FOG is a multitype zinc finger protein that is essential for megakaryopoiesis, binds to the amino-terminal finger of GATA-1, and modulates the transcription of GATA-1 target genes. Presently investigated are effects of FOG and GATA-1 on the transcription of the megakaryocytic integrin gene, αIIb. In GATA-1-deficient FDCER cells (in the presence of endogenous FOG), ectopically expressed GATA-1 activated transcription 3-10-fold both from αIIb templates and the endogenous αIIb gene. The increased expression of FOG increased reporter construct transcription 30-fold overall. Unexpectedly, αIIb gene transcription also was stimulated efficiently upon the ectopic expression of FOG per se. This occurred in the absence of any detectable expression of GATA-1 and was observed in multiple independent sublines for both the endogenous αIIb gene and transfected constructs yet proved to depend largely upon conserved GATA elements 457 and 55 base pairs upstream from the transcriptional start site. In 293 cells, FOG plus GATA-1 but not FOG alone moderately stimulated αIIb transcription, and no direct interactions of FOG with the αIIb promoter were detectable. Thus, FOG acts in concert with GATA-1 to stimulate αIIb expression but also can act via a GATA-1-independent route, which is proposed to involve additional hematopoietic-restricted cofactors (possibly GATA-2).

The course of development of hematopoietic progenitor cells is dictated, in part, by the differential expression of lineage-specifying transcription factors. Lymphopoiesis, myelopoiesis, granulopoiesis, erythropoiesis, and megakaryopoiesis, for example, are known from gene disruption experiments to depend on the expression of Ikaros (1), PU.1 (2), CCAAT/enhancer-binding protein-α (3), and GATA-1 (4), respectively. In addition, the abilities of such factors to control rates of target gene expression can involve interactions with additional lineage-restricted co-regulators. This is illustrated by roles for distinct Ikaros-Helios-Aiolos complexes in specifying developmental fates of T cells (1, 5, 6), by the regulation of Oct factor activity and Ikaros-Helios-Aiolos complexes in specifying developmental fates of T cells (1, 5, 6), and during erythropoiesis by the complexing of GATA-1 with FOG (friend of GATA-1) (8), C-terminal binding protein (9), and Tal1 (26) with Lmo2 (10, 11). GATA-1 is a Cys2/Cys2 zinc finger DNA-binding protein that binds preferentially to (A/T)GATAAVG elements via its carboxyl-terminal finger domain (12) and is expressed in erythrocytes, megakaryocytes, eosinophils, and mast cells (13–16). GATA-1 gene disruption in mice results in embryonic lethality due to anemia (4) and to an arrest in erythroid development at a late proerythroblast stage (17). During megakaryopoiesis, important roles for GATA-1 have been illustrated by experiments wherein the targeted disruption of an upstream activating element in the GATA-1 gene results in an accumulation of early megakaryocytic progenitor cells and a deficiency in platelet production (18). FOG is a 110,000-kDa multitype zinc finger protein that was discovered in a yeast two-hybrid screen based on its ability to interact specifically with the amino-terminal zinc finger of GATA-1 (8). In FOG−/− mice (and in FOG−/− embryonic stem (ES) cells (19), erythropoiesis is blocked at a pre-ultimate stage, while effects on megakaryopoiesis are more dramatic, and FOG−/− yolk sac and fetal liver cells give rise to few, if any, megakaryocytes (19). This broad defect indicates that FOG expression is either essential for early commitment to this lineage and/or that FOG acts subsequently to promote the transcription of late megakaryocytic genes.

Since FOG is a co-factor for GATA-1 (8) and since functional GATA elements occur within the promotors of most megakaryocytic genes studied to date (20–25), FOG might act as an obligatory GATA-1 co-factor. However, GATA-1 mutants that fail to bind FOG have been shown to activate the expression of the EKLF, heme-regulated eIF-α-kine, and FOG (26) genes in GATA-1-deficient ES cells. Thus, GATA-1 and/or FOG also may act in combination with alternate co-factors to regulate erythromegakaryocytic gene expression. With regards to megakaryocytic genes, investigations of roles for FOG are limited to two studies to date. In 416B cells, ectopically expressed FOG and GATA-1 increased the frequency of cells expressing acetylcholinesterase (8), and in 3T3 fibroblasts expression of FOG plus GATA-1 significantly activated transcription from a 7-kb upstream region of the erythromegakaryocytic gene p45 NF-E2 (8). To further determine how FOG might affect megakaryocytic gene expression, we presently have investigated whether FOG might regulate the expression of the megakaryocytic integrin subunit, αIIb. αIIb expression is restricted to megakaryocytes, platelets, and their progenitors (27) and, together with a more broadly expressed subunit β3, forms an integrin receptor that functions in platelet aggregation (28). In the promoter domains of the rat and human αIIb genes, upstream as well as TATA box-positioned GATA-1 ele-

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mements previously have been shown to be important for transcription (22, 29, 30). Flanking each of these two GATA ele-
mments are elements for Ets factor binding that likewise contribute to efficient transcription from the proximal promot-
elements of the rat and human aIb genes (28, 30, 31). Together with a 14 bp element for Sp1 (32), these elements (which lie within a 600-bp promoter domain) have been proposed to direct the lineage-specific expression of aIb, and similarly distributed elements also occur within the promoters of several additional megakaryocytic-specific genes including the Tpo receptor (23), chemokine PF4 (20), GPlba (24), and GPIX (25) genes. The present investigation focuses on aIb gene expression and pro-
vides evidence that FOG acts as an important positive regula-
tor via both GATA-1-dependent and independent routes.

EXPERIMENTAL PROCEDURES

Expression Vectors—pREP4-G1 was prepared by subcloning a wild-
type murine GATA-1 cDNA (1.6-kb XbaI–NcoI fragment) from pCIN-
neoGATA-1 (33) to pREP4 (Invitrogen, Palo Alto, CA). For pA2PuroEts1, a wild-type murine cts-1 cDNA (1.9-kb SmaI–BstXI fragment from pKCl) was blunt-ended, ligated to EcoRI adaptors, and cloned to pA2Puro (35). Vectors pXMGATA-1, pCINeoGATA-1, pXMER, and pEFNeoFOG have been described previously (8, 33).

Cell Lines—FCDER-FOG cells and independent clonal lines were prepared via the stable co-electrotransfection of FDCW2 cells (36) with 55 μg of pXM-190ER (37) plus 5 μg of pEFNeoFOG, stepwise selection in G418 (1 mg/ml) and erythropoietin (25 units/ml), and limiting dilu-
tion. FDCER, FDCER-G1, and FDCER-G1-pCG1 cell lines have been described previously (33). FDCER cell lines were maintained at 37 °C (5% CO2) in Opti-MEM I medium (Life Technologies, Inc.) supplemented with 8% fetal bovine serum, and 25 units of erythropoietin/ml. 293-G1, 293-FOG, and 293-Ets-1 cells were prepared by transfecting 293 cells with pREP4-G1, pEFNeoFOG, and pA2PuroEts1, respectively. Transfections were performed using calcium phosphate (Life Technol-
ologies) and thermal cycle: 5 min at 94 °C for 1 min, 60 °C for 30 s, and 72 °C for 60 s. Amplified products (30 cycles) were cloned to pCR-Script (Strat-
agene, La Jolla, CA) and sequenced. pXM-190ER (44); 3.0-kb EcoRI fragment of pSP-GAPDH (murine GAPDH); and 0.8-kb EcoRI fragment of pBOS-EKLF (murine EKLF) (44); 3.0-kb EcoRI fragment of pMT2A-Dua-
Ilb (human Ilb); 700-bp EcoRI fragment of pUC-GATA2 (5' region of murine GATA-2); and 0.8-kb KpnI–XhoI fragment of pSP-GAPDH (murine glyceraldehyde-3-phosphate dehydrogenase).

Northern and Western Blotting—Polydenylated RNA was isolated using Oligotex spin columns (Qiagen, Chatsworth, CA). RNA was elec-
trophoresed in 12% agarose, 6% formaldehyde gels, blotted to Nytran (Schleicher & Schuell), and fixed (312-nm exposure for 3 min plus 1 h at 68 °C under vacuum). Probes were prepared by random priming (33) using 25 ng of the following cDNA fragments: 1.8-kb KpnI–Nil fragment of pXM-190ER (44); 1.2-kb XhoI fragment of pBOS-EKLF; 3.0-kb EcoRI fragment of pMT2A-Dua-
ilb (human Ilb); an endogenous gene transcription were tested via

RESULTS

GATA-1-dependent Activation of aIb Gene Transcription in FDCER Cell Lines—In primary studies, roles for GATA-1 and FOG on endogenous aIb gene transcription were tested via their stable expression in FDCW2-derived cell lines. Recently, our laboratory has shown that these cells do not express GATA-1 at detectable levels, yet support the ability of exoge-
nous GATA-1 to (auto)activate the de novo expression of the endogenous GATA-1 gene (38). As shown in Fig. 1A, Northern blot analyses of FDCER-GATA-1 cell lines revealed that exogenous
are PCR cycle numbers and the positions of amplified m-IIb7a (endogenous GATA-1) and m-IIb910-Luc (exogenous GATA-1) transcripts also were assayed by reverse transcriptase-PCR. Shown is a representative result of Northern blots for these or other possible transfactors were apparent. Exogenous GATA-1 (versus parental FDCER cells) was expressed in FDCER-G1 cells (Fig. 5A). This result also was observed in repeated independent experiments in FDCER-G1-FOG cell lines. Based on these results (and the knowledge that FOG does not affect GATA-1’s DNA binding activity) (26), it was predicted that levels of FOG in GATA-1 cells might limit aIIb expression. If so, further increases in ectopic GATA-1 expression in FDCER-G1 cells might squelch rather than enhance the activity of FOG-GATA-1 complexes. To test this prediction, FDCER-G1 cells were transfected stably with a second GATA-1 expression vector (pCIneoG1), and effects on transcription from aIIb reporter constructs were assayed.

Increased expression of exogenous GATA-1 in FDCER-G1-PCG1 cells proved to inhibit transcription from pIIb545-Luc (and pIIb910-Luc) approximately 3-fold as compared with FDCER-G1 cells (Fig. 5B). Results are representative of three independent experiments (and increased levels of GATA-1 expression in FDCER-G1-PCG1 cells have been documented previously) (33). This apparent squelching effect demonstrates that levels of GATA-1 in FDCER-G1 cells do not limit aIIb transcription and is at least consistent with the notion that, when overexpressed, GATA-1 instead may sequester an apparently limiting co-factor such as FOG.

**FOG Activation of aIIb Gene Transcription via a GATA-1-independent Route**—In control experiments, FOG per se also was expressed in FDCER cells, and levels of aIIb gene transcription were assayed. Initially, this was tested using pIIb545-Luc. Somewhat unexpectedly, the expression of FOG at levels 2–3-fold above endogenous levels (see above; Fig. 4) increased rates of pIIb545 transcription in FDCER-FOG cells to levels essentially equivalent to those supported by GATA-1 in FDCER-G1 cells (Fig. 6A). This was observed in clonal as well as in polyclonal FDCER-FOG cell lines and suggested that FOG might promote aIIb gene transcription in the absence of GATA-1. To critically test this possibility, 32P-labeled reverse transcriptase-PCR was used to assay endogenous GATA-1 and aIIb transcript levels (Fig. 6B). In FDCER-FOG cells, no GATA-1 transcripts were detected. However, levels of endogenous aIIb gene expression in all clones tested were increased to levels approximating those induced by GATA-1 in FDCER-G1 cells.

The extent to which FOG-stimulated transcription of the

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**Fig. 1.** Endogenous m-aIIb gene expression is activated upon the ectopic expression of GATA-1 in FDCER cells. Upper panel, shown in Northern blots are levels of GATA-1, m-aIIb, and FOG transcript expression in GATA-1-expressing FDCER-G1 cells versus parental myeloid FDCER cells. Lower panel, endogenous m-aIIb and GAPDH transcripts in three independent clonal lines of FDCER-G1 cells (versus parental FDCER cells) also were assayed by reverse transcriptase-PCR. Shown are PCR cycle numbers and the positions of amplified m-aIIb and HPRT products.

GATA-1 expression also activated the expression of the endogenous aIIb gene. To confirm that this result was not a fortuitous clonal effect, aIIb transcript expression in FDCER-G1 clones c.10, c.9, and c.11 (i.e. three independent clones) was analyzed by 32P-labeled reverse transcriptase-PCR (Fig. 1B). In each clone, aIIb transcript expression was elevated severalfold due to the expression of exogenous GATA-1 (as compared directly with parental FDCER cells).

Next, to test whether this effect was mediated by cis elements within the aIIb promoter, an extended upstream region of the murine aIIb gene was cloned, sequenced, and used to prepare promoter-luciferase reporter constructs. Within this approximately 1000-bp promoter region, elements at approximately 5.4- and 2.9-fold, respectively. Maximal rates of transcription from each construct in FDCER-G1 cells were comparable, but transcription from pIIb910-Luc in parental FDCER cells was more pronounced. No effects of GATA-1 expression on low level transcription of the promoterless control template pGL2Basic were observed. For pIIb545-Luc, essentially equivalent results were obtained in repeated transfections of independent clonal lines of FDCER-G1 cells (Fig. 3B).

**FOG Amplifies GATA-1-dependent aIIb Gene Transcription in FDCER-G1 Cells**—In FDCER-G1 cells, possible effects of FOG on aIIb gene transcription next were tested by increasing FOG expression in these lines via stable transfection. In FDCER-G1-FOG, FDCER-FOG, FDCER-G1, and parental FDCER cells, Northern blotting first was used to assay levels of FOG and GATA-1 (as well as GATA-2) transcript expression (Fig. 4). As a point of comparison, levels of these transcripts in erythroid B6SUt.EP cells (and lymphoid CTLL2-ER cells) were co-analyzed. FOG transcript levels in FDCER cells were appreciable yet below those observed in B6SUt.EP cells. In FDCER-G1 cells, the ectopic expression of GATA-1 interestingly led to an estimated 3-fold increase in FOG transcript levels. In contrast, levels of GATA-2 transcript expression in FDCER-G1 and FDCER-G1-FOG cells were diminished. With regard to aIIb expression, ectopic expression of FOG in FDCER-G1 cells proved to stimulate rates of aIIb transcription to levels at least 5-fold above levels in FDCER-G1 cells and 38-fold above levels in parental FDCER cells (Fig. 5A). This result also was observed in repeated independent experiments in FDCER-G1-FOG cell lines. Based on these results (and the knowledge that FOG does not affect GATA-1’s DNA binding activity) (26), it was predicted that levels of FOG in GATA-1 cells might limit aIIb expression. If so, further increases in ectopic GATA-1 expression in FDCER-G1 cells might squelch rather than enhance the activity of FOG-GATA-1 complexes. To test this prediction, FDCER-G1 cells were transfected stably with a second GATA-1 expression vector (pCIneoG1), and effects on transcription from m-aIIb reporter constructs were assayed. Increased expression of exogenous GATA-1 in FDCER-G1-PCG1 cells proved to inhibit transcription from pIIb545-Luc (and pIIb910-Luc) approximately 3-fold as compared with FDCER-G1 cells (Fig. 5B). Results are representative of three independent experiments (and increased levels of GATA-1 expression in FDCER-G1-PCG1 cells have been documented previously) (33). This apparent squelching effect demonstrates that levels of GATA-1 in FDCER-G1 cells do not limit aIIb transcription and is at least consistent with the notion that, when overexpressed, GATA-1 instead may sequester an apparently limiting co-factor such as FOG.

**FOG Activation of aIIb Gene Transcription via a GATA-1-independent Route**—In control experiments, FOG per se also was expressed in FDCER cells, and levels of aIIb gene transcription were assayed. Initially, this was tested using pIIb545-Luc. Somewhat unexpectedly, the expression of FOG at levels 2–3-fold above endogenous levels (see above; Fig. 4) increased rates of pIIb545 transcription in FDCER-FOG cells to levels essentially equivalent to those supported by GATA-1 in FDCER-G1 cells (Fig. 6A). This was observed in clonal as well as in polyclonal FDCER-FOG cell lines and suggested that FOG might promote aIIb gene transcription in the absence of GATA-1. To critically test this possibility, 32P-labeled reverse transcriptase-PCR was used to assay endogenous GATA-1 and aIIb transcript levels (Fig. 6B). In FDCER-FOG cells, no GATA-1 transcripts were detected. However, levels of endogenous aIIb gene expression in all clones tested were increased to levels approximating those induced by GATA-1 in FDCER-G1 cells.

The extent to which FOG-stimulated transcription of the
m-allb gene depended upon intact −457 bp and/or −55 bp TATA box position GATA elements next was tested. First, roles for these elements in supporting polIIb545-Luc transcription in FDCER-G1 cells versus parental FDCER cells were examined. One, the other, or both GATA elements were mutated to the nonfunctional sequence CATA (46), and activities of the derived constructs polIIb545−Δ5 G-Luc, polIIb545−Δ3 G-Luc and polIIb545−Δ5Δ3 G-Luc were assayed. Mutation of the −457 bp GATA element inhibited transcription from the m-allb promoter in FDCER-G1 cells 5.5-fold (20,900- to 3800-unit decrease); mutation of the TATA box-positioned 55 bp GATA element inhibited transcription 2.7-fold (20,900- to 7800-unit decrease). Thus, in the presence of increased levels of FOG, while mutation of the TATA box-positioned GATA elements next was tested. First, roles for these elements in supporting pMCSIIb545-Luc transcription in transfected 293 fibroblasts were examined. As shown in Fig. 8A, only slightly increased rates of polIIb545−Δ5Δ3 G-Luc were assayed in 293-FOG cells. This result, together with the observed ability of FOG in the absence of GATA-1 to activate m-allb gene transcription in FDCER-FOG cells, raised the possibility that FOG might also stimulate m-allb gene expression via GATA-1-independent mechanisms. To further test this possibility, transcription from the mutant construct polIIb545−Δ5Δ3 G-Luc was assayed in FDCER-FOG cells (Fig. 7B). Residual transcription from polIIb545−Δ5Δ3 G-Luc in these GATA-1-deficient cells was somewhat higher than in FDCER cells, again suggesting that FOG might stimulate m-allb gene expression at least to a limited extent via a GATA element-independent route.

Finally, to test whether transcription from allb proximal promoter constructs would be stimulated efficiently by ectopically expressed FOG, FOG-1 and/or Ets-1 in nonhematopoietic cells, these factors first were expressed stably in 293 fibroblasts to yield 293-G1, 293-FOG, 293-G1-FOG, and 293-G1-FOG Ets-1 cells. Transfections with polIIb545-Luc then were performed, and activities were assayed in triplicate (with pCMV-βGal as a co-reporter). As shown in Fig. 8A (upper panel), ectopically expressed GATA-1 per se only slightly increased rates of polIIb545−Δ5 G-Luc transcription in 293-G1 cells (approximately 2-fold above parental 293-G1 cells), while ectopically expressed FOG (in 293-FOG cells) per se had no detectable positive effect. In combination, however, these factors in 293-G1-FOG cells reproductively stimulated transcription from the m-allb gene proximal promoter approximately 6-fold above levels in parental 293 cells. Ectopically expressed Ets-1, in contrast, did not significantly stimulate m-allb transcription in this reconstituted system in the absence or presence of GATA-1 (or GATA-1 plus FOG) (Fig. 8A, lower panel). Data shown are representative of three independent experiments in which essentially equivalent effects of these trans-factors on transcription from the m-allb proximal promoter were observed and similar activities were observed for polIIb910-Luc.2 In advance, Western and Northern blotting were used to identify matched sublines in which expression levels were highly comparable (Fig. 8B). These results demonstrate the positive co-action of
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FOG and GATA-1 in fibroblastic cells. These effects, however, were blunted as compared with those in FDCER-G1-FOG cells, and this is at least consistent with possible roles for alternate hematopoietic factors in activating the αIIb gene.

**DISCUSSION**

As introduced above, the disrupted expression of FOG in mice blocks the formation of megakaryocytes and erythrocytes. As shown initially in studies by Crispino et al. (26), however, FOG appears to be dispensable for the activation of at least certain GATA-1 target genes and has been proposed to act differentially with GATA-1 at distinct subsets of erythroid and megakaryocytic genes (19, 26). In addition, a FOG homologue in *Xenopus* recently has been discovered and demonstrated through ectopic expression and explant assays to repress the transcription of at least certain erythroid genes (possibly via interactions with C-terminal binding protein) (47). In separate studies, FOG also has been shown to inhibit GATA-1-dependent transcription from the eosinophil- and basophil-specific gene, eosinophil major basic protein (48). These reports suggest that FOG does not act simply as a GATA-1 co-activator and that its activity depends upon not only lineage but also promoter contexts. Despite FOG’s essential role in megakaryopoiesis (19), it also is noted that studies of its effects on megakaryocytic genes are limited to date to the demonstrated ability of FOG to stimulate transcription from a 7000-bp promoter domain of the erythromegakaryocytic *p45NF2* gene in transiently transfected 3T3 fibroblasts (8). Such considerations prompted the present investigations of roles for FOG (and GATA-1) in αIIb gene expression.

As a hematopoietic cell line that is GATA-1-deficient and expresses endogenous FOG at moderate levels, FDCER cells proved to be an advantageous model in which to test dosage effects of these (co)factors on αIIb gene expression. With regard to GATA-1 effects *per se*, with the exception of the observation in chicken HD50M myeloblastic cells that exogenous GATA-1 can promote the outgrowth of thromboblastic-like cells (including a subline that stained with an antibody thought to be specific for avian αIIbβ3 integrins) (49), the present study is the first to demonstrate GATA-1-dependent activation of endogenous αIIb gene expression. Consistent with the results of previous experiments, promoter-reporter transfection experiments in FDCER-G1 cells showed this to depend upon GATA elements positioned at −457 and −55 bp within the proximal αIIb promoter. In several additional megakaryocytic genes including *mpl* (25), *GP1ba* (24), *GP-IX* (25), and *PF4* (26), GATA elements likewise occur within 90 bp of transcription start sites and have been proposed to substitute for canonical TATA boxes by binding to a multisubunit TFIIID complex, which may contain GATA-1, an Ets factor, Sp1, and (based on the present findings) possibly FOG. In the present studies, however, this −55 GATA element contributed meaningfully to GATA-1-stimulated (and FOG-stimulated) αIIb transcription yet proved to
be somewhat less important than a 457 bp element. Flanking each of these GATA elements are sites for the binding of one or more Ets family transcription factors, and these sites also have been demonstrated to support transcription at the human and rat αIIb proximal promoters (22, 30–32, 50). In FDCER and FDCER-G1 cells, Northern blot analyses of Fli-1, Spi-1, and Ets-1 transcripts revealed each to be expressed at appreciable levels, and in FDCER-G1 lines Ets-1 levels were increased approximately 2-fold. Interestingly, Ets-1 (and Ets-2) recently has been shown to bind to C-terminal binding protein/p300 (51, 52), and based on the ability of GATA-1 to bind a nonequivalent region of C-terminal binding protein/p300 (9, 53), it is possible that FOG might also tether at least indirectly to one or another Ets factor.

More remarkable are, first, the overall 30–40-fold increase in levels of αIIb promoter transcription stimulated by ectopic co-expression of GATA-1 plus FOG in FDCER-G1-FOG cell lines and, second, the ability of FOG to activate αIIb gene transcription in GATA-1-deficient FDCER cells. Increases in αIIb transcription due to exogenous FOG in FDCER-G1-FOG cells are suggested to reflect FOG’s role as a limiting factor in GATA-1-dependent αIIb gene activation, and direct interactions between these co-factors are the most straightforward to propose as a mechanism underlying observed effects on αIIb transcription. However, opportunities also exist for GATA-1 and possibly FOG to modulate by secondary routes the expression of other potential regulators of αIIb gene expression. The case for direct action mechanisms is underlined by the apparent ability of exogenous GATA-1 to squelch αIIb transcription when expressed at elevated levels in FDCER-G1-pCG1 cells (see Fig. 5B) and by the major dependence of FOG activity in FDCER-G1-FOG cells on the intactness of 2457 and 255 bp GATA elements. Nonetheless, several observations also are consistent with alternate mechanisms of FOG action in addition to those mediated by interactions with GATA-1. These include FOG’s ability to activate αIIb transcription in the ap-
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**Fig. 7.** GATA-1 and FOG-induced transcription at the m-αIIb promoter depends (in part) on upstream and TATA-positioned GATA elements. To test the extent to which FOG-stimulated transcription depends on intact GATA elements at −457 and −55 bp within the m-αIIb promoter, polIIb545-ΔG5′-Luc (mutated −457 element), polIIIb545-ΔG5-ΔG3′-Luc (mutated −55 element), and polIIIb545-ΔG5′ΔG3′-Luc (both elements mutated to CATA) were constructed, and their activities in FDCER, FDCER-G1, FDCER-G1-FOG, and FDCER-FOG cell lines were assayed. A, upper panel, illustrated is the GATA-1-dependent activation of wild type and mutant polIIb545-Luc constructs in FDCER versus FDCER-G1 cells. Mean luciferase activities ± S.D. of triplicate transfections are shown. Shown in parentheses are -fold increases of activity in FDCER-G1 cells above FDCER cells transfected in parallel with the same construct. A, lower panel, illustrated is the FOG-enhanced activation of wild type and mutant polIIb544-Luc constructs in FDCER-G1-FOG cells. For each construct, shown in parentheses, are -fold increases in activity as compared with levels in FDCER-G1 cells (see upper panel). The hatched area in the wild-type histogram represents increases in m-αIIb transcription in FDCER-G1-FOG cells above levels in FDCER-G1 cells. Limited variability in transfection efficiencies was controlled for based on secreted alkaline phosphatase activities. Results in each panel are representative of three independent experiments.

B, the reporter constructs polIIb545-Luc (upper panel) and polIIb545-ΔG3′ G-Luc (lower panel) (together with pSEAP) were transfected into FDCER, FDCER-G1, and FDCER-FOG cells, and transcriptional activities were assayed. Shown are -fold increases in luciferase activities of the polIIb545-ΔG3′ G-Luc construct in FDCER-G1 or FDCER-FOG cells as compared with those in FDCER cells. Plotted are the means ± S.D. of triplicate transfections normalized against SEAP activities. Results in each panel are representative of three independent experiments.

**Fig. 8.** FOG and GATA-1-dependent transcription of the m-αIIb promoter in 293 fibroblasts. A, 293 cells were transfected stably with expression vectors for GATA-1 (pREP4GATA-1), FOG (pEFP-NeoFOG), and/or mEts1 (pAPuroEts-1), and the following cell lines were isolated: 293, 293-G1, 293-FOG, 293-G1-FOG, 293-Ets1, 293 Ets1-G1, and 293-Ets1-G1-FOG cells. The ability of each subline to support transcription from pαIIb545-Luc then was assayed. pCMVβgal was co-transfected, and samples were normalized for β-galactosidase activity to correct for limited variability in transfection efficiencies. Plotted are the activities (mean relative light units ± S.D.) of triplicate transfections. Shown in parentheses are -fold increases in luciferase activity supported by the specified transcription factors. B, levels of GATA-1, FOG, and/or Ets-1 expression in the above 293 cells and derived cell lines were assayed by Western blotting (for GATA-1; upper panel) or by Northern blotting (for FOG and Ets-1; lower panels). Equivalence in RNA loading was confirmed by hybridization to a 32P-labeled glyceraldehyde-3-phosphate dehydrogenase probe (GAPDH).
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hematopoietic factors (other than Ets-1). FDCER cells normally express FOG at readily detectable levels (see Fig. 4). Thus, the moderate ectopic increase in FOG expression, while not predicted (in the absence of GATA-1) to significantly affect m-αIIb gene expression, proved to stimulate the transcription of pIIb545-Luc and the endogenous m-αIIb gene in FDCER-FOG cells at levels comparable with those supported in FDCER-G1 cells by exogenous GATA-1. Recently, GATA-2 has been shown to be capable of binding via its amino-terminal zinc finger to FOG (8, 54) and is known to possess DNA binding properties highly similar to those of GATA-1 (55, 56). Also, GATA-2 is expressed at appreciable levels in FDCER cell lines (see Fig. 4), and it therefore presently is speculated that FOG activation of m-αIIb expression in FDCER-FOG cells might be facilitated by its partnering with GATA-2. This raises questions as to whether FOG might also interact with or otherwise regulate GATA-2 in other cells, including immature hematopoietic cells, which require high level GATA-2 expression for their early development (57). Consistent with this notion, Deconinck et al. recently have hypothesized that proliferation of hematopoietic progenitor cells in Xenopus might involve effects of FOG on GATA-2 expression (47). In this context, the down-regulation of GATA-2 and up-regulation of FOG due to GATA-1 expression in FDCER-G1 cells (see Fig. 4) is again noted. Finally, it also is possible that a presently identified E-box-like element immediately 3′ to the −457 bp GATA element in the murine human αIIb promoters (see Fig. 2) might also recruit FOG-GATA-1 (and/or FOG-GATA-2) complexes via its potential to bind Tal1-Lmo2 complexes (11). Each of the above-mentioned architectures is consistent with recently mapped interactions among these transcription factors (9, 11, 53), and in future experiments, it should be of interest to discover which of these architectures might provide for the selective high level expression of αIIb in megakaryocytic but not erythroid cells (each of which are believed to express all of the above-mentioned factors).

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