Building multifunctionality into a complex containing master regulators of hematopoiesis

Tohru Fujiwara[1], Hsiang-Ying Lee[1], Rajendran Sanalkumar[1], and Emery H. Bresnick[2]

Wisconsin Institutes for Medical Research, University of Wisconsin Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705

Edited by Stuart H. Orkin, Children's Hospital and The Dana Farber Cancer Institute, Harvard Medical School and The Howard Hughes Medical Institute, Boston, MA, and approved October 14, 2010 (received for review June 3, 2010)

Developmental control mechanisms often use multimeric complexes containing transcription factors, coregulators, and additional non-DNA binding components. It is challenging to ascertain how such components contribute to complex function at endogenous loci. We analyzed the function of components of a complex containing master regulators of hematopoiesis (GATA-1 and Scl/TAL1) and the non-DNA binding components ETO2, the LIM domain protein LMO2, and the chromatin looping factor LDB1. Surprisingly, we discovered that ETO2 and LMO2 regulate distinct target-gene ensembles in erythroid cells. ETO2 commonly repressed GATA-1 function via suppressing histone H3 acetylation, although it also regulated methylation of histone H3 at lysine 27 at select loci. Prior studies defined multiple modes by which GATA-1 regulates target genes with or without the coregulator Friend of GATA-1 (FOG-1). LMO2 selectively repressed genes that GATA-1 represses in a FOG-1–independent manner. As LMO2 controls hematopoiesis, its dysregulation is leukemogenic, and its influence on GATA factor function is unknown, this mechanistic link has important biological and pathophysiological implications. The demonstration that ETO2 and LMO2 exert qualitatively distinct functions at endogenous loci illustrates how components of complexes containing master developmental regulators can impart the capacity to regulate unique cohorts of target genes, thereby diversifying complex function.

blood | hematopoietic | genetic network | leukemia | progenitor cell

The regulatory machinery orchestrating development includes multimeric protein complexes containing transcription factors, coregulators, and other non-DNA binding proteins. Although a single complex can contain multiple enzymatic activities, including chromatin remodeling and modifying activities, and components capable of engaging in diverse protein-protein interactions, it is challenging to elucidate the contribution of the individual components to complex function at endogenous loci.

In the context of hematopoiesis, in which stem cells differentiate into progenitors and diverse blood-cell types (1), major progress has been made in defining the transcriptional drivers and their complexes. These drivers include the GATA factors, three of which (GATA-1 to -3) control hematopoiesis (2). GATA-1 regulates the differentiation of red blood cells, platelets, mast cells, and eosinophils (3–7). GATA-2 regulates the genesis and survival of hematopoietic stem cells (HSCs) and multipotent progenitors (8), whereas GATA-3 controls lymphopoiesis (9).

The canonical mechanism by which GATA-1 activates and represses transcription involves binding to the coregulator Friend of GATA-1 (FOG-1) (10). FOG-1 has nine zinc fingers, with four implicated in GATA-1 binding (11). Although FOG-1 does not appear to contact DNA, it facilitates GATA-1 chromatin occupancy at select sites (12, 13), GATA-1–mediated displacement of GATA-2 from chromatin (GATA switches) (12), and chromatin looping (14). FOG-1 binds NuRD (15) and CtBP corepressor complexes (16, 17), and NuRD binding mediates certain FOG-1 functions (18, 19). GATA-1 also activates and represses targets in a FOG-1–independent manner (20, 21). Because this poorly understood mechanism controls critical genes, including the HSC regulator LYL1 and the red cell cytoskeletal protein band 4.9 (21), it is important to elucidate the underlying mechanisms.

GATA-1 forms a complex with another master regulator of hematopoiesis, the basic-helix–loop–helix transcription factor Scl/TAL1 (22). Scl/TAL1 regulates HSC function, erythropoiesis, and is implicated in leukemogenesis (23–25). In vitro DNA binding results indicate that the GATA-1-Scl/TAL1 complex includes its heterodimeric partner E2A and the non-DNA binding components LMO2 and LDB1 (22). The LIM domain protein LMO2 is leukemogenic in humans (26). Retroviral vectors used for gene therapy of X-linked combined immunodeficiency syndrome commonly integrate into *LMO2*, activating its expression and inducing T-cell leukemia (26). LDB1 bears similarity to *Drosophila* Chip, both implicated in mediating transcriptional control over a long distance on a chromosome (27, 28). Additional interactors with the GATA-1-Scl/TAL1 complex include the non-DNA binding component ETO2 (29, 30), which binds histone deacetylases (HDACs) and participates in a chromosomal translocation in acute myeloid leukemia (31). Single-stranded DNA binding proteins 2 and 3 (32) and the chromatin remodeler BRG1 (33) also bind the GATA-1-Scl/TAL1 complex.

GATA-1 colocalizes with Scl/TAL1 at chromatin sites harboring a GATA motif and an E-box, recognized by Scl/TAL1-E2A heterodimers, or solely a GATA motif (34–36). Before GATA-1–mediated displacement of GATA-2 from chromatin, GATA-2 colocalizes with Scl/TAL1 (34). Thus, GATA-2 may also assemble a complex with Scl/TAL1. Many questions remain unanswered regarding the functions of the GATA-Scl/TAL1 complex components, and the activities bestowed on the complex by these components. Given the GATA-1-Scl/TAL1 paradigm, it is instructive to consider the consequences of perturbing individual constituents of the complex. Interfering with any component might elicit similarly devastating consequences. Alternatively, the components might function uniquely, and individual perturbations would therefore yield qualitatively distinct phenotypes. Herein, we test these models by knocking-down ETO2 and LMO2 in a genetic complementation assay in GATA-1–null cells. These studies identified striking differences in the ETO2- and LMO2-regulated target-gene ensembles and important mechanistic differences, which highlight how master regulators of development coinhabiting the same complex exert qualitatively distinct activities.

Results and Discussion

Discriminatory Functions of a Non-DNA Binding Component of a Complex Containing Master Regulators of Hematopoiesis. The contribution of non-DNA binding components of the GATA-1-Scl/TAL1 complex to activity of the complex is not well defined. We analyzed the role of

Author contributions: T.F., H.-Y.L., R.S., and E.H.B. designed research; T.F., H.-Y.L., and R.S. performed research; T.F., H.-Y.L., R.S., and E.H.B. analyzed data; and T.F., H.-Y.L., R.S., and E.H.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: Gene array datasets have been deposited in the GEO database, www.ncbi.nlm.nih.gov/geo (accession no. GSE24359).

[1]T.F., H.-Y.L., and R.S. contributed equally to this work.

[2]To whom correspondence should be addressed. E-mail: ehbresni@wisc.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1007804107/-/DCSupplemental.

GENETICS

one of these components, ETO2, in GATA-1 activity using GATA-1–null G1E cells (37) stably expressing GATA-1 fused to the estrogen receptor ligand-binding domain (G1E-ER-GATA-1 cells) (38, 39). We tested whether reducing ETO2 levels influences the capacity of ER-GATA-1 to regulate target genes. After transfection of G1E-ER-GATA-1 cells with anti-*Cbfa2t3* [*Cbfa2t3* encodes ETO2 (40)] siRNA, ER-GATA-1 was activated with β-estradiol (Fig. 1*A*). *Cbfa2t3* mRNA (Fig. S1*A*) and protein (Fig. S1*B*, *Upper*) expression strongly decreased, and ER-GATA-1 levels were unaltered (Fig. S1*B*, *Lower*).

We compared expression profiles in β-estradiol-induced G1E-ER-GATA-1 cells transfected with control and anti-*Cbfa2t3* siRNA. This analysis revealed altered expression of established GATA-1 targets and those not known to be GATA-1-regulated (Fig. 1*B*). The ETO2-regulated genes included those in which ER-GATA-1–mediated activation (Fig. 1*C*) and repression (Fig. 1*D*) were significantly enhanced by the knockdown ($P < 0.05$). An additional cohort of GATA-1 targets was unaffected (Fig. 1*E*). The reduction in ETO2 levels therefore derepressed ER-GATA-1 activity, increasing its activation and repression of targets. ETO2 occupied GATA switch sites bound by GATA-2 (Fig. S2*E*) and GATA-1 (Fig. S2*A*) in uninduced and induced G1E-ER-GATA-1 cells, respectively, at representatives of this group (Fig. S2*C*), indicating a direct function at these loci. As certain GATA-1 targets were unaffected, ETO2 mediates GATA-1 function in a context-dependent manner.

The gene exhibiting the greatest sensitivity to ETO2 knockdown was *Slc4a1* (Fig. 1*C*, *Left*), a GATA-1 target gene encoding an anion exchanger with pivotal roles in red cells (41). GATA-1 (Fig. S3*B*) and ETO2 (Fig. S3*C*) occupies the *Slc4a1* promoter and intron 1 (+1.4 kb) (Fig. S3*A*) and regulates *Slc4a1* transcription in a FOG-1–dependent manner (36, 42), but other aspects of this transcriptional mechanism are unknown.

To establish how knocking-down ETO2 renders *Slc4a1* hypersensitive to ER-GATA-1, we analyzed the epigenetic landscape at *Slc4a1* in untreated and β-estradiol–treated G1E-ER-GATA-1 cells. Histone H3 di-acetylated at K9/K14 was considerably higher at sites of ER-GATA-1 occupancy, whereas tetra-acetylated histone H4 was distributed throughout the locus. ER-GATA-1–mediated activation of *Slc4a1* transcription was accompanied by increased H3-diacK9/K14 and tetraacetylated H4 (Fig. S3 *D* and *E*). Analogous to H3-diacK9/K14, H3-dimeK4 was highest at ER-GATA-1 occupancy sites and increased upon activation, albeit not at the promoter (Fig. S3*F*). Two marks associated with repression, H3-trimeK9 and H3-trimeK27 (43), were unusually high (comparable or higher than the inactive *Necdin* promoter) at the inactive and active *Slc4a1* locus (Fig. S3 *G* and *H*). The presence of active and repressive marks resembles bivalent chromatin domains,

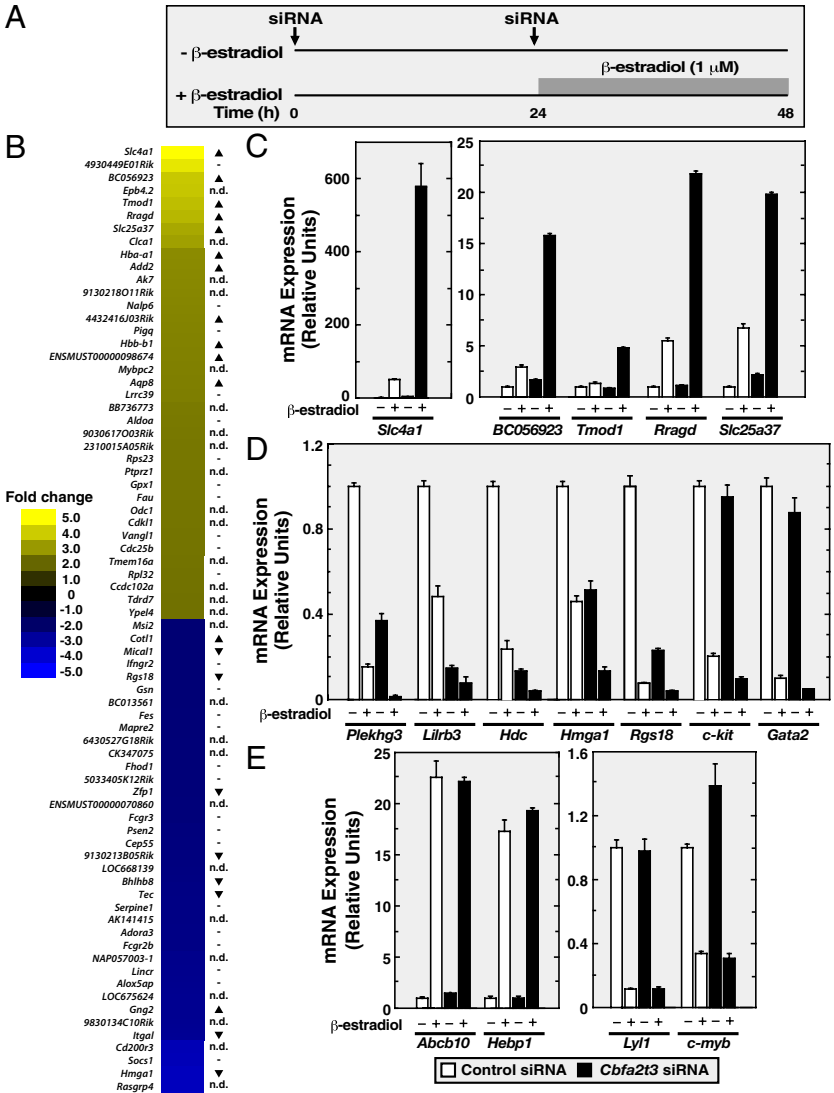

Fig. 1. ETO2 controls a small subset of GATA-1 target genes. (*A*) Experimental strategy for siRNA-mediated knockdown of *Cbfa2t3* in G1E-ER-GATA-1 cells. (*B*) Profiles were compared in β-estradiol-treated G1E-ER-GATA-1 cells transfected with control or *Cbfa2t3* siRNA. The heat map depicts the mean fold-change resulting from ETO2 knockdown ($n = 2$). The GATA-1 responsiveness in G1E-ER-GATA-1 cells, based on prior array analyses (36, 56), is indicated on the right: ▲, GATA-1 activated; ▼, GATA-1-repressed; –, not GATA-1 regulated; n.d., expression not detected. (*C–E*) Quantitative RT-PCR validation of array results. (*C*) *Cbfa2t3* siRNA-activated genes; (*D*) *Cbfa2t3* siRNA-repressed genes; (*E*) *Cbfa2t3* siRNA-insensitive genes (mean ± SE, $n = 4$).

which may reflect a state in which the gene is poised to be activated (44), but such domains have not been described in erythroid cells.

We asked whether the ETO2 knockdown altered all or specific components of this nucleoprotein architecture in untreated and β-estradiol–treated G1E-ER-GATA-1 cells. In untreated cells, knocking-down ETO2 significantly, but modestly, increased ER-GATA-1 (Fig. 2*A*) and Scl/TAL1 (Fig. 2*B*) occupancy at the promoter and the +1.4-kb site. ER-GATA-1 (Fig. 2*A*) and Scl/TAL1 (Fig. 2*B*) occupancy was unaffected in β-estradiol–treated cells. The knockdown significantly increased H3-diacK9/K14 at the promoter and intron 1, without influencing this mark at the active and inactive *RPII215* and *Necdin* promoters, respectively (Fig. 2*C*). Whereas the knockdown did not affect H3-dimeK4 (Fig. 2*D*) and H3-trimeK9 (Fig. 2*E*) at *Slc4a1* and other targets, in the presence of active ER-GATA-1, the knockdown strongly reduced H3-trimeK27 at the promoter and intron 1, without affecting this mark at control promoters (Fig. 2*F*). Although ETO2 regulation of histone modifications had not been described, given its binding to HDACs (45), increased H3-diacK9/K14 is consistent with this mechanism. In contrast, its regulation of H3-trimeK27 was surprising. It is attractive to consider that high-level H3-trimeK27 might uniquely characterize ETO2-regulated GATA-1 targets. Although ETO2-regulated targets (*BC056923*, *Tmod1*, and *Rragd*), but not the nonresponsive target (*Abcb10*), have high-level H3-trimeK27 (Fig. S4), ETO2 did not regulate H3-trimeK27 at all targets (Fig. 2*F*, *Right*). ETO2 suppressed H3-diacK9/K14 at all responsive targets, but not the nonresponsive targets (Fig. 2*C*, *Right*). ETO2 also suppressed ER-GATA-1–mediated repression at select loci, and this action at *Plekhg3* involved suppressing H3-trimeK27 and maximizing H3-dimeK4 (Fig. S5). Thus, ETO2-dependent transcriptional repression involves a widely used epigenetic mechanism to suppress H3-diacK9/K14 and a locus-selective action to regulate H3-trimeK27 (Fig. 2*G*).

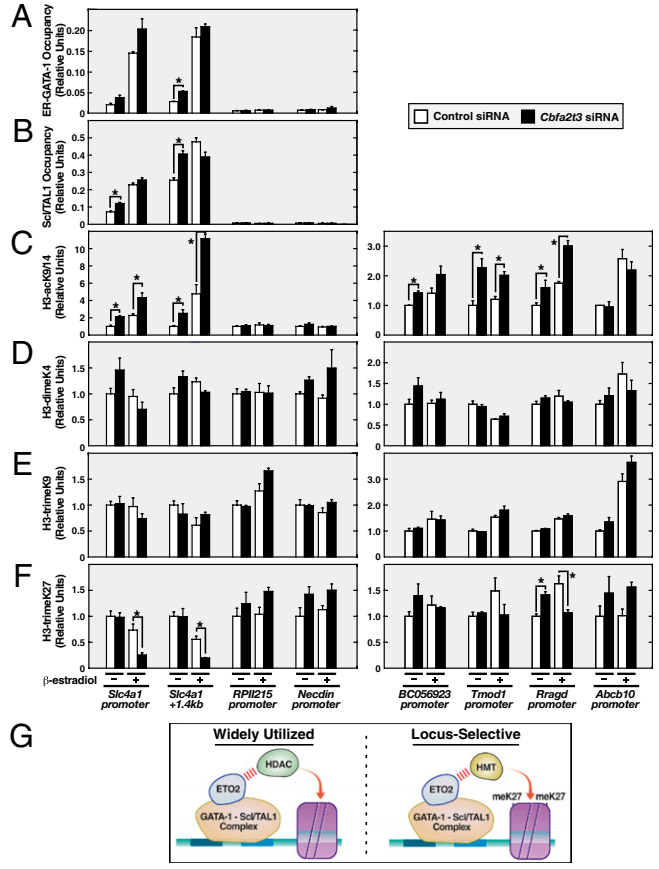

Fig. 2. ETO2-dependent epigenetic mechanisms. (*A*–*F*) Quantitative ChIP analysis at ETO2-regulated genes (*Slc4a1*, *BC056923*, *Tmod1*, and *Rragd*) and ETO2-insensitive genes (*Abcb10*, *RPII215*, and *Necdin*) in G1E-ER-GATA-1 cells transfected with control and *Cbfa2t3* siRNA: GATA-1 (*A*), Scl/TAL1 (*B*), acetyl H3 (*C*), H3-dimeK4 (*D*), H3-trimeK9 (*E*), and H3-trimeK27 (*F*). For histone modifications (*C*–*F*), the result of uninduced G1E-ER-GATA-1 cells transfected with control siRNA was set to 1.0 (mean ± SE, $n = 3$). $*P < 0.05$. (*G*) Models depicting the broadly used mechanism in which ETO2 suppresses H3-diacK9/K14 near the ETO2-associated GATA-1-Scl/TAL1 complex and the locus-selective mechanism at *Slc4a1* in which ETO2 confers high-level H3-trimeK27. HDAC, histone deacetylase; HMT, histone methyltransferase. Preimmune values did not exceed 0.0035.

GATA-1-Scl/TAL1 Complex Structure/Function: Qualitatively Distinct Mechanistic and Biological Functions of Individual Complex Components. Although ETO2, LMO2, and LDB1 are components of GATA-1-Scl/TAL1 complexes (22, 29, 30, 46), whether these components function as a unit or independently to regulate GATA-1 target genes is unknown. We knocked-down LMO2 mRNA (Fig. S1*C*) and protein (Fig. S1*D*, *Upper*), which did not alter ER-GATA-1 levels (Fig. S1*D*, *Lower*), and conducted expression profiling to establish the LMO2-regulated gene ensemble (Fig. 3*A*). Surprisingly, the knockdown significantly up-regulated all but five of the LMO2-regulated genes, indicating an unexpected predominant repressive function of LMO2. As LMO2 overexpression inhibited erythropoiesis, our loss-of-function analysis provides a conceptual framework for understanding this result (47). Genes encoding prototypical erythroid proteins (e.g., heme biosynthetic enzymes and red cell cytoskeletal proteins) were conspicuously absent from this gene ensemble. LMO2 targets included genes shown previously to be FOG-1–independent, GATA-1–repressed genes (21), including *Lyl1*, *Rgs18*, *Clec4d*, *Vim*, *Sept9*, *Lgals9*, and *Klf10* (Fig. 3*A*). Because the mechanism underlying FOG-1–independent GATA-1–mediated repression has been elusive, and these targets include the important regulator of HSCs LYL1 (48), we focused on this set of genes.

Quantitative RT-PCR analysis confirmed that the LMO2 knockdown up-regulated FOG-1–independent, GATA-1–repressed genes (*Rgs18*, *Vim*, *Clec4d*, *Lyl1*) (Fig. 3*B*). Even in uninduced G1E-ER-GATA-1 cells before GATA-1 occupancy, the knockdown up-regulated *Rgs18*, *Vim*, *Clec4d*, and *Lyl1* (Fig. 3*B*). In contrast, the ETO2 knockdown decreased *Rgs18* expression (Fig. 1*D*). Although knocking down ETO2 yielded ER-GATA-1–mediated hyperinduction of *Slc4a1* (Fig. 1*C*), knocking-down LMO2 significantly inhibited GATA-1–mediated induction of *Slc4a1* (Fig. 3*C*, *Left*). Although ETO2 and LMO2 exert qualitatively distinct responses, both occupy *Lyl1*, *Rgs18*, and *Slc4a1* (Fig. S2 *C* and *D*). Because LMO2 facilitates ER-GATA-1–mediated repression of FOG-1–independent genes, we tested whether it also affects FOG-1–independent activation of genes. The LMO2 knockdown did not affect ER-GATA-1–mediated induction of the prototypical FOG-1–independent gene *Fog1* nor repression of the prototypical FOG-1–dependent gene *Gata2* (Fig. 3*C*, *Right*).

Because ETO2 and LMO2 knockdowns yielded qualitatively distinct outputs, and both factors bind Scl/TAL1, we asked whether the factor-specific activities are Scl/TAL1-dependent or -independent. If Scl/TAL1 is critical for ETO2 or LMO2 to regulate their gene ensembles, knocking-down Scl/TAL1 should recapitulate the gene dysregulation resulting from ETO2 or LMO2 knockdowns. Knocking-down Scl/TAL1 (Fig. S1 *E* and *F*), which did not alter ER-GATA-1 levels (Fig. S1*F*), significantly reduced ER-GATA-1–mediated activation of *Slc4a1*, resembling the LMO2 knockdown (Fig. 3*C*, *Left*), although the reduction was greater with the Scl/TAL1 knockdown (Fig. 3*D*, *Left*). Also resembling the LMO2 knockdown, knocking-down Scl/TAL1 significantly reduced ER-GATA-1–mediated repression of the FOG-1–independent gene *Lyl1*, but not the FOG-1–dependent gene *Gata2* (Fig. 3*D*, *Right*). As LMO2 and Scl/TAL1 exert qualitatively similar influences on GATA-1 targets, this finding supports the notion that LMO2 and Scl/TAL1 function collectively in the FOG-1–independent GATA-1 repression mechanism. In contrast, knocking-down Scl/

TAL1 did not yield hyperactivation of *Slc4a1* in response to ER-GATA-1 activation. The distinct influences of ETO2 and Scl/TAL1 on the FOG-1–dependent, GATA-1–activated gene *Slc4a1* are inconsistent with ETO2 contributing to a solitary function of the GATA-1-Scl/TAL1 complex.

The different ETO2 and LMO2 mechanisms (Fig. 4*A*) raises the question as to whether the respective gene ensembles mediate similar or distinct biological pathways. Gene Ontology analysis revealed that the majority of ETO2- and LMO2-regulated genes control distinct pathways (Fig. 4*B*). Most ETO2- and LMO2-regulated genes (61 and 138, respectively) differed, but both factors coregulated only 16 genes (Fig. 4*C*). The coregulated genes included four ER-GATA-1–activated (*Hba-a1*, *Hbb-b1*, *Cotl1*, *Gng2*), four ER-GATA-1–repressed (*Mical1*, *Hdc*, *Lilrb3*, *Plekhg3*), and eight not regulated by ER-GATA-1 (*Aldoa*, *Fau*, *Gsn*, *Fes*, *Fcgr3*, *Fcgr2b*, *Alox5ap*, and *Ptprz1*). ETO2 and LMO2 coregulate a minority of GATA-1–regulated and –insensitive genes (Fig. 4*C*). Thus, a comparable reduction in the level of two components of the GATA-1-Scl/TAL1 complex dysregulated distinct gene ensembles, and the genes comprising these ensembles differed considerably vis-à-vis their cellular functions.

LMO2 had not been implicated in FOG-1–independent GATA factor pathways or any other mode of GATA-1 function. We tested whether LMO2 occupies FOG-1–independent, GATA-1–repressed genes, which would imply a direct function at these loci. ER-GATA-1 occupied the *Lyl1* and *Rgs18* promoters, but not the control *Necdin* promoter (Fig. S2*A*). Scl/TAL1 occupied the promoters similarly in uninduced and induced cells (Fig. S2*B*), consistent with its colocalization with GATA-2, before GATA-1 occupancy (34, 36). LMO2 occupancy resembled that of Scl/TAL1 (Fig. S2*D*), and coupled with dysregulated transcription upon LMO2 knockdown, these results indicate that LMO2 functions directly to control these FOG-1–independent, GATA-1–repressed genes.

Multimeric Complexes Containing Master Developmental Regulators: Mechanistic Principles and Biological Implications. Critical developmental regulators that establish and modulate genetic networks often reside in multimeric complexes. Many questions remain unanswered regarding how individual components contribute to complex function. Perhaps the simplest mechanism involves the coordination of the actions of all components of a complex to yield a sole molecular output, such as activation or repression. Individual components may have dedicated functions to mediate recruitment of the complex to factors occupying target genes, to stabilize intracomplex interactions, to catalyze chromatin modification/remodeling, to interact with the transcriptional machinery, or to integrate cell signals. Given these intertwined activities, perturbations of individual components would yield qualitatively similar defects. Alternatively, distinct components may function in unique pathways. Rather than collectively mediating complex recruitment and activity, individual components might selectively mediate recruitment to distinct cohorts of targets. Similarly, components may have intrinsic enzymatic activities essential for the control of select

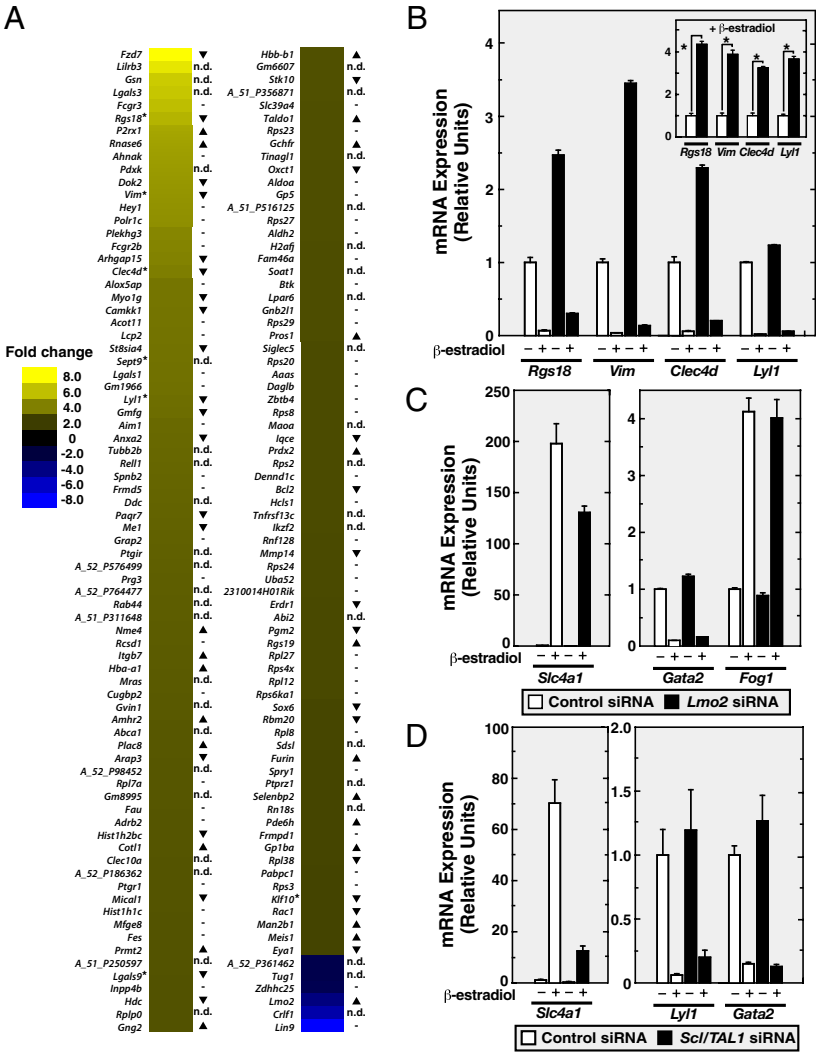

Fig. 3. A distinct component of the GATA-Scl/TAL1 complex, LMO2, regulates a unique target gene ensemble. (*A*) Expression profiles were compared in β-estradiol-treated G1E-ER-GATA-1 cells transfected with control or *Lmo2* siRNA. The heat map depicts the mean fold-change resulting from LMO2 knockdown (n = 2). The GATA-1 responsiveness in G1E-ER-GATA-1 cells, based on prior array analyses (36, 56), is indicated on the right: ▲, GATA-1 activated; ▼, GATA-1 repressed; –, not GATA-1 regulated; n.d., expression not detected. *Genes demonstrated previously (21) to be FOG-1–independent, GATA-1–repressed genes. (*B* and *C*) Quantitative RT-PCR validation of array results. (*B*) *Lmo2* siRNA-activated genes; *$P < 0.05$; (*C*) *Lmo2* siRNA-repressed (*Slc4a1*) and -insensitive (*Gata2*, *Fog1*) genes. (*D*) Quantitative RT-PCR analysis of *Slc4a1*, *Lyl1*, and *Gata2* mRNA in G1E-ER-GATA-1 cells transfected with siRNA against *Scl/TAL1* or control siRNA. *Gapdh* mRNA was analyzed as a control.

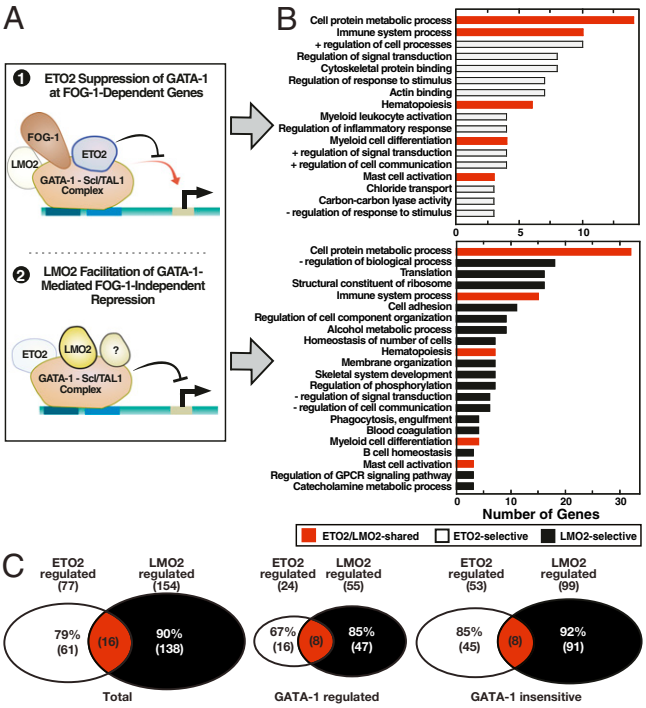

Fig. 4. Mechanistic and biological diversity of ETO2 versus LMO2 target genes. (*A*) Models of ETO2 and LMO2 function. The models depict the qualitatively distinct activities of ETO2 and LMO2 within the GATA-1-Scl/TAL1 complex at different target genes. Because knocking down LMO2 reduces but does not prevent FOG-1–independent repression, an unidentified factor may also mediate repression. (*B*) Gene Ontology analysis. Genes showing ≥ 2.2-fold change based on microarray analysis (Figs. 1*B* and 3*A*) were analyzed using DAVID Bioinformatics Program (http://david.abcc.ncifcrf.gov/). A *P* value of 0.05 was used as the standard cutoff level. (*C*) Relationship between ETO2- and LMO2-regulated genes, based on expression changes ≥ 2.2-fold. The 16 coregulated genes are fewer than that suggested by *B*, as the ETO2/LMO2-shared GO category contains genes coregulated by ETO2- and LMO2 and those uniquely regulated by ETO2 or LMO2.

targets, and therefore building multiple components with specialized activities into the complex would diversify complex function.

Because the GATA-1-Scl/TAL1 complex has been defined biochemically (15, 22, 29, 46, 47, 49), its components colocalize at chromatin sites (36, 50, 51), and it has critical biological functions (1), this is a particularly attractive system for addressing mechanisms underlying the function of multimeric complexes. High-efficiency knockdowns of two components of this complex revealed major differences in their activities to control target genes. Gene Ontology analysis revealed extreme differences in the spectrum of biological activities attributed to ETO2 vs. LMO2 targets. ETO2 and LMO2 therefore exert qualitatively distinct functions, inconsistent with a model in which the tightly linked actions of these components confer a solitary activity. The qualitatively distinct activities indicate that these components can convey independent molecular and cellular functions, entirely unexpected based on the GATA-1-Scl/TAL1 complex paradigm.

Our results illustrate how *trans*-acting factors that bind DNA sequence specifically can serve as a scaffold to build a complex with multifunctionality. Although ETO2 and LMO2 clearly exert independent functions, ETO2 and LMO2 coregulate a small cohort of genes (Fig. 4*C*). Thus, the independent actions of these factors do not preclude their collective function, at least not at select loci. In principle, an alternative possibility is that ETO2 and LMO2 function both within and independent of the GATA-1-Scl/TAL1 complex. ETO2 interacts with the intracellular domain of Notch1 and the Notch-regulated transcription factor CBF1, thereby repressing Notch target genes (52).

Considering parameters dictating the composition of multiprotein complexes at endogenous chromatin sites, at the most rudimentary level, the requisite DNA and protein interaction modules are critically important. The precise configuration of *cis*-elements may influence the conformation of DNA-bound components, favoring or disfavoring nucleation of scaffold constituents. Regarding the GATA-1-Scl/TAL1 complex, a deeper understanding of the molecular structure of the complex in solution and when bound to chromatin is required to assess the relative importance of *cis*-element–instigated conformational transitions. Differences in the signaling milieu characteristic of distinct subnuclear compartments may also control scaffold composition in a spatially restricted manner. Although GATA-1 target genes regulated via distinct mechanisms can reside in different subnuclear compartments (53), whether subnuclear organization and spatially restricted signaling affect GATA-1-Scl/TAL1 complex structure and function is unknown.

Given our strong evidence that components of a multimeric complex containing master regulators of development exert qualitatively distinct functions, it is instructive to consider the pathophysiological implications of this work. As disruptions of different GATA-1-Scl/TAL1 complex constituents yielded qualitatively distinct phenotypes, this may explain why different complex components are linked to the genesis of distinct leukemias (26, 31, 54). To understand how perturbations of individual components of a complex containing multiple developmental regulators yield diverse pathophysiologies, it will be important to define the structure and function of the complex in distinct cellular contexts, and importantly in distinct neighborhoods within the 3D confines of the nucleus.

Materials and Methods

Cell Culture. Cultures were grown as described in *SI Materials and Methods*.

Antibodies. Antibodies are described in *SI Materials and Methods*.

Quantitative ChIP Assay. Real-time PCR-based quantitative ChIP analysis was conducted as described (55) and in *SI Materials and Methods*.

Small Interfering RNA-Mediated Knockdown. ETO2 knockdown in G1E-ER-GATA-1 cells was as described previously (36). The SMARTpool siRNA sequences are described in *SI Materials and Methods*. The siGENOME Non-Targeting siRNA pool (D-001206–13, Dharmacon/Thermo Fisher Scientific) was used as a control. Small interfering RNA was transfected into 3×10^6 G1E-ER-GATA-1 cells by Amaxa Nucleofector kit R (Amaxa Inc.). The siRNA was transfected twice at 0 and 24 h, cells were induced with β-estradiol at 24 h, and harvested after 48 h (Fig. 1*A*).

Quantitative RT-PCR Analysis and Transcription Profiling. Total RNA was analyzed as described in *SI Materials and Methods*.

Western Blotting. Western blotting was conducted as described in *SI Materials and Methods*.

ACKNOWLEDGMENTS. This work was supported by National Institutes of Health Grants DK68634 and DK50107 (to E.H.B.). H.-Y.L. was supported by a predoctoral fellowship from the American Heart Association.

1. Orkin SH, Zon LI (2008) Hematopoiesis: An evolving paradigm for stem cell biology. *Cell* 132:631–644.
2. Bresnick EH, Lee HY, Fujiwara T, Johnson KD, Keles S (2010) GATA switches as developmental drivers. *J Biol Chem* 285:31087–31093.
3. Tsai SF, et al. (1989) Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. *Nature* 339:446–451.
4. Simon MC, et al. (1992) Rescue of erythroid development in gene targeted GATA-1- mouse embryonic stem cells. *Nat Genet* 1:92–98.
5. Shivdasani RA, Fujiwara Y, McDevitt MA, Orkin SH (1997) A lineage-selective knockout establishes the critical role of transcription factor GATA-1 in megakaryocyte growth and platelet development. *EMBO J* 16:3965–3973.
6. Migliaccio AR, et al. (2003) GATA-1 as a regulator of mast cell differentiation revealed by the phenotype of the GATA-1 low mouse mutant. *J Exp Med* 197:281–296.
7. Yu C, et al. (2002) Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo. *J Exp Med* 195: 1387–1395.

8. Tsai FY, et al. (1994) An early haematopoietic defect in mice lacking the transcription factor GATA-2. *Nature* 371:221–226.
9. Ting CN, Olson MC, Barton KP, Leiden JM (1996) Transcription factor GATA-3 is required for development of the T-cell lineage. *Nature* 384:474–478.
10. Tsang AP, et al. (1997) FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation. *Cell* 90: 109–119.
11. Cantor AB, Katz SG, Orkin SH (2002) Distinct domains of the GATA-1 cofactor FOG-1 differentially influence erythroid versus megakaryocytic maturation. *Mol Cell Biol* 22: 4268–4279.
12. Pal S, et al. (2004) Coregulator-dependent facilitation of chromatin occupancy by GATA-1. *Proc Natl Acad Sci USA* 101:980–985.
13. Letting DL, Chen YY, Rakowski C, Reedy S, Blobel GA (2004) Context-dependent regulation of GATA-1 by friend of GATA-1. *Proc Natl Acad Sci USA* 101:476–481.
14. Vakoc CR, et al. (2005) Proximity among distant regulatory elements at the beta-globin locus requires GATA-1 and FOG-1. *Mol Cell* 17:453–462.
15. Hong W, et al. (2005) FOG-1 recruits the NuRD repressor complex to mediate transcriptional repression by GATA-1. *EMBO J* 24:2367–2378.
16. Turner J, Crossley M (1998) Cloning and characterization of mCtBP2, a co-repressor that associates with basic Krüppel-like factor and other mammalian transcriptional regulators. *EMBO J* 17:5129–5140.
17. Snow JW, Orkin SH (2009) Translational isoforms of FOG1 regulate GATA1-interacting complexes. *J Biol Chem* 284:29310–29319.
18. Miccio A, et al. (2010) NuRD mediates activating and repressive functions of GATA-1 and FOG-1 during blood development. *EMBO J* 29:442–456.
19. Gao Z, et al. (2010) FOG-1-mediated recruitment of NuRD is required for cell lineage re-enforcement during haematopoiesis. *EMBO J* 29:457–468.
20. Crispino JD, Lodish MB, MacKay JP, Orkin SH (1999) Use of altered specificity mutants to probe a specific protein-protein interaction in differentiation: The GATA-1:FOG complex. *Mol Cell* 3:219–228.
21. Johnson KD, et al. (2007) Friend of GATA-1-independent transcriptional repression: A novel mode of GATA-1 function. *Blood* 109:5230–5233.
22. Wadman IA, et al. (1997) The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins. *EMBO J* 16:3145–3157.
23. Porcher C, et al. (1996) The T cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. *Cell* 86:47–57.
24. Mikkola HK, et al. (2003) Haematopoietic stem cells retain long-term repopulating activity and multipotency in the absence of stem-cell leukaemia *SCL/tal-1* gene. *Nature* 421:547–551.
25. Shivdasani RA, Mayer EL, Orkin SH (1995) Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL. *Nature* 373:432–434.
26. Hacein-Bey-Abina S, et al. (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 302:415–419.
27. Morcillo P, Rosen C, Baylies MK, Dorsett D (1997) Chip, a widely expressed chromosomal protein required for segmentation and activity of a remote wing margin enhancer in *Drosophila*. *Genes Dev* 11:2729–2740.
28. Song SH, Hou C, Dean A (2007) A positive role for NLI/Ldb1 in long-range beta-globin locus control region function. *Mol Cell* 28:810–822.
29. Schuh AH, et al. (2005) ETO-2 associates with SCL in erythroid cells and megakaryocytes and provides repressor functions in erythropoiesis. *Mol Cell Biol* 25: 10235–10250.
30. Meier N, et al. (2006) Novel binding partners of Ldb1 are required for haematopoietic development. *Development* 133:4913–4923.
31. Hiebert SW, Lutterbach B, Amann JM (2001) Role of co-repressors in transcriptional repression mediated by the t(8;21), t(16;21), t(12;21), and inv(16) fusion proteins. *Curr Opin Hematol* 8:197–200.
32. Xu Z, et al. (2007) Single-stranded DNA-binding proteins regulate the abundance of LIM domain and LIM domain-binding proteins. *Genes Dev* 21:942–955.
33. Xu Z, Meng X, Cai Y, Koury MJ, Brandt SJ (2006) Recruitment of the SWI/SNF protein Brg1 by a multiprotein complex effects transcriptional repression in murine erythroid progenitors. *Biochem J* 399:297–304.
34. Wozniak RJ, et al. (2008) Molecular hallmarks of endogenous chromatin complexes containing master regulators of hematopoiesis. *Mol Cell Biol* 28:6681–6694.
35. Tripic T, et al. (2009) SCL and associated proteins distinguish active from repressive GATA transcription factor complexes. *Blood* 113:2191–2201.
36. Fujiwara T, et al. (2009) Discovering hematopoietic mechanisms through genome-wide analysis of GATA factor chromatin occupancy. *Mol Cell* 36:667–681.
37. Weiss MJ, Yu C, Orkin SH (1997) Erythroid-cell-specific properties of transcription factor GATA-1 revealed by phenotypic rescue of a gene-targeted cell line. *Mol Cell Biol* 17:1642–1651.
38. Gregory T, et al. (1999) GATA-1 and erythropoietin cooperate to promote erythroid cell survival by regulating bcl-xL expression. *Blood* 94:87–96.
39. Grass JA, et al. (2003) GATA-1-dependent transcriptional repression of GATA-2 via disruption of positive autoregulation and domain-wide chromatin remodeling. *Proc Natl Acad Sci USA* 100:8811–8816.
40. Chyla BJ, et al. (2008) Deletion of Mtg16, a target of t(16;21), alters hematopoietic progenitor cell proliferation and lineage allocation. *Mol Cell Biol* 28:6234–6247.
41. Peters LL, et al. (1996) Anion exchanger 1 (band 3) is required to prevent erythrocyte membrane surface loss but not to form the membrane skeleton. *Cell* 86:917–927.
42. Kim S-I, Bultman SJ, Jing H, Blobel GA, Bresnick EH (2007) Dissecting molecular steps in chromatin domain activation during hematopoietic differentiation. *Mol Cell Biol* 27:4551–4565.
43. Campos EI, Reinberg D (2009) Histones: Annotating chromatin. *Ann Rev Genet* 43: 559–599.
44. Bernstein BE, Meissner A, Lander ES (2007) The mammalian epigenome. *Cell* 128: 669–681.
45. Amann JM, et al. (2001) ETO, a target of t(8;21) in acute leukemia, makes distinct contacts with multiple histone deacetylases and binds mSin3A through its oligomerization domain. *Mol Cell Biol* 21:6470–6483.
46. Goardon N, et al. (2006) ETO2 coordinates cellular proliferation and differentiation during erythropoiesis. *EMBO J* 25:357–366.
47. Visvader JE, Mao X, Fujiwara Y, Hahm K, Orkin SH (1997) The LIM-domain binding protein Ldb1 and its partner LMO2 act as negative regulators of erythroid differentiation. *Proc Natl Acad Sci USA* 94:13707–13712.
48. Souroullas GP, Salmon JM, Sablitzky F, Curtis DJ, Goodell MA (2009) Adult hematopoietic stem and progenitor cells require either Lyl1 or Scl for survival. *Cell Stem Cell* 4:180–186.
49. Rodriguez P, et al. (2005) GATA-1 forms distinct activating and repressive complexes in erythroid cells. *EMBO J* 24:2354–2366.
50. Cheng Y, et al. (2009) Erythroid GATA1 function revealed by genome-wide analysis of transcription factor occupancy, histone modifications, and mRNA expression. *Genome Res* 19:2172–2184.
51. Im H, et al. (2005) Chromatin domain activation via GATA-1 utilization of a small subset of dispersed GATA motifs within a broad chromosomal region. *Proc Natl Acad Sci USA* 102:17065–17070.
52. Engel ME, Nguyen HN, Mariotti J, Hunt A, Hiebert SW (2010) Myeloid translocation gene 16 (MTG16) interacts with Notch transcription complex components to integrate Notch signaling in hematopoietic cell fate specification. *Mol Cell Biol* 30:1852–1863.
53. Lee H-Y, et al. (2009) Controlling hematopoiesis through sumoylation-dependent regulation of a GATA factor. *Mol Cell* 36:984–995.
54. Wechsler J, et al. (2002) Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. *Nat Genet* 32:148–152.
55. Im H, et al. (2004) Measurement of protein-DNA interactions in vivo by chromatin immunoprecipitation. *Methods Mol Biol* 284:129–146.
56. Welch JJ, et al. (2004) Global regulation of erythroid gene expression by transcription factor GATA-1. *Blood* 104:3136–3147.