutation of GATA-54 within the IIb777 construct resulted in a CAT activity of 108 ± 16%, indicating a null contribution of this GATA site to the promoter activity. A plasmid construct, IIb414, which did not contain the enhancer region was tested for its transcriptional activity. This 414-bp region of the promoter was able to direct the expression of the CAT enzyme at a lower but significant level (32% compared to 100% with the enhancer containing fragment). When this IIb414 construct was mutated at position −54, a drop of the CAT activity from 32 to 4% was observed. These results suggested that the −54 GATA site is active only when the −463 GATA site was not functional. In support to this conclusion was the observation that mutations at −54 and −463 reduced the CAT activity down to 15 ± 3%, compared to 39% obtained with the single mutation at −463.
Finally, mutation of the four GATA sites induced an important decrease of the CAT activity 100% to 26 ± 4%. This drop of activity was less marked than that observed with the construct where only GATA -463 and -54 were mutated (26% compared to 15%). This can be explained by the negative effect of the -243 GATA site, and the very weak contribution of the GATA -376 site.

**DISCUSSION**

GATA-1 has been detected in several early myeloid cell lines but this expression is turned off in the presence of myeloid growth factors (29). This suggests that GATA-1 is probably expressed at an early stage of hematopoiesis in different pluripotent precursors and that this expression is only maintained in selected lineages. The role of GATA-1 in the development of erythroid cells has been extensively investigated. Implication of this factor in the regulation of erythroid genes has been demonstrated at the level of promoters (7), enhancers (30), or erythroid dominant region, DCR (31-33). Knockout experiments have established the crucial role of GATA-1 in the erythroid differentiation process (34).

Although the role of GATA-1 in the regulation of megakaryocytic genes is less understood, recent observations indicate that it may also be implicated in the control of the differentiation of this lineage. GATA-1 was found in megakaryocytes, and it was shown that overexpression of this factor in the myeloid cell line 416B induces megakaryocytic markers (35). This suggests that GATA-1 can act as an important regulator of megakaryocytogenesis, and its implication in the transcriptional regulation of megakaryocytic genes has been proposed (9, 13, 36, 37). To verify this hypothesis we have studied the interaction of GATA-1 with the promoter region of the megakaryocyte-specific GPIIb gene.

To clearly identify GATA-1 as the implicated factor, a polyclonal anti-GATA-1 antibody was produced, using a synthetic peptide containing 20 amino acid residues of a GATA-1-specific hydrophilic domain, with no homology with other members of the GATA family. The specificity of this antibody was analyzed using ELISA and mobility shift experiments and was based on the following observations: 1) on ELISA, the antibody reacted with the GATA-1 peptide but did not interact with an irrelevant peptide; 2) the antibody was able to form a super-shifted DNA-protein complex in mobility shift assays using a probe containing the GATA-1-binding site of the erythroid-specific PBGD promoter and HEL nuclear proteins; 3) the antibody failed to react with a DNA-protein complex formed between a specific GATA-2 probe of the PPET-1 gene and nuclear proteins of HUVEC which express GATA-2 but do not produce GATA-1. Since GATA-1 and GATA-2 but not GATA-3 are present in HEL cells (4, 5, 38), we concluded from these results that the antibody was specific to GATA-1. Although this antibody was able to form a supershifted complex with a WGATAR probe, the intensity of the shifted band was considerably reduced, indicating that the antibody was also able to inhibit the formation of the complex.

The promoter domain of the GPIIb gene contains four potential GATA-binding sites centered at -54, -243, -376, and -463. Mobility shift assays have shown that each GATA site interacted with a GATA protein present in HEL nuclear extracts. This protein was identified as GATA-1 using the specific anti-GATA-1 antibody in supershift experiments.

The relative contribution of each GATA-1-binding site to the transcriptional activity of the promoter was established by the mutation of each site either alone or in combination, and the effect of these mutations was examined in CAT assays.

We have already shown that the -463 GATA site is contained within a specific erythromegakaryocytic enhancer (13). Consistent with this observation we found that this GATA site is critical. Its mutation induces a drop of 60% in the promoter activity. The present study shows that one of the critical factors that interacts with this enhancer is GATA-1.
and establishes the role of this transcription factor in the activity of a megakaryocyte-specific promoter.

Mutation of the −376 site produced a drop of about 15% in the CAT activity, suggesting that the binding of GATA-1 to this site does not directly contribute to the promoter function. The sequence of this site contains a GATTAG motif which diverges from the consensus WGATAR motif and may not be functionally active within the GPIIb promoter context.

In previous CAT assays using a 77-bp sequence upstream from the transcription start site of the GPIIb promoter, we have shown that the GATA site at position −54 was functional (9). Mutation of this site, however, within the context of the whole promoter, did not affect the transcriptional activity. This suggests that the −54 GATA site has a minor contribution to the overall activity. Nevertheless, the mutation of this site in the promoter deleted from its enhancer region reduced considerably the residual activity. These results indicate that the site at −54 may be functional and responsible for the residual activity of about 30%, providing the enhancer is deleted. One possible interpretation of this observation could be that the site at position −463 has a dominant effect on the −54 site. Observation that a double mutation at position −463 and −54 resulted in a 85% inhibition of the promoter activity supports this conclusion.

Recent studies on the β-globin and the PF4 promoters suggest that GATA sites located at the expected position for a TATA box could be implicated in the control of the cell specificity (36, 39). Timothy and Emerson (39) propose that interactions between a GATA site located at −30 and a distal enhancer containing another GATA site controls the erythroid specificity of the β-globin promoter. Such a situation is encountered on GPIIb promoter, with the proximal −54 GATA site and the erythromegakaryocytic enhancer that have been previously identified. This enhancer contains the −463 GATA site that is essential for the enhancer function. We have checked if the megakaryocytic specificity of GPIIb promoter depends on a cooperativity between the −54 and the −463 GATA sites by changing the −54 GATA sequence but not in the −463 GATA site.

In summary, the results presented in this paper demonstrate that GATA-1 is a major regulator of GPIIb gene expression in vivo. Not all GATA sites are functionally equivalent, and they may differentially modulate the promoter activity during megakaryocytopoiesis.

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REFERENCES

1. Metcalf, D. (1989) Nature 339, 27–30
2. Evans, T., and Felsenfeld, G. (1989) Cell 58, 877–885
3. Orkin, S. H. (1990) Cell 63, 655–672
4. Tsai, F. M., Martin, D. J., Zen, L. I., D’Andrea, A. D., Wong, G. G., and Orkin, S. H. (1989) Nature 344, 440–451
5. Lee, M. K., Temenoff, D. H., Clifford, J. A., and Quertermous, T. (1991) J. Biol. Chem. 266, 16186–16192
6. Ho, T. C., Vorhees, O., Marin, N., Oakley, B. K., Tsai, S. F., Orkin, S. H., and Leiden, J. M. (1991) EMBO J. 10, 1187–1192
7. Evans, T., Reitman, M., and Felsenfeld, G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5979–5986
8. Martin, D. K., Zen, L. I., Matter, G., and Orkin, S. H. (1990) Nature 344, 444–446
9. Romeo, P.-H., Prandini, M.-H., Joulin, V., Mignotte, V., Prenant, M., Vainchenker, W., Marguerie, G., and Uzan, G. (1990) Nature 344, 447–449
10. Prandini, M.-H., Denarier, E., Frachet, P., Uzan, G., and Marguerie G. (1989) Biochem. Biophys. Res. Commun. 166, 595–601
11. Moll, A., Andrieux, A., Chapel, A., Schweitzer, A., Berthier, R., and Marguerie, G. (1992) Br. J. Haematol. 83, 635–639
12. Uzan, G., Prenant, M., Franchi, M.-H., Martin, F., and Marguerie, G. (1991) J. Biol. Chem. 266, 8032–8039
13. Prandini, M.-H., Uzan, G., Martin, F., Thevenon, D., and Marguerie, G. (1992) J. Biol. Chem. 267, 10370–10374
14. Mignotte, V., Wall, L., deBoer, E., Grosfeld, F. and Romeo, P.-H. (1989) Nucleic Acids Res. 17, 37–54
15. Inoue, A., Yamasawa, M., Takawa, Y., Mitsuji, Y., Kobayashi, M., and Masaki, T. (1989) J. Biol. Chem. 264, 14545–14559
16. Courtois, G., Morgan, J. G., Campbell, L. A., Fourel, G., and Crabtree, G. D. (1987) Science 238, 680–692
17. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1471–1489
18. Jaffe, E. A., Nachman, R. L., Becker, C. G., and Minick, C. R. (1973) J. Clin. Invest. 52, 2745–2756
19. Schreiber, E., Matthaios, P., Müller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419
20. Hoffigan, B. D., and Desiderio, S. V. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7019–7023
21. Singhe, H., Sen, R. Baltimore, D., and Sharp, P. A. (1986) Nature 319, 373–388
22. Luckow, B., and Schütz, G. (1987) Nucleic Acids Res. 15, 1311–1326
23. De Wet, J., Wood, K. V., De Loucas, M., Helinski, D. R., and Subramani, S. (1987) Mol. Cell. Biol. 7, 725–737
24. Korman, C., Moffat, L. F., and Howard, B. H. (1982) Mol. Cell. Biol. 2, 1051–1053
25. Clinton, G. M., and Brown, N. A. (1991) Methods Enzymol. 200, 463–473
26. Kage, H., and Glick, M. (1979) in Methods of Hormone Radioimmunoassay (Lebovitz, R. M., and Behrman, H. R., eds) pp. 329–329, Academic Press, New York
27. Orkin, S. H. (1992) Blood 80, 573–581
28. Wilson, D. J., Dorfman, D. M., and Orkin, S. H. (1990) Mol. Cell. Biol. 10, 4854–4862
29. Crotta, S., Nicols, S., Ronchi, A., Ottolenghi, S., Ruzzi, L., Shimada, Y., Migliaccio, A. R., and Migliaccio, G. (1990) Nucleic Acids Res. 18, 6863–6869
30. Wall, L., deBoer, E., and Grosfeld, P. (1986) Genes & Dev. 2, 1098–1100

2 F. Martin, M.-H. Prandini, D. Thevenon, G. Marguerie, G. Uzan, unpublished results.

3 E. Denarier, personal communication.
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31. Grosveld, F., van Assendelft, G. B., Greaves, D. R., and Kollias, B. (1987) Cell 61, 975–985
32. Talbot, D., and Grosveld, F. (1991) EMBO J. 10, 1291–1298
33. Talbot, D., Philipson, S., Farser, P., and Grosveld, F. (1990) EMBO J. 9, 2169–2177
34. Pevey, L., Simon, M. C., Robertson, E., Klein, W. H., Tsai, S. F., D'Agati, V., Orkin, S. H., and Costantini, F. (1991) Nature 349, 257–260
35. Visvader, J. E., Elefanty, A. G., Strasser, A., and Adams J. M. (1992) EMBO J. 11, 4557–4564
36. Ravid, K., Dot, T., Beeler, D. L., Kuter, D. J., and Rosenberg, R. D. (1991) Mol. Cell Biol. 11, 6116–6127
37. Lemarchandel, V., Ghysdael, J., Mignotte, V., Rahuel, C., and Roméo, P.-H. (1993) Mol. Cell Biol. 13, 668–676
38. Joulin, V., Bories, D., Eléouet, J.-F., Labastie, M.-C., Chrétien, S., Mattéi, M.-G., and Roméo, P.-H. (1991) EMBO J. 10, 1809–1816
39. Fong, C. T., and Emmerson, B. M. (1992) Genes & Dev. 6, 521–532
40. Macleod, K., Leprince, D., and Stelelin, D. (1991) Trends Biochem. Sci. 17, 251–256
41. Hadker, K., Beug, H., Kornfeld, S., and Graf, T. (1983) Cell 31, 643–653
42. Moscovici, M. L., Juricic, P., Samarut, J., Mura, C. V., and Moscovici, C. (1983) Virology 129, 65–78
43. Ben-David, Y., Giddens, E. B., Letwin, K., and Bernstein, A. Genes & Dev. 5, 908–918
44. Moreau-Gachelin, F., Ray, D., de Both, N. J., van der Feltz, M. J. M., Tambourin, P., and Tavitian, A. (1996) Leukemia 4, 20–25
45. Schwartzbauer, G., Schlesinger, K., and Evans, T. (1992) Nucleic Acids Res. 20, 4429–4436
46. Heidenreich, R., Eisman, R., Surrey, S., Delgrosso, K., Bennett, J. S., Schwartz, E., and Poncz, M. (1990) Biochemistry 29, 1232–1244