Coregulator-dependent facilitation of chromatin occupancy by GATA-1

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Coregulator recruitment by DNA-bound factors results in chromatin modification and protein-protein interactions, which regulate transcription. However, the mechanism by which the Friend of GATA (FOG) coregulator mediates GATA factor-dependent transcription is unknown. We showed previously that GATA-1 replaces GATA-2 at an upstream region of the *GATA-2* locus, and that this GATA switch represses *GATA-2*. Genetic complementation analysis in FOG-1-null hematopoietic precursors revealed that FOG-1 is not required for establishment or maintenance of the active *GATA-2* domain, but is critical for the GATA switch. Analysis of GATA factor binding to additional loci also revealed FOG-1-dependent GATA switches. Thus, FOG-1 facilitates chromatin occupancy by GATA-1 at sites bound by GATA-2. We propose that FOG-1 is a prototype of a new class of coregulators termed chromatin occupancy facilitators, which confer coregulation in certain contexts via enhancing trans-acting factor binding to chromatin *in vivo*.

A paradigm has emerged in which coregulator proteins are recruited to chromatin templates by DNA-bound activators and repressors, thereby regulating transcription (1–3). Once recruited, coregulators commonly catalyze chromatin modifications, such as histone acetylation and methylation, which control DNA accessibility and binding of the transcriptional machinery (4). Although this mechanism is used by diverse trans-acting factors, such factors can recruit more than one coregulator, creating complex scenarios involving multiple biochemical reactions. For example, the highly conserved GATA family of transcription factors (5, 6), which recognize WGATAR DNA motifs (7, 8), associate with the histone acetyltransferase (HAT) cAMP response element-binding (CREB)-binding protein (9) and the coregulator Friend of GATA-1 (FOG-1) (10).

FOG-1 is the founding member of the FOG family of coregulators (10) and mediates both activation and repression of GATA-1, GATA-2, and GATA-3 target genes (10–12). Despite the definitive evidence that FOG-1 is a GATA factor coregulator, the mechanism by which FOG-1 functions is unclear. FOG-1 contains nine zinc fingers, but sequence-specific DNA binding activity of FOG-1 has not been detected. Abrogation of FOG-1 coregulator activity requires mutation of multiple amino acid residues from distinct regions of FOG-1 (13). Thus, sequences mediating protein–protein interactions with typical coregulators, such as HATs, histone methyltransferases, histone deacetylases (HDACs), and chromatin remodeling complexes, have not been defined. Furthermore, the mouse knock-in of a FOG-1 mutant lacking a conserved binding site for the corepressor C-terminal binding protein (CtBP) has no obvious phenotype, inconsistent with CtBP mediating essential functions of FOG-1 (14). Based on these findings and the absence of related coregulators, mechanisms underlying FOG-1 coregulator activity have remained elusive.

Here, we investigated the mechanism by which FOG-1 functions as a GATA factor coregulator, specifically in the context of GATA-1- and GATA-2-mediated regulation of *GATA-2* transcription. GATA-1 is a critical regulator of erythroid, megakaryocytic, eosinophil, and mast cell differentiation (15–21), whereas GATA-2 is essential for hematopoietic stem and progenitor cell function and mast cell differentiation (22, 23). Disruption of murine *GATA-2* results in embryonic lethality characterized by a major loss of blood cells and reductions in hematopoietic precursors (22, 23). As GATA-1 levels increase during erythroid differentiation, GATA-2 levels decrease (19, 24, 25). Taken together with the fact that GATA-2 is derepressed in GATA-1-null cells (19), GATA-1 and GATA-2 are reciprocally expressed during hematopoiesis.

The reciprocal relationship between GATA-1 and GATA-2 expression is explained in part by the direct GATA-1-mediated transcriptional repression of *GATA-2* (26). GATA-1 binds a conserved upstream region (−2.8 kb) of the *GATA-2* locus, displacing GATA-2 from this region (26). This "GATA switch" is tightly coupled with repression and is accompanied by a broad reduction in histone acetylation throughout the *GATA-2* locus. We proposed a bimodal repression model in which GATA-1 induces the GATA switch, abrogates positive autoregulation and results in the assembly of repressive nucleoprotein complexes at the −2.8-kb region. Deacetylation would lock the locus into an inactive state. Because both GATA-1 and GATA-2 functionally interact with FOG-1 (27), we asked whether GATA-2 utilizes FOG-1 to establish or maintain the active state of the *GATA-2* locus and whether GATA-1 requires FOG-1 to repress *GATA-2*. These studies revealed mechanistic insights regarding how FOG-1 mediates GATA factor function, which have broad relevance to coregulator mechanisms and the control of FOG-1-dependent developmental processes.

Materials and Methods

Cell Culture. FOG-1$^{-/-}$ HOX-11 immortalized cells (13) were maintained in Iscove's Modified Dulbecco's Medium (IMDM) (GIBCO/BRL) containing 15% FBS (GIBCO/BRL), 100 units/ml penicillin/streptomycin (GIBCO/BRL), and 10 ng/ml interleukin 3 (R & D Systems). G1E cells (33) were maintained in IMDM containing 2% penicillin/streptomycin (GIBCO/BRL), 2 units/ml erythropoietin, 120 nM monothioglycerol (Sigma), 0.6% conditioned medium from a Kit ligand producing Chinese hamster ovary (CHO) cell line, and 15% FBS (GIBCO/BRL). G1E–ER–GATA-1 cells (28, 29), which stably express an estrogen receptor (ER) hormone binding domain fusion to GATA-1 (ER–GATA-1), were maintained identical to G1E cells except media contained 1 μg/ml puromycin. FOG-1$^{-/-}$–ER–GATA-1 cells, which stably express ER–GATA-1 (12), were generated by retroviral infection. Wild-type GATA-1 cDNA was cloned in the pGD-G1ER-puro construct (10, 12, 30), in which GATA-1 cDNAs were fused in-frame to the ligand-binding domain of the ER. FOG-1$^{-/-}$ and G1E cells (5×10^6) were incubated with the appropriate retroviral supernatants, and cells were selected with puromycin (1 μg/ml).

Abbreviations: ALAS-2, aminolevulinate synthase 2; ChIP, chromatin immunoprecipitation; COF, chromatin occupancy facilitator; ER, estrogen receptor; FOG-1, Friend of GATA-1; HAT, histone acetyltransferase; HS, hypersensitive site.

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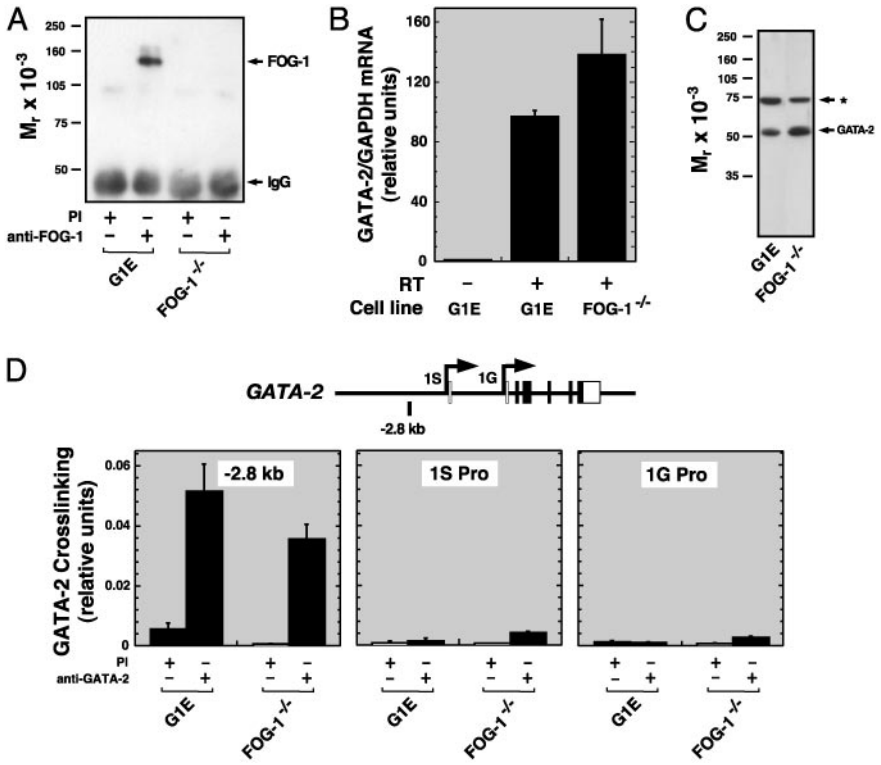

Fig. 1. *GATA-2* transcription is FOG-1 independent. (*A*) Western blot analysis of *FOG-1* expression in G1E and FOG-1$^{-/-}$ cells. Whole cell extracts were immunoprecipitated with anti-FOG-1 polyclonal antibody or preimmune (PI) serum and were analyzed by Western blotting with anti-FOG-1 antibody. (*B*) Quantitative real-time RT-PCR was used to measure *GATA-2* mRNA expression in G1E and FOG-1$^{-/-}$ cells. Exon 3/exon 4 primers amplified *GATA-2* transcripts arising from usage of both 1S and 1G promoters (46). GAPDH mRNA was measured as a control. The plots depict the mean GATA-2/GAPDH ratios (mean ± SEM, three independent experiments). (*C*) Western blot analysis of GATA-2 expression in whole cell lysates from G1E and FOG-1$^{-/-}$ cells. A broadly expressed cross-reactive band is denoted by the asterisk. (*D*) Quantitative ChIP analysis of GATA-2 binding to the *GATA-2* locus in G1E and FOG-1$^{-/-}$ cells (mean ± SEM of three independent experiments). The diagram at the top of the graph represents the murine *GATA-2* locus. The vertical line below the locus indicates the position of the −2.8-kb amplicon, upstream of the 1S exon.

Independent clones were isolated by limiting dilution. Stable cell lines were cultured in the presence of 1 μM tamoxifen for 48 h, and expression of ER–GATA-1 was measured by Western blotting. For FOG-1-rescued cells (13), FOG-1$^{-/-}$ cells (10^7) were infected with murine myeloproliferative (MMP) (31) retroviruses packaged with a FOG-1 cDNA retroviral vector or an empty retroviral vector. Wild-type FOG-1 cDNA was cloned between the viral ATG and an internal ribosome entry site–GFP element. Control cells were infected with empty vector. Cells were washed and incubated in FOG-1$^{-/-}$ growth medium for 2 days. GFP+ cells were isolated by FACS to >90% purity by using a Beckman Coulter high-speed sorter. Sorted cells were grown for 2 h in FOG-1$^{-/-}$ growth medium containing erythropoietin (2 units/ml) and thrombopoietin (5 ng/ml) and analyzed by chromatin immunoprecipitation (ChIP).

Quantitative ChIP Assay. Real-time PCR-based quantitative ChIP analysis was conducted as described (26, 29, 32). Cells were grown in media containing 15% FBS with or without 1 μM tamoxifen (Sigma) for 24 h. Protein–DNA crosslinking was conducted by treating cells with formaldehyde at a final concentration of 0.4% (1% for FOG-1 ChIP) for 10 min at room temperature with gentle agitation. Glycine (0.125 M) was added to quench the reaction. ChIP was conducted as described in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site. Primers and antibodies are described in *Supporting Materials and Methods*.

Quantitative RT-PCR. Real-time RT-PCR methodology and sequences of forward and reverse primers are indicated in *Supporting Materials and Methods*.

Protein Analysis. To detect FOG-1 expression, whole cell lysates were prepared in Nonidet P-40 lysis buffer (50 mM Tris, pH 8.0/150 mM NaCl/1% Nonidet P-40/2 mM DTT/0.2 mM PMSF/20 μg/ml leupeptin). Lysates were cleared by centrifugation at 13,000 × *g* for 30 min at 4°C. Proteins were analyzed as described in *Supporting Materials and Methods*.

Results and Discussion

FOG-1 Is Not Required for Establishment or Maintenance of the Active *GATA-2* Domain. GATA-2 binds the −2.8-kb region of the *GATA-2* locus when the locus is transcriptionally active, and GATA-1-dependent displacement of GATA-2 instigates transcriptional repression (26). These studies were conducted in GATA-1-null G1E hematopoietic cells, which express endogenous GATA-2 (33). Because GATA-2 physically and functionally interacts with FOG-1 (27), we used G1E and FOG-1-null hematopoietic precursors (13) to ask whether FOG-1 is required to establish and/or maintain the active *GATA-2* domain. FOG-1 is expressed in G1E cells, and as expected, is undetectable in the null cells (Fig. 1*A*). GATA-2 mRNA (Fig. 1*B*) and protein (Fig. 1*C*) were expressed at slightly higher levels in FOG-1$^{-/-}$ versus G1E cells, demonstrating that FOG-1 is not required for *GATA-2* transcription.

One explanation for the lack of a FOG-1 requirement for *GATA-2* transcription is that GATA-2 might not function through the −2.8-kb region in this system. Although GATA-2 binds the −2.8-kb region of the *GATA-2* domain in G1E cells (26), binding has not been examined in other cell contexts. Quantitative ChIP analysis revealed GATA-2 binding to the −2.8-kb region in FOG-1$^{-/-}$ cells, but not to the 1S and 1G promoters, identical to that seen in G1E cells (Fig. 1*D*). Thus, despite the occupancy of the −2.8-kb region by GATA-2 in FOG-1$^{-/-}$ cells and the functional interaction between GATA-2 and FOG-1 (27), FOG-1 is not required for *GATA-2* transcription. Moreover, quantitative ChIP analysis was used to define the patterns of acetylated histones H3 (acH3) and H4 (acH4) and H3 methylated at lysine 4 (H3-meK4) at the *GATA-2* domain in FOG-1$^{-/-}$ versus G1E cells. We previously showed that the pattern in G1E cells is diagnostic of the transcriptionally active state (26). The *GATA-2* domain in FOG-1$^{-/-}$ and G1E cells had indistinguishable patterns (Fig. 7, which is published as supporting information on the PNAS web site). These results are consistent with the finding that a knock-in of a GATA-2 mutant defective in FOG-1 binding in a GATA-2-null background supports normal steady-state hematopoiesis (27).

FOG-1 Is Critical for the GATA Switch That Represses *GATA-2* Transcription. A V205G mutant of GATA-1, impaired in FOG-1 binding but retaining normal DNA binding activity *in vitro*, failed to repress *GATA-2* transcription in the G1E system (12). To address whether elevation of GATA-1 levels can bypass the apparent FOG-1 requirement for repression, we tested whether high-level overexpression of GATA-1 fused to an ER hormone-binding domain (ER–GATA-1) in FOG-$1^{-/-}$ cells represses *GATA-2* transcription. Stable clonal cell lines were derived (FOG-$1^{-/-}$–ER–GATA-1), which express ER–GATA-1 at levels far greater than endogenous GATA-1 (Fig. 2*A*). Tamoxifen treatment of FOG-$1^{-/-}$–ER–GATA-1 cells induced a small decrease in *GATA-2* primary transcripts (≈30% decrease) (Fig. 2*B*). By contrast, tamoxifen-mediated activation of ER–GATA-1 in G1E–ER–GATA-1 cells induced a 91% decrease in *GATA-2* primary transcript levels (Fig. 2*B*) (26). The levels of GATA-2 protein paralleled the transcript levels (Fig. 2*C*). Thus, highly overexpressed ER–GATA-1 is insufficient to silence *GATA-2*, implicating FOG-1 as a mediator of ER–GATA-1-dependent repression of *GATA-2* transcription.

We reasoned that FOG-1 might be required for GATA-1 binding to chromatin, the GATA switch, GATA-1-dependent reduction in histone acetylation, or abrogation of polymerase (Pol) II recruitment. Our previous work implicated the GATA switch as an early event in repression (26). Thus, we examined whether FOG-1 is required for ER–GATA-1 to displace GATA-2 from the −2.8-kb region of the *GATA-2* locus. Quantitative ChIP analysis revealed GATA-1 and GATA-2 occupancy at the −2.8-kb region in FOG-$1^{-/-}$ cells, consistent with the expression of endogenous GATA-1 and GATA-2 in these cells (Fig. 2*D*). High-level overexpression of ER–GATA-1 in the FOG-$1^{-/-}$ cells, at levels greater than ER–GATA-1 in G1E–ER–GATA-1 cells (Fig. 2*A*), had no effect on GATA-1 and GATA-2 occupancy (Fig. 2*D*). Tamoxifen-mediated activation of ER–GATA-1 was accompanied by a small increase in GATA-1 occupancy and essentially no change in GATA-2 occupancy. By comparison, activation of less ER–GATA-1 in the G1E system results in at least a 3- to 4-fold increase in ER–GATA-1 binding and abrogation of GATA-2 binding (26).

GATA factor occupancy was also analyzed at *GATA-1* hypersensitive site (HS)-1, α-globin HS-26, and *aminolevulinate synthase* (*ALAS-2*) intron 8. These functionally important regions contain consensus GATA-1 motifs, which have been implicated in GATA-1-mediated transcriptional regulation (34–38). Endogenous GATA-1 and GATA-2 occupied these sites in FOG-$1^{-/-}$ cells (Fig. 2*E*). ER–GATA-1 activation had little or no effect on GATA-1 and GATA-2 occupancy.

Because the GATA switch is a proximal event in GATA-1-mediated *GATA-2* repression, disruption of the switch should abrogate subsequent events in the repression mechanism. To test this prediction, we asked whether ER–GATA-1 overexpression in FOG-$1^{-/-}$ cells induces a domain-wide reduction in histone acetylation. Comparison of the patterns of acetylated histones H3 and H4 and H3-meK4 at the *GATA-2* locus in FOG-$1^{-/-}$ cells, with or without activated ER–GATA-1, revealed no differences in the modifications (Fig. 8, which is published as supporting information on the PNAS web site). Thus, molecular events instigated by the GATA switch are defective, because FOG-1 is required for the switch.

The experiments described above involved comparative analyses in FOG-$1^{-/-}$ cells (13) and FOG-1-expressing G1E cells (33). It was critical to determine whether defects observed in FOG-$1^{-/-}$ cells can be rescued via reintroduction of FOG-1. Retroviral-mediated expression of FOG-1 in the FOG-$1^{-/-}$ cells induces differentiation over a time course of 5 days, with day 0 representing 2 days after infection (13). Because the FOG-1 retroviral expression vector is bicistronic with an internal ribosome entry site controlling GFP expression, FACS can be used to isolate FOG-1-expressing cells 2 days after infection. No significant accumulation of benzidine positive cells is apparent at this time (13), indicating that terminal differentiation has not occurred.

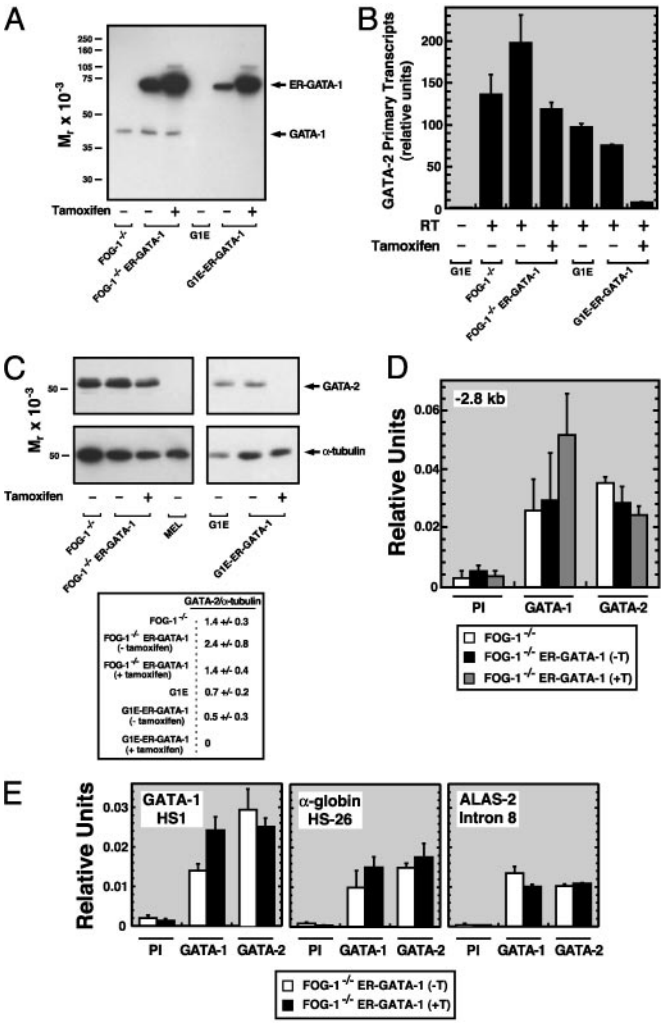

Fig. 2. High-level overexpression of ER–GATA-1 does not efficiently repress *GATA-2* transcription in FOG-$1^{-/-}$ cells. (*A*) Western blot analysis of GATA-1 and ER–GATA-1 expression in whole cell lysates from untreated and tamoxifen-treated (1 μM, 24 h) FOG-$1^{-/-}$, FOG-$1^{-/-}$–ER–GATA-1, G1E, and G1E–ER–GATA-1 cells. (*B*) Relative expression of *GATA-2* primary transcripts expressed from both 1S and 1G promoters. Quantitative real-time RT-PCR was used to measure relative *GATA-2* primary transcript levels, which were normalized by the levels of GAPDH transcripts (mean ± SEM, three independent experiments). (*C*) (*Upper*) Western blot analysis of GATA-2 in whole cell lysates from the same samples as those analyzed by RT-PCR. Lysates from DMSO-induced mouse erythroleukemia cells were used as a negative control for GATA-2 expression. Blots were probed with anti-GATA-2 antibody and then stripped and reprobed with anti-α-tubulin antibody. A representative blot of GATA-2 and α-tubulin is shown. (*Lower*) The GATA-2/α-tubulin ratios, which were quantitated via densitometric analysis (mean ± SEM of three independent experiments). (*D*) Quantitative ChIP analysis of GATA-1 and GATA-2 binding to the −2.8 kb region of *GATA-2* locus in untreated and tamoxifen-treated (1 μM, 24 h) FOG-$1^{-/-}$ and FOG-$1^{-/-}$-ER–GATA-1 cells (mean ± SEM, five independent experiments). (*E*) Quantitative ChIP analysis of GATA-1 and GATA-2 binding to *GATA-1* HS1, α-globin HS-26, and *ALAS-2* intron 8 in untreated and tamoxifen-treated (1 μM, 24 h) FOG-$1^{-/-}$ and FOG-$1^{-/-}$-ER–GATA-1 cells (mean ± SEM of three independent experiments).

The mRNA was detected in cells infected with the FOG-1 retrovirus (Fig. 3*A*). FOG-1 expression strongly reduced *GATA-2* primary transcripts (Fig. 3*B*) and abrogated GATA-2 expression (Fig. 3*C*). GATA-1 levels increased ≈3-fold (Fig. 3*C*), which was considerably lower than the level of ER–GATA-1 in the

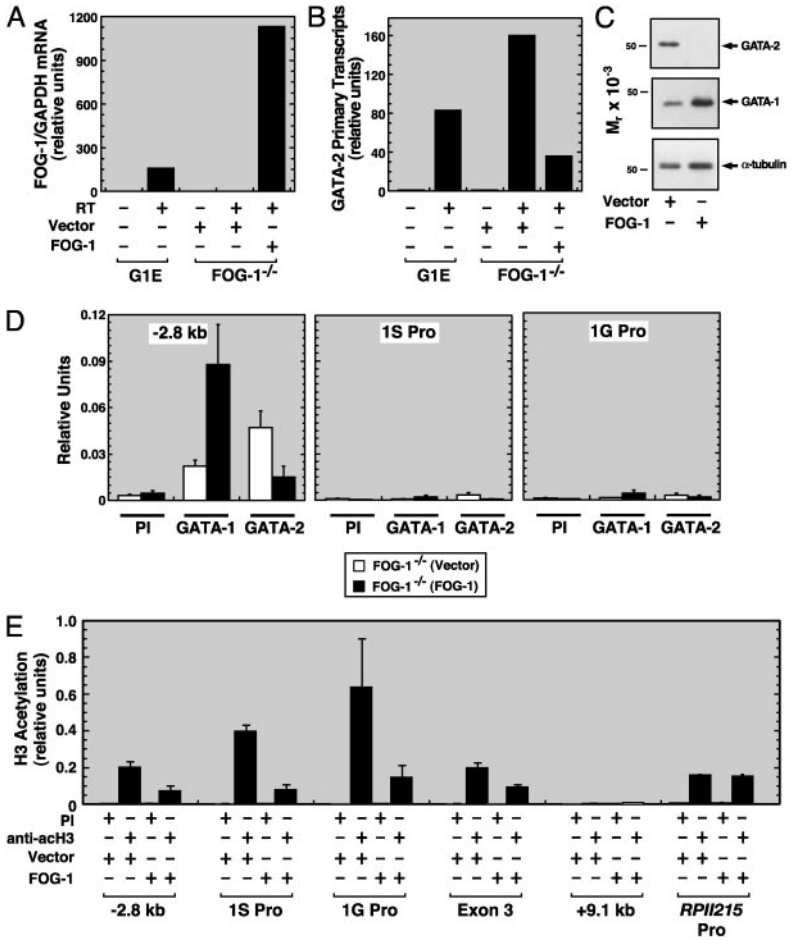

Fig. 3. FOG-1 is required for the GATA-switch, for broad histone deacetylation, and for repression of *GATA-2* transcription. (*A*) Quantitative real-time RT-PCR analysis of *FOG-1* mRNA expression in G1E cells and in FOG-1$^{-/-}$ cells infected with empty or FOG-1-expressing retroviral vectors. Relative expression levels were normalized by GAPDH expression (mean ± SEM of two independent experiments) and plotted with respect to the values of control G1E cell samples lacking RT. (*B*) Quantitative RT-PCR analysis of *GATA-2* primary transcripts in G1E cells, FOG-1$^{-/-}$ cells containing empty vector, and FOG-1 rescued FOG-1$^{-/-}$ cells (mean from two independent experiments). (*C*) Western blot analysis of GATA-2 (*Top*) and GATA-1 (*Middle*) protein levels. Whole cell lysates from FOG-1$^{-/-}$ cells infected with empty or FOG-1-expressing retrovirus were subjected to Western blot analysis with anti-GATA-2 or anti-GATA-1 antibodies. Blots were stripped and reprobed with anti-α-tubulin antibody. A representative Western blot of α-tubulin is shown (*Bottom*). (*D*) Quantitative ChIP analysis of GATA-1 and GATA-2 binding to the *GATA-2* locus in FOG-1$^{-/-}$ cells infected with empty or FOG-1-expressing retrovirus (mean ± SEM of five independent experiments). (*E*) Quantitative ChIP analysis of histone H3 acetylation at the *GATA-2* locus and the *RPII215* promoter after infection of FOG-1$^{-/-}$ cells with empty or FOG-1-expressing retrovirus (mean ± SEM of three independent experiments).

FOG-1$^{-/-}$–ER–GATA-1 cells (Fig. 2*A*). Importantly, the much higher expression of ER–GATA-1 in FOG-1$^{-/-}$–ER–GATA-1 cells resulted in only a small increase in ER–GATA-1 binding and did not induce the GATA switch or repress *GATA-2* transcription. By contrast, quantitative ChIP analysis of FOG-1$^{-/-}$ cells infected with the FOG-1 retrovirus revealed a 4-fold increase in GATA-1 binding with a concomitant 3-fold decrease in GATA-2 binding to the −2.8-kb region (Fig. 3*D*). No binding was detected at the 1S and 1G promoters. Thus, FOG-1 expression rescued the GATA switch and *GATA-2* repression in FOG-1$^{-/-}$ cells, demonstrating a FOG-1 requirement for the replacement of endogenous GATA-2 by endogenous GATA-1 at the −2.8-kb region. GATA factor binding was not absolutely FOG-1 dependent, however, as endogenous GATA-1 and GATA-2 binding was detected in FOG-1$^{-/-}$ cells.

Based on the bimodal repression model (26), the GATA switch precedes a domain-wide reduction in histone acetylation. We examined the acetylation state of sites within the *GATA-2* locus in FOG-1$^{-/-}$ cells infected with a control retrovirus versus a FOG-1-expressing retrovirus (Fig. 3*E*). Reductions in histone H3 acetylation were detected at the −2.8-kb region, the 1S and 1G promoters, and at exon 3, whereas hypoacetylation at a site 9.1 kb downstream of *GATA-2* remained unchanged. Enriched histone H3 acetylation at the constitutively active promoter of *RPII215*, which encodes the large subunit of RNA polymerase II, was unchanged. These results show that FOG-1 expression in the FOG-1$^{-/-}$ cells rescues both the GATA switch and the domain-wide reduction in histone acetylation, the defining steps of the bimodal repression model.

Facilitation of Chromatin Occupancy by GATA-1 Is a General Function of FOG-1. To determine whether FOG-1 facilitates GATA-1 occupancy at other loci, we measured endogenous GATA factor occupancy in FOG-1$^{-/-}$ cells infected with empty or FOG-1-expressing retroviruses. We also measured ER–GATA-1 and GATA-2 occupancy in untreated and tamoxifen-treated G1E–ER–GATA-1 cells. FOG-1 expression in FOG-1$^{-/-}$ cells and tamoxifen treatment of G1E–ER–GATA-1 cells induced α-globin and *ALAS-2* transcripts (Fig. 4*A*). Similarly, FOG-1 expression in FOG-1$^{-/-}$ cells and tamoxifen treatment of G1E–ER–GATA-1 cells induced GATA-1 occupancy and GATA-2 displacement at *GATA-1* HS1, α-globin HS-26, and *ALAS-2* intron 8 (Fig. 4*B*). Thus, FOG-1 is required for GATA-1 to access chromatin sites bound by GATA-2.

Because high-level ER–GATA-1 overexpression did not displace GATA-2 from chromatin (Fig. 2 *D* and *E*), it is highly unlikely that the FOG-1-dependent ≈3-fold induction of GATA-1 generates sufficient levels of GATA-1 to displace GATA-2. If FOG-1 directly mediates the GATA switch, one would predict that FOG-1 would localize to the switch site. Quantitative ChIP was conducted with the anti-FOG-1 antibody used in Fig. 1*A* to immunoprecipitate endogenous FOG-1. FOG-1 was crosslinked solely to the −2.8-kb region of the *GATA-2* locus in G1E and in tamoxifen-treated G1E–ER–GATA-1 cells, in which *GATA-2* is transcriptionally active and inactive, respectively (Fig. 5*A*). No crosslinking was detected in FOG-1$^{-/-}$ cells. Because GATA-2 occupied the −2.8-kb region in the active state, the results suggest that GATA-2 recruits FOG-1. FOG-1 occupancy was also detected in G1E cells at *GATA-1* HS1, α-globin HS-26, and *ALAS-2* intron 8, but not at the neural-specific *Necdin* promoter (Fig. 5*B*). Ectopically expressed FOG-1 in FOG-1$^{-/-}$ cells occupied sites identical to endogenous FOG-1 and did not occupy the *GATA-2* 1S and the *necdin* promoters (Fig. 5*C*). The association of FOG-1 at the GATA switch sites provides strong evidence that FOG-1 directly mediates the displacement of GATA-2 by GATA-1.

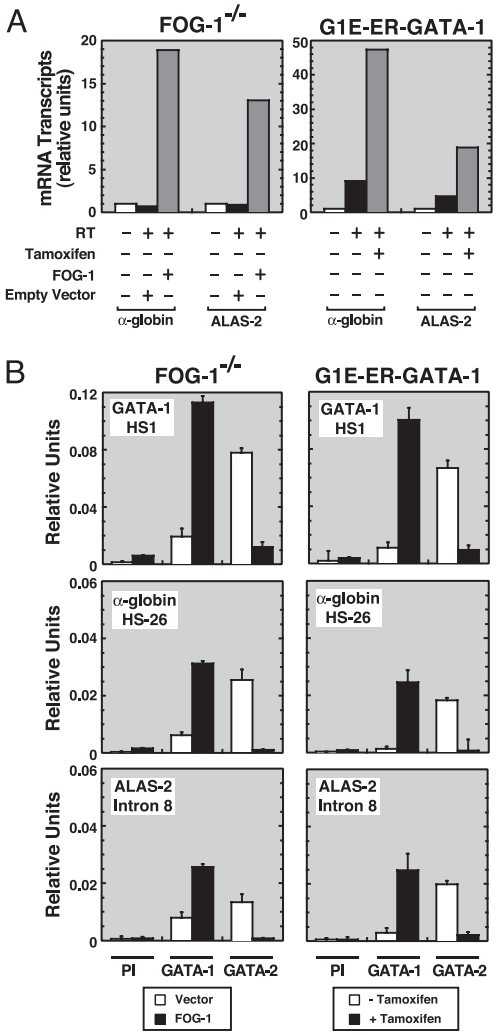

Fig. 4. FOG-1 is required for GATA switches at the *GATA-1*, α-globin, and *ALAS-2* loci. (*A*) Quantitative RT-PCR analysis of α-globin and ALAS-2 mRNA transcripts in FOG-1$^{-/-}$ cells infected with an empty or FOG-1-expressing retrovirus and in untreated and tamoxifen-treated (10 h) G1E–ER–GATA-1 cells (mean of two independent experiments) (*B*) Quantitative ChIP analysis of GATA-1 and GATA-2 binding in FOG-1$^{-/-}$ cells infected with an empty or FOG-1-expressing retrovirus and in untreated and tamoxifen-treated G1E–ER–GATA-1 cells (mean ± SEM of three independent experiments).

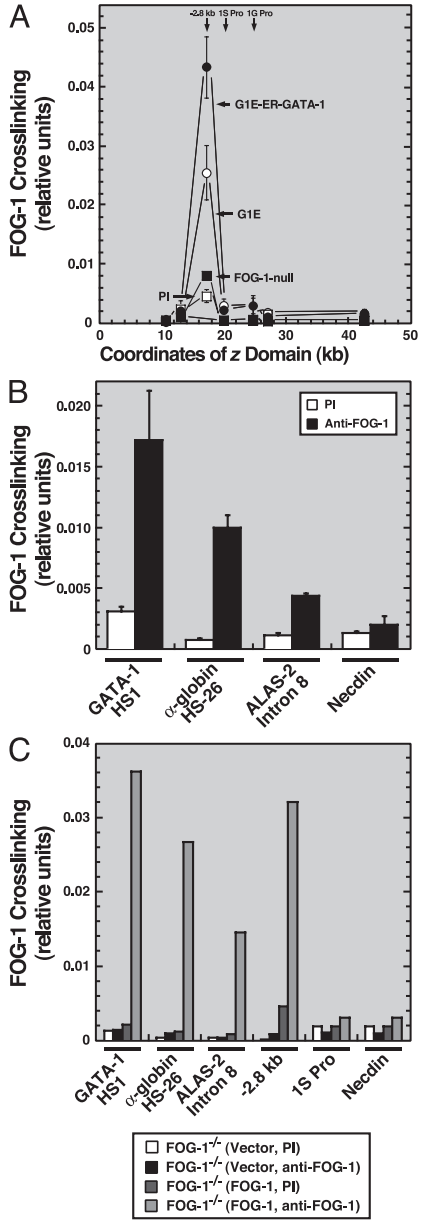

Fig. 5. FOG-1 occupies sites in which the FOG-1-dependent GATA switches occur. Quantitative ChIP analysis was conducted with anti-FOG-1 antibody in FOG-1$^{-/-}$, G1E, and tamoxifen-treated G1E–ER–GATA-1 cells. (*A*) The graph depicts the pattern of endogenous FOG-1 crosslinking at various sites of the *GATA-2* locus (mean ± SEM of four independent experiments). The positions of the −2.8 kb, 1S promoter, and 1G promoter amplicons are shown by arrows at the top. (*B*) Occupancy of chromatin sites by endogenous FOG-1. FOG-1 crosslinking in G1E cells was detected at *GATA-1* HS1, α-globin HS-26, *ALAS-2* intron 8, but not at the *Necdin* promoter (mean ± SEM of three independent experiments). (*C*) Occupancy of chromatin sites by ectopically expressed FOG-1. FOG-1$^{-/-}$ cells were infected with an empty or FOG-1-expressing retrovirus, and FOG-1 occupancy was measured by quantitative ChIP.

The Chromatin Occupancy Facilitator (COF) Paradigm. Although many examples exist in which DNA-bound factors recruit coregulators that directly induce chromatin modification, we are unaware of situations whereby a coregulator facilitates chromatin occupancy by the recruiting trans-acting factor. These are not mutually exclusive mechanisms, as a coregulator that facilitates chromatin occupancy by the recruiting factor might do so via local chromatin modification. However, this mechanism has not been reported. We describe herein experiments demonstrating that GATA-1 occupancy of chromatin sites bound by GATA-2 is facilitated by FOG-1. We propose that the interaction of GATA-1 with FOG-1 tethers GATA-1 to the chromatin with an affinity considerably higher than that endowed by the equilibrium binding constant of the GATA-1–WGATAR interaction. One can envision two modes in which COF activity is conferred. GATA-1 might encounter FOG-1 at the chromatin template, forming a complex that expels GATA-2 (Fig. 6*A*). Alternatively, a GATA-1–FOG-1 complex formed before recruitment might displace a GATA-2–FOG-1 complex (Fig. 6*B*).

Why is FOG-1 required to facilitate chromatin binding by GATA-1? Chicken GATA-1 forms a stable complex with a reconstituted nucleosome containing six GATA motifs *in vitro* (39), although the binding affinity is reduced relative to the naked DNA template. Furthermore, GATA-4 binds and regulates the structures of a reconstituted nucleosome array in which the albumin enhancer containing GATA motifs and a minimal promoter were flanked by five copies of sea urchin 5S rDNA sequences (40). Thus, it appears that GATA factors do not completely lack the ability to access

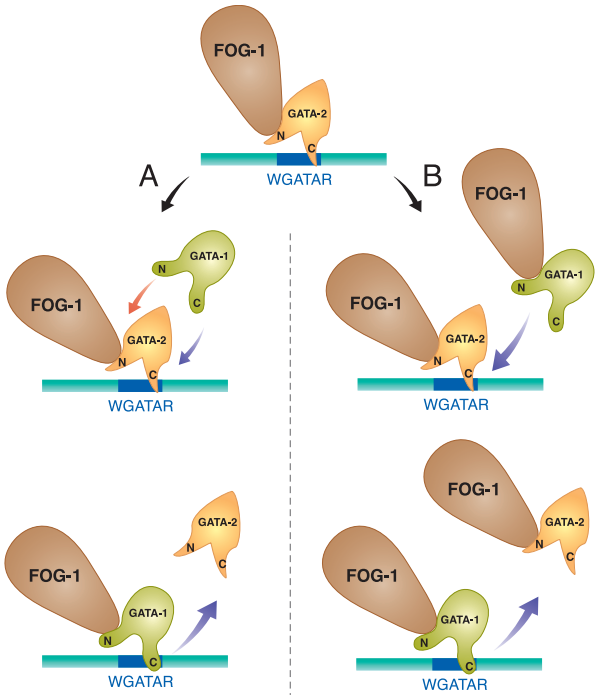

Fig. 6. Model of chromatin occupancy facilitator activity of FOG-1. FOG-1 colocalizes with GATA-2 at chromatin sites containing WGATAR motifs. FOG-1 facilitate chromatin occupancy of GATA-1 at such sites. Model A assumes that GATA-1 encounters a GATA-2–FOG-1 complex at the chromatin template. Via interactions between the N-terminal zinc finger of GATA-1 and FOG-1, GATA-1 displaces GATA-2 from the chromatin site. Model B assumes that a GATA-1–FOG-1 complex encounters a GATA-2–FOG-1 complex at the chromatin template. Following a complex switch, the GATA-1–FOG-1 complex would stably occupy the chromatin site.

chromatin sites *in vitro*. However, endogenous GATA-1 in nuclear extracts cannot stably associate with the −2.8-kb region reconstituted into a mononucleosome (S.P. and E.H.B., unpublished data), indicating that GATA-1 cannot readily access all nucleosomal sites.

Because DNA binding is often necessary but insufficient for conferring transcriptional control, COF activity might be coupled with traditional coregulator activities, which collectively activate or repress transcription. However, no such activities have been identified for FOG-1. Major efforts involving *in vitro* DNA binding assays with naked DNA to determine whether FOG-1 has DNA binding activity or whether it modulates the affinity or specificity of GATA-1 DNA binding have not yielded positive results. Fingers 2–4 of FOG-1 have low-affinity DNA binding activity, but DNA binding has not been demonstrated with intact FOG-1 (A. Tsang and S.H.O., unpublished data). It is therefore unlikely that COF activity can be explained by the enhanced affinity or altered specificity of GATA-1 binding to naked DNA. It is conceivable that one or more of the five C_2HC and four C_2H_2 zinc fingers of FOG-1 contact DNA *in vivo*, because the prototypical nine C_2H_2 zinc finger protein transcription factor IIIA (TFIIIA) binds a ≈40-bp DNA sequence of the 5S ribosomal RNA gene internal control region (41, 42). Other multizinc finger proteins, such as MyT1 (43), promyelocytic leukemia zinc finger (PLZF) (44), and neuron-restrictive silencer factor/repressor element-1 silencing factor (NRSF/REST) (45), containing six, nine, and nine, zinc fingers, respectively, also have sequence-specific DNA binding activity.

Is COF activity unique to FOG-1, or is it common to coregulators? Because chromatin occupancy by trans-acting factors has not been examined in cells lacking cognate coregulators, one can only speculate in this regard. It will be of considerable interest to determine whether FOG-1 directly contacts DNA *in vivo*, anchoring GATA-1 to a GATA motif, and whether COF activity collaborates with distinct biochemical functions of FOG-1 or an associated factor to control hematopoiesis.

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