Differential sensitivities of transcription factor target genes underlie cell type-specific gene expression profiles

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Changes in transcription factor levels and activities dictate developmental fate. Such a change might affect the full ensemble of target genes for a factor or only uniquely sensitive targets. We investigated the relationship among activity of the hematopoietic transcription factor GATA-1, chromatin occupancy, and target gene sensitivity. Graded activation of GATA-1 in GATA-1-null cells revealed high-, intermediate-, and low-sensitivity targets. GATA-1 activity requirements for occupancy and transcription often correlated. A GATA-1 amino-terminal deletion mutant severely deregulated the low-sensitivity gene *Tac-2*. Thus, cells expressing different levels of a cell type-specific activator can have qualitatively distinct target gene expression patterns, and factor mutations preferentially deregulate low-sensitivity genes. Unlike other target genes, GATA-1-mediated *Tac-2* regulation was bimodal, with activation followed by repression, and the coregulator Friend of GATA-1 (FOG-1) selectively mediated repression. A GATA-1 mutant defective in FOG-1 binding occupied a *Tac-2* regulatory region at levels higher than wild-type GATA-1, whereas FOG-1 facilitated chromatin occupancy at a distinct target site. These results indicate that FOG-1 is a determinant of GATA factor target gene sensitivity by either facilitating or opposing chromatin occupancy.

chromatin | erythroid | GATA | hematopoietic

Changes in transcription factor levels and activities underlie the regulation of key processes such as cell differentiation, proliferation, and survival. Transcription factors often occupy variants of their cognate cis elements at target genes, and such variants can have distinct binding affinities *in vitro*. Thus, changes in transcription factor activities might result in differential occupancy of target genes commensurate with *in vitro* binding affinities. However, affinities measured with oligonucleotides might not correlate with chromatin occupancy, and rules governing chromatin occupancy are poorly understood. Considerable progress has been made in defining chromatin target sites for GATA transcription factor family members.

GATA-1 and GATA-2 have distinct and shared roles to regulate hematopoiesis (1). GATA-2 promotes the proliferation and survival of multipotent precursors (2, 3), whereas GATA-1 regulates erythroid, megakaryocyte, and eosinophil differentiation (4–7). GATA factors engage DNA and coregulators via a dual zinc finger domain (8), and other regions modulate activity (9, 10). GATA-1 increases histone acetylation, dimethylation of histone H3 at lysine 4 (11–13), and RNA polymerase II recruitment (14) and induces higher-order chromatin changes (15).

GATA-1 and GATA-2 preferentially bind (A/T)GATA(A/G) *in vitro* (16, 17) but also recognize GATC (16, 18). GATA motifs are abundant in DNA and reside at most erythroid-specific genes (8, 19). GATA-1 and GATA-2 occupy a small subset of the conserved GATA motifs at the β-globin locus with qualitatively similar patterns (13, 14). Sites can differ in apparent affinities, as β*major* promoter occupancy requires a greater activity/level of an estrogen receptor (ER) ligand binding domain fusion to GATA-1 (ER-GATA-1) vs. the locus control region (13). At the *Gata2* and α*-globin* loci, GATA-1 and GATA-2 have common and unique target sites. GATA-2 occupies sites 3.9, 2.8, and 1.8 kb upstream of the active *Gata2* 1S promoter (20, 21). GATA-1-induced *Gata2* repression involves "GATA switches" in which GATA-1 replaces GATA-2 at −3.9- and −2.8-kb regions. GATA-1-mediated loss of GATA-2 occupancy at the −1.8-kb site occurs without high-level GATA-1 occupancy. At the α*-globin* locus, GATA-2 occupies DNaseI hypersensitive site-12 (HS-12) and HS-26, whereas GATA-1 occupies these and additional regions later in development (22).

GATA factor chromatin occupancy is likely to be regulated by GATA motif features, chromatin, neighboring cis elements, and GATA factor concentration (23, 24). GATA-1 DNA binding activity is 10-fold greater in primitive vs. definitive chicken erythroid cells (25), although the consequence of this difference *vis-à-vis* chromatin occupancy is unknown. In the GATA-1-null mouse embryonic stem cell-derived erythroid cell line G1E, ER-GATA-1 preferentially occupies sites at the β*-globin* locus in which high-level histone acetylation and chromatin accessibility are preestablished (13). GATA-1 binds the acetyltransferases CBP/p300 (26) and induces histone acetylation (11, 12), but whether CBP/p300 enhance GATA-1 occupancy is unknown. The GATA-1 and GATA-2 coregulator Friend of GATA-1 (FOG-1), which mediates activation and repression (27), facilitates GATA-1 occupancy (28, 29) and GATA switches (28). FOG-1 represses GATA-1 target genes via the NuRD corepressor complex (30). GATA-1 binds Sp1 and EKLF transcription factors (31, 32), and their DNA binding sites can coreside with GATA motifs, but it is unclear whether these factors regulate GATA-1 occupancy.

How GATA-1 concentration relates to chromatin occupancy and gene expression is of considerable interest. The absence (33) or severe depletion (34) of GATA-1 in mice is embryonic-lethal because of defective primitive erythropoiesis, and a 5-fold reduction in GATA-1 delays erythropoiesis (35). Mutations that reduce GATA-1 activity uncouple survival, proliferation, and differentiation functions of GATA-1. As little as 5% GATA-1 expression protects cells from apoptosis and induces leukemogenesis (36). In humans with Down syndrome, expression of a "short" GATA-1 (GATA-1s) lacking amino-terminal sequences is linked to a transient myeloproliferative disorder and acute megakaryoblastic leukemia (37–39). GATA-1s expression in megakaryoblast precursors

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Abbreviations: ER, estrogen receptor; FOG-1, Friend of GATA-1; HS, DNaseI hypersensitive site.

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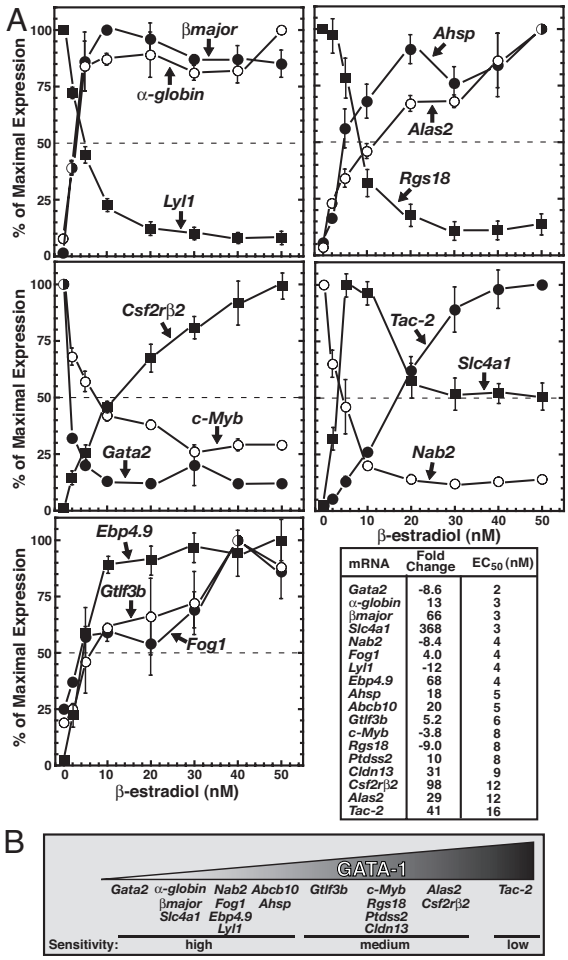

mRNA	Fold Change	EC_{50} (nM)
Gata2	-8.6	2
α-globin	13	3
βmajor	66	3
Slc4a1	368	3
Nab2	-8.4	4
Fog1	4.0	4
Lyl1	-12	4
Ebp4.9	68	4
Ahsp	18	5
Abcb10	20	5
Gtlf3b	5.2	6
c-Myb	-3.8	8
Rgs18	-9.0	8
Ptdss2	10	8
Cldn13	31	9
Csf2rβ2	98	12
Alas2	29	12
Tac-2	41	16

Fig. 1. Differential sensitivities of GATA-1 target genes. Shown is real-time RT-PCR quantitation of mRNA in G1E-ER-GATA-1 cells treated with varying concentrations of β-estradiol for 16 h. Values representing the highest activities were designated as 100 (mean ± SE; three independent experiments). Fold changes in mRNA levels and EC_{50} values are indicated in the table in *Bottom Right*. Graphs depicting *Abcb10*, *Ptdss2*, and *Cldn13* expression are not shown. (*B*) Summary of target gene sensitivities.

deregulates genes that promote proliferation, which are normally repressed by GATA-1 (39, 40).

The findings described above highlight the importance of understanding the relationship between GATA factor levels, chromatin occupancy, and target gene regulation. Herein, we segregate GATA-1 target genes based on differential sensitivities to GATA-1 levels and provide evidence that various factor levels/activities establish qualitatively distinct expression profiles. Furthermore, analysis of the low-sensitivity GATA-1 target gene *Tac-2* revealed mechanistic insights into GATA-1 and FOG-1 function.

Results and Discussion

Differential Sensitivities of GATA-1 Target Genes. Conditional activation of ER-GATA-1 stably expressed in GATA-1-null G1E cells (G1E-ER-GATA-1 cells) induces differentiation, recapitulating a normal window of erythropoiesis (41). Treating G1E-ER-GATA-1 cells with increasing concentrations of β-estradiol or tamoxifen yields graded ER-GATA-1 occupancy at chromatin sites (13, 21). ER-GATA-1 activation involves both increased ER-GATA-1 levels (Fig. 6, which is published as supporting information on the PNAS web site) and increased activity. Using this approach, we tested whether GATA-1 target genes respond similarly or distinctly to changes in the GATA-1 level/activity. G1E-ER-GATA-1 cells were treated with increasing concentrations of β-estradiol for 16 h, and GATA-1 target gene expression was quantitated (Fig. 1*A*). A low ER-GATA-1 level/activity repressed *Gata2*, *Nab2*, and *Lyl1* and activated *α-globin*, *βmajor*, *Slc4a1*, *Fog1*, *Abcb10*, *Ebp4.9*, and *Ahsp*. A moderate level/activity activated *Gtlf3b*, *Ptdss2*, *Cldn13*, *Alas2*, and *CsF2rβ2* and repressed *c-Myb* and *Rgs18*. A high level/activity activated *Tac-2*. These results demonstrating differential sensitivities of GATA-1 target genes (Fig. 1*B*) were not predictable based on the kinetics of GATA-1 target gene expression upon maximal ER-GATA-1 activation, because *Gata2* repression and *βmajor* activation are early and late events, respectively (42).

A fundamental determinant of the sensitivity of target loci to a trans-acting factor is the ease with which the factor accesses chromatin sites. To establish whether differential chromatin access underlies the distinct sensitivities, the ER-GATA-1 level/activity was titrated with 5, 10, or 40 nM β-estradiol for 16 h, and chromatin occupancy was measured at sites defined previously (13, 14, 20, 22, 28, 43–45). ER-GATA-1 occupied β-globin HSs1–4 maximally and the *βmajor* promoter nearly maximally in 10 nM β-estradiol-treated cells (Fig. 2*A*). *βmajor* expression was also maximal at this con-

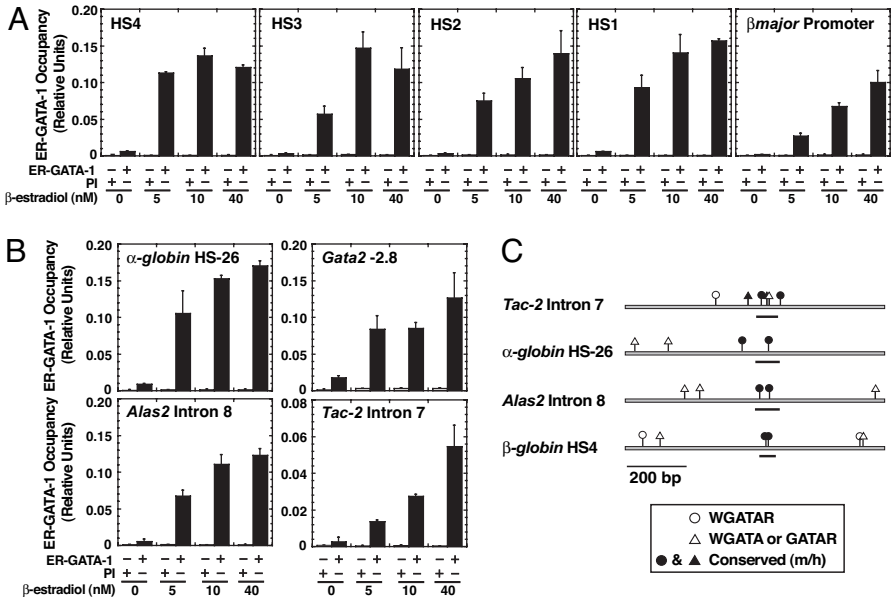

Fig. 2. Differential ER-GATA-1 activity requirements for occupancy of endogenous chromatin sites. Shown are quantitative ChIP analyses of ER-GATA-1 occupancy in G1E-ER-GATA-1 cells treated for 16 h with 0, 5, 10, or 40 nM β-estradiol (mean ± SE; three independent experiments). (*A*) ER-GATA-1 occupancy at previously characterized β-globin locus sites (13). (*B*) ER-GATA-1 occupancy at *α-globin* HS-26, *Alas2* intron 8, the *Gata2* −2.8-kb region, and *Tac-2* intron 7. (*C*) Distribution of WGATAR (A/TGATAA/G), WGATA, and GATAR sites located within 500 bp on either side of the region analyzed by ChIP. Black bars represent ChIP amplicons. Conservation is between mouse and human. PI, preimmune sera.

centration (Fig. 1*A*). Previously (13) we demonstrated that *βmajor* promoter occupancy requires a greater GATA-1 level/activity than occupancy at HSs1–4; the 16-h β-estradiol treatment in Fig. 2 was not optimal for revealing this difference. Consistent with the high ER-GATA-1 sensitivity of *α-globin* and *Gata2*, 5 nM β-estradiol induced nearly maximal ER-GATA-1 occupancy at *α-globin* HS-26 and the *Gata2* −2.8-kb region (Fig. 2*B*). Maximal ER-GATA-1 occupancy at the *Alas2* intron 8 enhancer required 10 nM β-estradiol, and *Alas2* activation required moderate ER-GATA-1 activity (Fig. 1*A*). ER-GATA-1 occupancy at *Tac-2* intron 7 required a high ER-GATA-1 level/activity, similar to the *βmajor* promoter and differing from other sites (Fig. 2*B*).

Despite having a similar number of A/TGATAA/G, nGATAA/G, and A/TGATAn motifs, including conserved motifs, vs. other sites tested (Fig. 2*C*), *Tac-2* activation requires a considerably higher ER-GATA-1 level/activity than other genes. *Tac-2* is highly induced upon differentiation of human peripheral blood erythroid precursors and is also expressed in mouse Ter119+ bone marrow erythroid cells (45).

Deletion of the GATA-1 Amino Terminus Severely Deregulates a Low-Sensitivity Target Gene. GATA-1 amino-terminal sequences are required for maximal transactivation (9), but the underlying mechanisms are unknown. Gene expression profiling in megakaryoblasts from GATA-1s transgenic mice demonstrated that GATA-1 target genes are differentially deregulated (39, 46). We reasoned that mutations impairing GATA-1 activity preferentially affect target genes requiring high GATA-1 activity. To test this prediction, GATA-1 target gene expression was measured in G1E clones stably expressing ER-GATA-1(ΔN) or ER-GATA-1 at levels differing by <2-fold (Fig. 3). ER-GATA-1(ΔN) activation with 1 μM β-estradiol for 24 h induced several GATA-1 targets, but at levels lower than with ER-GATA-1. ER-GATA-1(ΔN) activated *βmajor* 417-fold, 5-fold lower than with ER-GATA-1. ER-GATA-1(ΔN) activated *α-globin*, *Ahsp*, and *Alas2* <4-fold lower than with ER-GATA-1. ER-GATA-1(ΔN) repressed *Gata2* (20) and *c-Kit* (47) 3.5-fold and 2.6-fold lower, respectively, vs. ER-GATA-1. ER-GATA-1(ΔN) was almost completely defective in activating *Tac-2*. *Tac-2* expression increased only in clone 34 (4.6-fold), whereas activation by ER-GATA-1 averaged 29-fold. The capacity of ER-GATA-1(ΔN) to partially regulate most GATA-1 target genes analyzed, yet fail to activate *Tac-2*, is consistent with the greater GATA-1 level/activity requirement for *Tac-2* activation. Because high-level GATA-1(Δ1–83) expression is required to rescue hematopoiesis in mice with reduced GATA-1 levels (10), low-sensitivity targets might have important functions.

GATA-1 zinc fingers are sufficient for DNA binding with naked DNA templates (9, 48), but it is unclear whether other GATA-1 sequences regulate chromatin occupancy. To determine whether the impaired activity of ER-GATA-1(ΔN) arises from defective chromatin occupancy, we compared ER-GATA-1(ΔN) and ER-GATA-1 chromatin occupancy. ER-GATA-1(ΔN) and ER-GATA-1 occupied HS3 and HS4 and the −2.8-kb region of *Gata2* similarly, demonstrating that ER-GATA-1(ΔN) fully occupies certain sites (Fig. 7, which is published as supporting information on the PNAS web site). ER-GATA-1(ΔN) occupancy at *α-globin* HS-26, *Alas2* intron 8, and *Tac-2* intron 7 was ≈2-fold lower, whereas occupancy at the *βmajor* promoter was 4-fold lower vs. GATA-1. Thus, deletion of the amino terminus variably affected chromatin occupancy. The ≈2-fold reduction of ER-GATA-1(ΔN) occupancy at *Tac-2* intron 7 could, in principle, reduce ER-GATA-1 below a critical threshold required for activation, considering that the absolute level of ER-GATA-1 occupancy at intron 7 is lower than at other sites (Fig. 2 *A* and *B*). Alternatively, the amino terminus might enhance *Tac-2* activation after DNA binding, consistent with its transactivation activity (9).

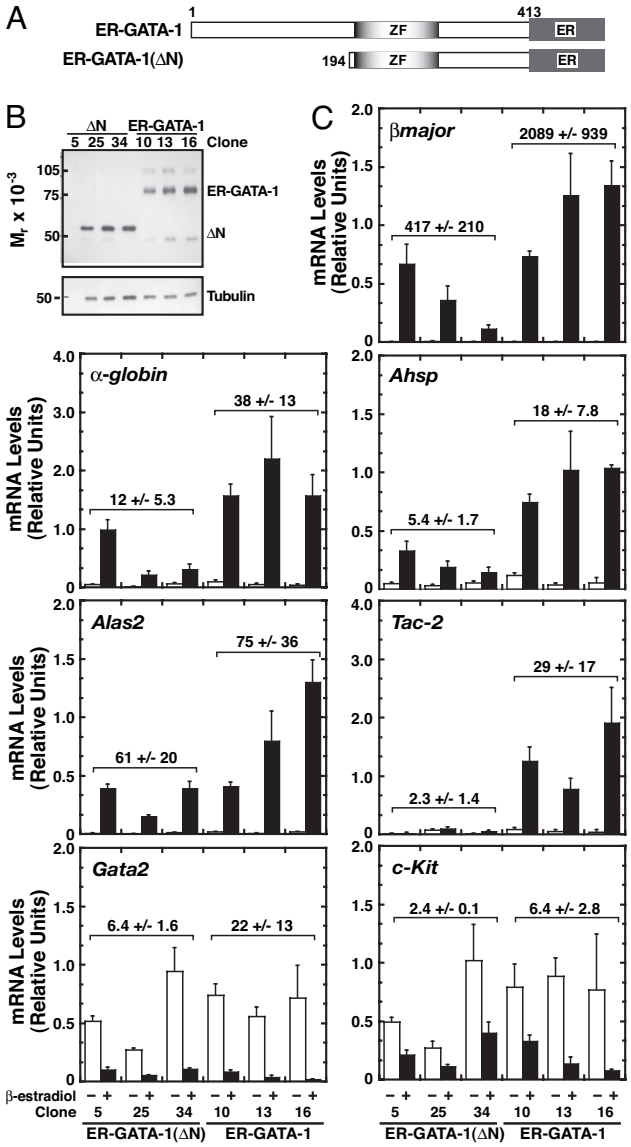

Fig. 3. Truncation of the GATA-1 amino terminus preferentially ablates *Tac-2* activation. (*A*) Proteins analyzed. (*B*) Western blot analysis of ER-GATA-1(ΔN) and ER-GATA-1 stably expressed in G1E clonal cell lines. (*C*) GATA-1 target gene expression was compared in G1E clones expressing ER-GATA-1(ΔN) or ER-GATA-1. Real-time RT-PCR analysis was conducted after induction with 1 μM β-estradiol for 24 h (mean ± SE; three independent experiments). The β-estradiol-induced fold change in mRNA was averaged for the three clones of ER-GATA-1(ΔN) or ER-GATA-1; the differences between values from cells expressing ER-GATA-1(ΔN) and values from cells expressing ER-GATA-1 were statistically significant ($P = 0.003, 0.001, 0.002, 0.002, 0.006$, and 0.023 for *βmajor*, *α-globin*, *Ahsp*, *Tac-2*, *Gata2*, and *c-Kit*, respectively) for all genes except *Alas2*.

Unique Biphasic *Tac-2* Expression Pattern. *Tac-2* encodes a neurokinin-B (NK-B) precursor protein. *Tac-2* is expressed during erythropoiesis (45), in the placentas of women with preeclampsia (49), and by certain neuronal cells (50). Analysis of ER-GATA-1 occupancy at conserved GATA motifs within an ≈100-kb chromosomal region containing *Tac-2* revealed high-level ER-GATA-1 occupancy at *Tac-2* intron 7 (45). To investigate the mechanism underlying GATA-1-mediated *Tac-2* activation, we tested whether the kinetics of ER-GATA-1-induced *Tac-2* expression resembles that of other GATA-1 target genes. *Tac-2* mRNA increased during the 24-h β-estradiol treatment (Fig. 4*A*), but, in contrast to other

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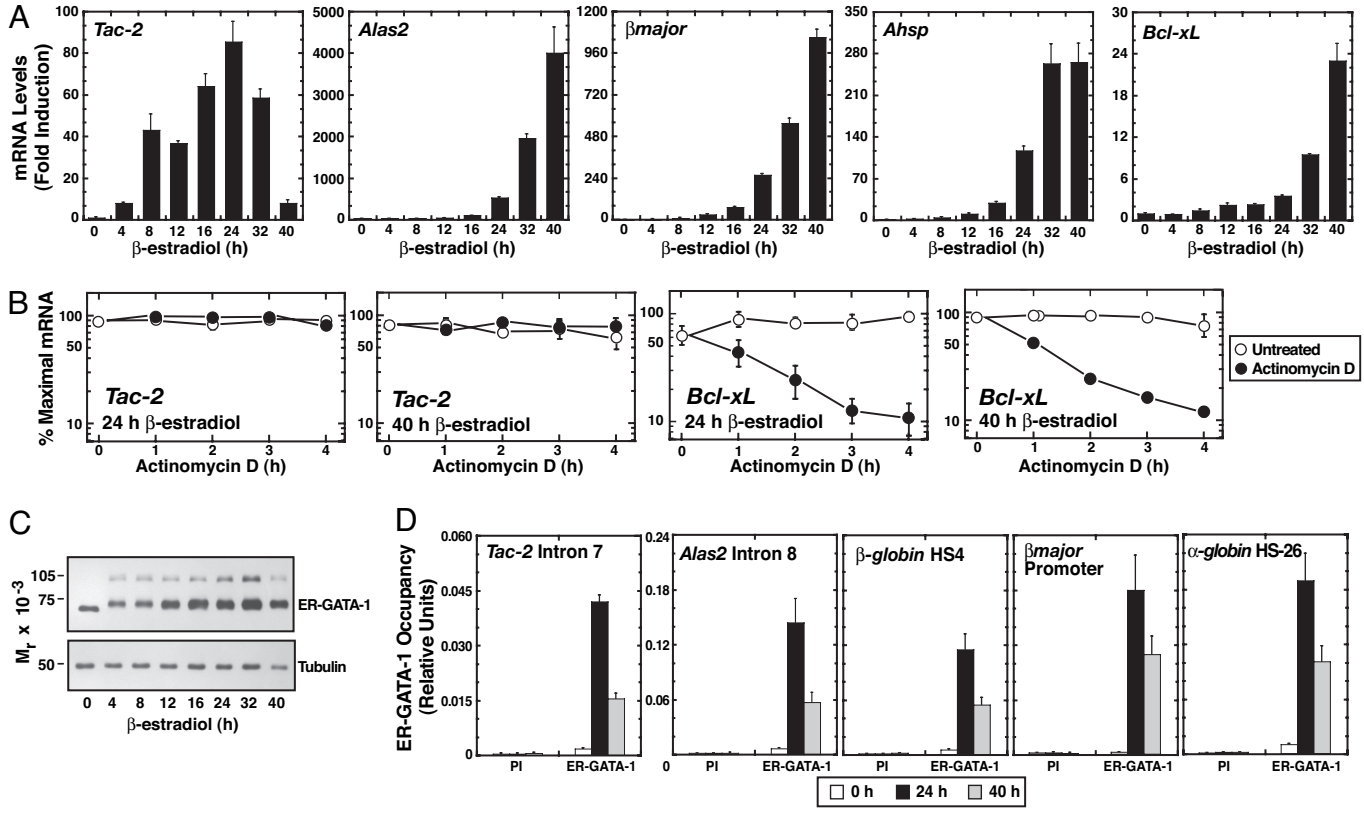

Fig. 4. Unique biphasic pattern of GATA-1-mediated *Tac-2* regulation. (*A*) Real-time RT-PCR analyses of GATA-1 target gene mRNA in G1E-ER-GATA-1 cells treated with 1 μM β-estradiol for up to 40 h (mean $\pm$ SE; three independent experiments). (*B*) Real-time PCR analysis of *Tac-2* and *Bcl-xL* mRNA stability. G1E-ER-GATA-1 cells were treated with 1 μM β-estradiol for 24 or 40 h before adding actinomycin D (10 μg/ml) for up to 4 h (mean $\pm$ SE; three independent experiments). (*C*) Western blot analysis of ER-GATA-1 in G1E-ER-GATA-1 cells. (*D*) ChIP analysis of ER-GATA-1 occupancy after 24 or 40 h of β-estradiol treatment (mean $\pm$ SE; three independent experiments). PI, preimmune sera. Decreased ER-GATA-1 occupancy at 40 vs. 24 h was statistically significant ($P < 0.05$) in all cases except the *βmajor* promoter.

targets, *Tac-2* mRNA declined 11-fold by 40 h. This expression pattern occurred in two independent G1E-ER-GATA-1 clonal lines that varied ≈4-fold in ER-GATA-1 expression (data not shown).

To test whether biphasic expression results from transcriptional activation, followed by mRNA destabilization, *Tac-2* mRNA stability was measured after 24 or 40 h of β-estradiol treatment. *Tac-2* mRNA was constant after 4 h of actinomycin D treatment regardless of the duration of β-estradiol treatment (Fig. 4*B*). Thus, *Tac-2* mRNA is not unstable or destabilized. Because *Bcl-xL* mRNA, which increases late in G1E-ER-GATA-1 differentiation (42, 51), had a $t_{1/2}$ of ≈1 h, the assay discriminates between unstable and stable mRNAs. Rapid turnover of *Tac-2* mRNA therefore does not explain the reduced mRNA levels, indicating that *Tac-2* is transcriptionally repressed.

Because *Tac-2* activation requires a high ER-GATA-1 level/activity and the modestly impaired activity of ER-GATA-1(ΔN) catastrophically reduces *Tac-2* expression, *Tac-2* repression might result from a small reduction in ER-GATA-1 level/activity late in differentiation. Western blot analysis after 40 h of β-estradiol treatment revealed a small decrease in ER-GATA-1, although the ER-GATA-1/tubulin ratio was constant (Fig. 4*C*). Under these conditions, 2- to 3-fold losses in ER-GATA-1 occupancy were observed at all chromatin sites tested (Fig. 4*D*). Because *Tac-2* activation requires the highest GATA-1 level/activity, comparable decreases in occupancy at all sites might preferentially deregulate *Tac-2*.

Dual GATA-1-Mediated Activation and Repression of a Target Gene: Selective FOG-1 Utilization for Repression. FOG-1 facilitates GATA-1 chromatin occupancy at certain sites, is required for GATA switches (28, 29), and mediates activation and repression. The GATA-1 amino-terminal zinc finger binds FOG-1 (52, 53), and mutation of V205 within this finger disrupts binding (27, 53). ER-GATA-1(V205) mutants fail to complement GATA-1 loss in GATA-1-null G1E cells and do not regulate most GATA-1 target genes (27). GATA-1 increases *Eklf* (27) and *Fog1* (42) transcription in a FOG-1-independent manner, but GATA-1-null G1E cells express these genes, and GATA-1 only modestly increases their mRNAs. Thus, although GATA-1 up-regulates *Eklf* and *Fog1*, GATA-1 is dispensable for establishing their active state. This finding contrasts with FOG-1-dependent GATA-1 targets, in which GATA-1 is often critical for high-level activation or repression.

To determine whether *Tac-2* activation is FOG-1-dependent, we tested whether ER-GATA-1(V205G) can activate *Tac-2* (Fig. 5*A*). ER-GATA-1(V205G) activated *Tac-2* 3- to 4-fold greater than with ER-GATA-1. Unlike ER-GATA-1, the induction was sustained for 40 h (Fig. 5*B*). Thus, V205, which is known only to mediate FOG-1 binding, is required for *Tac-2* repression but not activation. Furthermore, ER-GATA-1 activated *Tac-2* expression 124-fold in FOG-1-null cells (54) overexpressing ER-GATA-1 (28), whereas *Ahsp* and *Alas2* were induced only 2.4- and 15-fold, respectively (Fig. 5*C*). Combined with the V205G mutant data, the results establish that *Tac-2* activation is FOG-1-independent. *Tac-2* activation in G1E and FOG-1-null cells in which differentiation is blocked, the rapid activation kinetics, and GATA-1 occupancy of the conserved GATA motif-containing intron 7 indicate that *Tac-2* is a direct target. *Tac-2* is therefore the only FOG-1-independent GATA-1 target gene reported that critically requires GATA-1 for transcriptional activity.

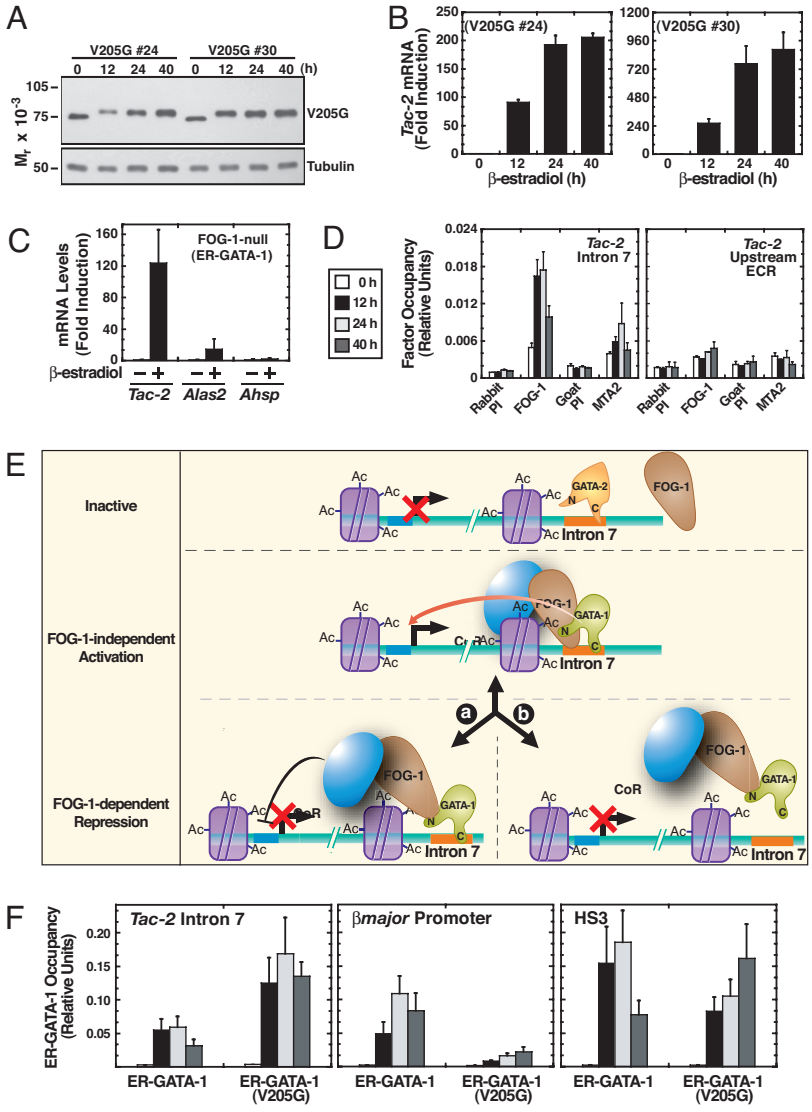

Fig. 5. FOG-1-independent and -dependent components of GATA-1-mediated *Tac-2* regulation. (*A*) Western blot analysis of ER-GATA-1(V205G) stably expressed in clonal lines 24 and 30 at 0, 12, 24, and 40 h after β-estradiol treatment. (*B*) Kinetics of *Tac-2* activation by ER-GATA-1(V205G). (*C*) *Tac-2* activation in FOG-1-null cells expressing ER-GATA-1 treated with tamoxifen for 24 h (mean ± SD; two independent experiments). (*D*) ChIP analysis of FOG-1 and MTA2 occupancy at *Tac-2* intron 7 in G1E-ER-GATA-1 cells (mean ± SE; six and two independent experiments for FOG-1 and MTA2, respectively). (*E*) The two nonmutually exclusive models illustrate FOG-1-independent activation followed by FOG-1-dependent repression. FOG-1 utilizes a corepressor complex to repress the promoter (a), or FOG-1 directly opposes GATA-1 chromatin occupancy (b). (*F*) ChIP analysis of ER-GATA-1 and ER-GATA-1(V205G) occupancy (mean ± SE; four immunoprecipitations and two independent experiments). Preimmune signals did not exceed 0.003. (See key in *D*.) The differences in ER-GATA-1 vs. V205G occupancy at intron 7 and *βmajor* promoter were statistically significant ($P < 0.05$) except for the 12-h *Tac-2* intron 7 values.

Because FOG-1 selectively mediated *Tac-2* repression, we tested whether ER-GATA-1 occupancy at *Tac-2* intron 7 resulted in delayed FOG-1 accumulation at this site during the repression phase. However, FOG-1 occupied intron 7, but not an upstream evolutionarily conserved region, concomitant with ER-GATA-1 occupancy (Fig. 5*D*). Similar to ER-GATA-1, FOG-1 occupancy significantly decreased by 40 h. The FOG-1-associated NuRD complex component MTA2 (metastasis-associated protein 2) similarly occupied intron 7 before repression. Although the NuRD complex contains histone deacetylase subunits (30), diacetylated histone H3 and multiacetylated H4 at *Tac-2* intron 7 and promoter did not decline upon repression (Fig. 8, which is published as supporting information on the PNAS web site).

Given the FOG-1 requirement for repression, why does maximal FOG-1 occupancy precede the major decline in *Tac-2* mRNA? We reasoned that either a FOG-1–corepressor complex initiates repression and ER-GATA-1-FOG-1 complexes are evicted from repressed templates, or FOG-1 actively opposes GATA-1 chromatin occupancy (Fig. 5*E*). Both models assume that ER-GATA-1(V205G) defective in FOG-1 binding would occupy *Tac-2* intron 7 to a greater extent than ER-GATA-1. ER-GATA-1(V205G) occupancy at intron 7 was ≈3-fold greater than ER-GATA-1 (Fig. 5*F*), even though ER-GATA-1(V205G) was expressed slightly less than ER-GATA-1 (data not shown). Almost no ER-GATA-1(V205G) occupancy occurred at the *βmajor* promoter, consistent with FOG-1 chromatin occupancy facilitator activity (28, 29) (Fig. 5*F*). ER-GATA-1(V205G) and ER-GATA-1 occupied HS3 similarly. Thus, FOG-1 uniquely suppresses GATA-1 chromatin occupancy at *Tac-2* intron 7.

Mechanistic Insights. Analysis of target gene sensitivity to GATA-1 levels identified a high GATA-1 level/activity requirement for occupancy and activation of *Tac-2*. Unlike other genes studied, *Tac-2* activation is followed by repression. FOG-1 mediates *Tac-2* repression but not activation, representing the first example in which GATA-1 activates and represses a target gene while selectively using FOG-1 for repression.

FOG-1 and MTA2 occupy intron 7 before repression, suggesting that FOG-1–MTA2 repressive activity is initially suppressed or insufficient to counter coactivator functions, or that repression is a relatively slow process. As ER-GATA-1(V205G) occupies intron 7 and activates *Tac-2* greater than ER-GATA-1, FOG-1 suppresses ER-GATA-1 occupancy at *Tac-2* and activation. Acting like a rheostat, FOG-1 is therefore a key determinant of *Tac-2* sensitivity to GATA-1 levels. The subsequent engagement of FOG-1 repressive activity ensures transient activation. Thus, FOG-1 has context-dependent activity to facilitate GATA-1 chromatin occupancy (28, 29) and to restrict

GATA-1 occupancy. The anti-GATA-1 activity might be related to FOG-1-mediated repression of erythropoiesis in *Xenopus laevis* (55) and blood cell development in *Drosophila* (56).

The demonstration that GATA-1 target genes are differentially sensitive to the GATA-1 level/activity emphasizes the importance of defining parameters underlying GATA factor selection of functional target sites in a genome littered with GATA motifs. Target sensitivity involves not only parameters that determine chromatin occupancy but also those regulating chromatin-bound factors. It will be particularly instructive to assess the contribution of individual parameters and combinations thereof to establishing and regulating target sensitivities and the impact of altering these parameters on GATA factor-instigated gene networks.

Methods

Cell Culture. G1E cells expressing ER-GATA-1 constructs were cultured as described (13).

Quantitative Real-Time RT-PCR. Total RNA was purified with TRIzol (GIBCO/BRL, Invitrogen, Carlsbad, CA), and cDNA was prepared from 1.5 μg of RNA as described (28). Relative expression levels were determined by real-time RT-PCR from a standard curve of serial dilutions of cDNA samples and were normalized to GAPDH expression. Primer sequences are described in *Supporting Materials and Methods*.

Quantitative ChIP Assay. ChIP analyses were conducted as described (57) and as described in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

RNA Stability Assay. G1E-ER-GATA-1 cells were pretreated with 1 μM β-estradiol for 24 or 40 h. Cultures were then treated with 10 μg/ml actinomycin D (A1410; Sigma, St. Louis, MO) for up to 4 h. Total RNA was purified and analyzed by real-time RT-PCR analysis.

Protein Analysis. Total protein was prepared by boiling cells for 5 min in SDS sample buffer (50 mM Tris, pH 6.8/100 mM DTT/2% SDS/0.1% bromophenol blue/10% glycerol) (1×10^7 cells per milliliter of buffer). Proteins were analyzed by Western blotting using ECL-Plus (GE Healthcare, Piscataway, NJ). ER-GATA-1 fusions were detected with anti-ER antibody AB16.

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1. Fujiwara Y, Chang AN, Williams AM, Orkin SH (2004) *Blood* 103:583–585.
2. Tsai FY, Keller G, Kuo FC, Weiss M, Chen J, Rosenblatt M, Alt FW, Orkin SH (1994) *Nature* 371:221–226.
3. Tsai F-Y, Orkin SH (1997) *Blood* 89:3636–3643.
4. Hirasawa R, Shimuzu R, Takahashi S, Osawa M, Takayanagi S, Kato Y, Onodera M, Minegishi N, Yamamoto M, Fukao K, *et al.* (2002) *J Exp Med* 195:1379–1386.
5. Pevny L, Lin CS, D'Agati V, Simon MC, Orkin SH, Costantini F (1995) *Development (Cambridge, UK)* 121:163–172.
6. Pevny L, Simon MC, Robertson E, Klein WH, Tsai SF, D'Agati V, Orkin SH, Costantini F (1991) *Nature* 349:257–260.
7. Shivdasani RA, Fujiwara Y, McDevitt MA, Orkin SH (1997) *EMBO J* 16:3965–3973.
8. Cantor AB, Orkin SH (2002) *Oncogene* 21:3368–3376.
9. Martin DI, Orkin SH (1990) *Genes Dev* 4:1886–1898.
10. Shimizu R, Takahashi S, Ohneda K, Engel JD, Yamamoto M (2001) *EMBO J* 20:5250–5260.
11. Letting DL, Rakowski C, Weiss MJ, Blobel GA (2003) *Mol Cell Biol* 23:1334–1340.
12. Kiekhaefer CM, Grass JA, Johnson KD, Boyer ME, Bresnick EH (2002) *Proc Natl Acad Sci USA* 99:14309–14314.
13. Im H, Grass JA, Johnson KD, Kim S-I, Boyer ME, Imbalzano AN, Bieker JJ, Bresnick EH (2005) *Proc Natl Acad Sci USA* 102:17065–17070.
14. Johnson KD, Grass JD, Boyer ME, Kiekhaefer CM, Blobel GA, Weiss MJ, Bresnick EH (2002) *Proc Natl Acad Sci USA* 99:11760–11765.
15. Vakoc CR, Letting DL, Gheldof N, Sawado T, Bender MA, Groudine M, Weiss MJ, Dekker J, Blobel GA (2005) *Mol Cell* 17:453–462.
16. Ko LJ, Engel JD (1993) *Mol Cell Biol* 13:4011–4022.
17. Merika M, Orkin SH (1993) *Mol Cell Biol* 13:3999–4010.
18. Newton A, MacKay JP, Crossley M (2001) *J Biol Chem* 276:35794–35801.
19. Evans T, Felsenfeld G, Reitman M (1990) *Annu Rev Cell Biol* 6:95–124.
20. Grass JA, Boyer ME, Paul S, Wu J, Weiss MJ, Bresnick EH (2003) *Proc Natl Acad Sci USA* 100:8811–8816.
21. Martowicz ML, Grass JA, Boyer ME, Guend H, Bresnick EH (2005) *J Biol Chem* 280:1724–1732.
22. Anguita E, Hughes J, Heyworth C, Blobel GA, Wood WG, Higgs DR (2004) *EMBO J* 23:2841–2852.
23. Bresnick EH, Martowicz ML, Pal S, Johnson KD (2005) *J Cell Physiol* 205:1–9.
24. Bresnick EH, Johnson KD, Kim S-I, Im H (2006) *Prog Nucleic Acids Res Mol Biol* 81:435–471.
25. Minie ME, Kimura T, Felsenfeld G (1992) *Development (Cambridge, UK)* 115:1149–1164.
26. Blobel GA, Nakajima T, Eckner R, Montminy M, Orkin SH (1998) *Proc Natl Acad Sci USA* 95:2061–2066.
27. Crispino JD, Lodish MB, MacKay JP, Orkin SH (1999) *Mol Cell* 3:219–228.
28. Pal S, Cantor AB, Johnson KD, Moran T, Boyer ME, Orkin SH, Bresnick EH (2004) *Proc Natl Acad Sci USA* 101:980–985.
29. Letting DL, Chen YY, Rakowski C, Reedy S, Blobel GA (2004) *Proc Natl Acad Sci USA* 101:476–481.
30. Hong W, Nakazawa M, Chen YY, Kori R, Vakoc CR, Rakowski C, Blobel GA (2005) *EMBO J* 24:67–78.
31. Gregory RC, Taxman DJ, Seshasayee D, Kensinger MH, Bieker JJ, Wojchowski DM (1996) *Blood* 87:1793–1801.
32. Merika M, Orkin SH (1995) *Mol Cell Biol* 15:2437–2447.
33. Fujiwara Y, Browne CP, Cunniff K, Goff SC, Orkin SH (1996) *Proc Natl Acad Sci USA* 93:12355–12358.
34. Takahashi S, Onodera K, Motohashi H, Suwabe N, Hayashi N, Yanai N, Nabesima Y, Yamamoto M (1997) *J Biol Chem* 272:12611–12615.
35. McDevitt MA, Fujiwara Y, Shivdasani RA, Orkin SH (1997) *Proc Natl Acad Sci USA* 94:7696–7681.
36. Shimizu R, Kuroha T, Ohneda O, Pan X, Ohneda K, Takahashi S, Philipsen S, Yamamoto M (2004) *Mol Cell Biol* 24:10814–10825.
37. Wechsler J, Greene M, McDevitt MA, Anastasi J, Karp JE, LeBeau MM, Crispino JD (2002) *Nat Genet* 32:148–152.
38. Xu G, Nagano M, Kanezaki R, Toki T, Hayashi Y, Taketani T, Taki T, Mitui T, Koike K, Kato K, *et al.* (2003) *Blood* 102:2960–2968.
39. Li Z, Godinho FJ, Klusmann JH, Garriga-Canut M, Yu C, Orkin SH (2005) *Nat Genet* 37:613–619.
40. Muntean AG, Crispino JD (2005) *Blood* 106:1223–1231.
41. Weiss MJ, Yu C, Orkin SH (1997) *Mol Cell Biol* 17:1642–1651.
42. Welch JJ, Watts JA, Vakoc CR, Yao Y, Wang H, Hardison RC, Blobel GA, Chodosh LA, Weiss MJ (2004) *Blood* 104:3136–3147.
43. Surinya KH, Cox TC, May BK (1998) *J Biol Chem* 273:16798–16809.
44. Gallagher PG, Liem RI, Wong E, Weiss MJ, Bodine DM (2005) *J Biol Chem* 280:39016–39023.
45. Pal S, Nemeth MJ, Bodine DM, Miller JL, Svaren J, Thein SL, Lowry PJ, Bresnick EH (2004) *J Biol Chem* 279:31348–31356.
46. Bourquin JP, Subramanian A, Langebrake C, Reinhardt D, Bernard O, Ballerini P, Baruchel A, Cave H, Dastugue N, Hasle H, *et al.* (2006) *Proc Natl Acad Sci USA* 103:3339–3344.
47. Munugalavadla V, Dore LC, Tan BL, Hong L, Vishnu M, Weiss MJ, Kapur R (2005) *Mol Cell Biol* 25:6747–6759.
48. Omichinski JG, Trainor C, Evans T, Gronenborn AM, Clore GM, Felsenfeld G (1993) *Proc Natl Acad Sci USA* 90:1676–1680.
49. Page NM, Woods RJ, Gardiner SM, Lomthaisong K, Gladwell RT, Butlin DJ, Manyonda IT, Lowry PJ (2000) *Nature* 405:797–800.
50. Page NM, Woods RJ, Lowry PJ (2001) *Regul Pept* 98:97–104.
51. Gregory T, Yu C, Ma A, Orkin SH, Blobel GA, Weiss MJ (1999) *Blood* 94:87–96.
52. Tsang AP, Visvader JE, Turner CA, Fujuwara Y, Yu C, Weiss MJ, Crossley M, Orkin SH (1997) *Cell* 90:109–119.
53. Fox AH, Liew C, Holmes M, Kowalski K, MacKay JP, Crossley M (1999) *EMBO J* 18:2812–2822.
54. Cantor AB, Katz SG, Orkin SH (2002) *Mol Cell Biol* 22:4268–4279.
55. Deconinck AE, Mead PE, Tevosian SG, Crispino JD, Katz SG, Zon LI, Orkin SH (2000) *Development (Cambridge, UK)* 127:2031–2040.
56. Fossett N, Tevosian SG, Gajewski K, Zhang Q, Orkin SH, Schultz RA (2001) *Proc Natl Acad Sci USA* 98:7342–7347.
57. Im H, Grass JA, Johnson KD, Boyer ME, Wu J, Bresnick EH (2004) *Methods Mol Biol* 284:129–146.