Genetic framework for GATA factor function in vascular biology

Amelia K. Linnemann[a], Henriette O'Geen[b], Sunduz Keles[c], Peggy J. Farnham[d], and Emery H. Bresnick[a,1]

[a]Department of Cell and Regenerative Biology, Wisconsin Institutes for Medical Research, University of Wisconsin Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705; [b]Genome Center, University of California, Davis, CA 95616; [c]Department of Statistics and Biostatistics and Medical Informatics, University of Wisconsin School of Medicine and Public Health, Madison, WI 53706; and [d]University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, CA 90089

Vascular endothelial dysfunction underlies the genesis and progression of numerous diseases. Although the GATA transcription factor GATA-2 is expressed in endothelial cells and is implicated in coronary heart disease, it has been studied predominantly as a master regulator of hematopoiesis. Because many questions regarding GATA-2 function in the vascular biology realm remain unanswered, we used ChIP sequencing and loss-of-function strategies to define the GATA-2–instigated genetic network in human endothelial cells. In contrast to erythroid cells, GATA-2 occupied a unique target gene ensemble consisting of genes encoding key determinants of endothelial cell identity and inflammation. GATA-2–occupied sites characteristically contained motifs that bind activator protein-1 (AP-1), a pivotal regulator of inflammatory genes. GATA-2 frequently occupied the same chromatin sites as c-JUN and c-FOS, heterodimeric components of AP-1. Although all three components were required for maximal AP-1 target gene expression, GATA-2 was not required for AP-1 chromatin occupancy. GATA-2 conferred maximal phosphorylation of chromatin-bound c-JUN at Ser-73, which stimulates AP-1–dependent transactivation, in a chromosomal context-dependent manner. This work establishes a link between a GATA factor and inflammatory genes, mechanistic insights underlying GATA-2–AP-1 cooperativity and a rigorous genetic framework for understanding GATA-2 function in normal and pathophysiological vascular states.

endothelium | genomics | genome-wide | transcriptome

An important approach to understanding the biology and pathophysiology of the vascular system involves dissecting transcriptional mechanisms underlying the development and function of endothelial cells. Transcription factors implicated in endothelial transcriptional mechanisms (1) include GATA binding protein 2 (GATA-2), which is required to generate and/or maintain multipotent hematopoietic precursors during embryogenesis (2, 3). GATA transcription factors function as master regulators of development and have important functions in differentiated cells (4). Although GATA-2 is expressed in endothelial cells (5, 6), the GATA-2–driven genetic network in endothelium and its role in vascular biology are unclear.

The pathophysiological importance of GATA-2 in the vascular system is supported by the finding that *GATA2* polymorphisms correlate with coronary artery disease (7). GATA-2 regulates genes that encode important mediators of endothelial cell function, including angiopoietin-2 (8), matrix metalloproteinase-2 (9), and vascular cell adhesion molecule-1 (10). In vitro and in vivo studies indicate that GATA-2 functions in human umbilical vein endothelial cells (HUVEC) and retinal endothelial cells to mediate mechano-signaling–dependent angiogenesis, which involves GATA-2–dependent induction of VEGF receptor II (11). Despite this compelling evidence, *Gata2*-null mice die at embryonic day 10.5 (E10.5) because of severe anemia, although their vasculature appears to be morphologically normal (3). However, the absence of an overt vasculogenesis defect does not negate the importance of GATA-2 in adult endothelium.

We used ChIP sequencing (ChIP-seq) and expression profiling to describe a GATA-1– and GATA-2–driven genetic network in human K562 erythroleukemia cells, which resemble primitive erythroid cells (12). This dataset, which was validated in primary erythroid cells (12), lacked genes that impart a unique identity to endothelial cells. We hypothesize that the GATA-2 target gene ensembles in endothelial and hematopoietic cells differ considerably, but analogous to its hematopoietic functions, GATA-2 exerts important activities in endothelium.

Herein we use ChIP-seq and expression profiling in HUVEC to define a GATA-2–driven genetic network that differs greatly from that of K562 cells. The composition of the network indicated that GATA-2 functions in concert with activator protein-1 (AP-1), a pivotal regulator of inflammation (13, 14), thus linking GATA-2 with inflammatory processes. Given the dearth of mechanistic information regarding vascular functions of GATA-2 and the broad scope of pathophysiologies involving vascular dysfunction with associated inflammation (15), linking endothelial GATA-2 to inflammatory genes establishes an important framework for understanding the role of GATA-2 in normal and pathological vascular states.

Results and Discussion

Exquisite Cell Type-Specificity of GATA-2 Chromatin Occupancy. To establish a framework for understanding GATA-2 function in endothelium, we used ChIP-seq to define the GATA-2 chromatin target sites in HUVEC. The ChIP assay was validated by quantitating GATA-2 occupancy at the *GATA2* +9.5 kb enhancer (16), which activates a linked *LacZ* reporter in the vasculature of transgenic mouse embryos (17). GATA-2 occupancy at the +9.5 kb site was ~15-fold greater than the preimmune control, whereas no significant occupancy was detected at the negative control *necdin* promoter (Fig. S1). The ChIP-seq analysis was conducted via the standard mode of analysis for the ENCODE Consortium. Immunoprecipitated DNA from two biological replicates was analyzed by ChIP-seq. Because the replicates met established criteria for reproducibility, the datasets were merged, and peaks were called again. Unique sequences were mapped to the genome (HG19). Replicates A and B yielded 9.9 million and 12.6 million unique sequences, respectively. Using a false-discovery rate of 0.0001, replicates A and B yielded 18,233 and 8,830 GATA-2–occupied loci (peaks), respectively. Comparison of replicates revealed major overlap, with 7,619 (86%) of the B peaks present in A. Merging the replicates yielded 15,529 peaks corresponding
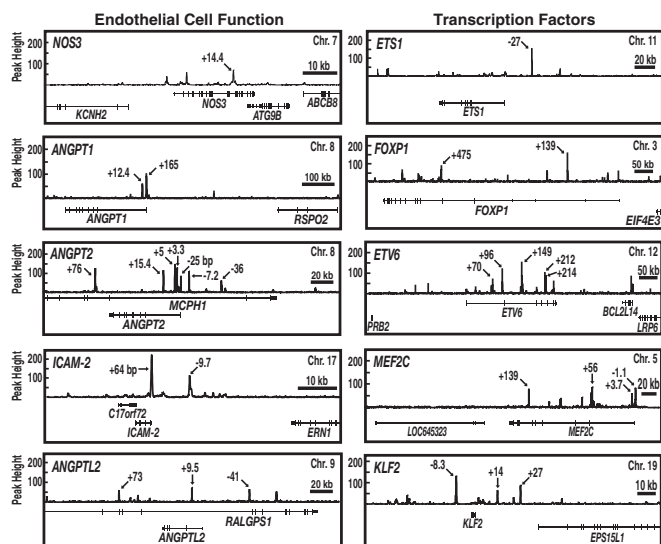
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Fig. 1. Representative GATA-2 target genes in HUVEC. Signal maps of GATA-2 occupancy sites in HUVEC identified by ChIP-seq. Genes important for endothelial cell function and transcription factors are depicted. Arrows indicate ChIP-seq peak locations relative to the transcription start site of the respective GATA-2 target gene (kb).

to 6,081 genes (Dataset S1). The mean peak height was 75 bp (range: 14–229 bp), and the mean width was 417 bp.

The GATA-2–occupied genes encode proteins known to be important for endothelial cell function, including endothelial NOS3, angiopoietins 1, 2, and 3, and intercellular adhesion molecule-2 (Fig. 1). Certain genes encoded transcription factors, including Kruppel-like factor-2 (*KLF2*) (Fig. 1). *KLF2* is induced by shear stress, mediates shear stress signaling (18), determines vascular tone (19), mediates endothelial cell thrombotic function (20), and promotes vessel maturation (21). *KLF2* is repressed by the proinflammatory cytokine IL-1β (18) and mediates the anti-inflammatory actions of statins (22). Additional transcription factors included the forkhead factor FOXP1 and multiple v-ets erythroblastosis virus E26 oncogene homolog (ETS)/ETS-variant (ETV) factors (Dataset S2), including ETV6, which is required for hematopoiesis and maintenance of the vascular network (23, 24). GATA-2 occupied myocyte-specific enhancer factor 2C (*MEF2C*), which is essential for vascular development (25). *MEF2C* contains an endothelial enhancer, corresponding to the GATA-2 peak at +139 kb, which directs endothelial cell-specific expression of a reporter in E7.5–E9.5 mice (26). The ChIP-seq analysis revealed a rich set of targets, including numerous genes not linked previously to endothelial cell biology.

The majority of GATA-2–occupied peaks (13,256 peaks, 85%) were not occupied in our prior K562 cell analysis (12) (Fig. 2A). In HUVEC and K562 cells (12), the majority of peaks resided distal to the genes in putative enhancer regions (Fig. 2B). The unique chromatin-bound sites in HUVEC vs. K562 cells indicates that GATA-2 occupies a very small subset of genomic GATA motifs in a cell type-specific manner. To explore the relationship between GATA-2 occupancy and the epigenome, we evaluated histone marks in HUVEC at occupied sites. This analysis revealed that 91% of GATA-2–occupied sites overlap with H3K4me1 (Fig. 2C), a diagnostic enhancer mark (27). A smaller subset of occupied sites (35%) overlaps with H3K4me3 (Fig. 2D). As expected for a mark associated with transcription (28), 61% of these overlapping peaks reside in introns and exons. A small cohort of occupied sites overlaps with H3K27me3 (Fig. 2E), H3K36me3 (Fig. 2F), and H4K20me1 (Fig. 2G) (1%, 3%, and 1%, respectively).

Linking GATA-2 with the Inflammatory Regulator AP-1. We assessed whether the GATA-2 cistrome in HUVEC resembles that of K562 cells, in which the majority of GATA-2–occupied peaks reside at sites containing the GATA motif (12). Of the 15,529 GATA-2 peaks, 12,930 (83%) contained at least one WGATAR motif. Of the 6,976,111 GATA motifs in the human genome, 25,823 (0.37%) resided within the 15,529 peaks. Constrained de novo motif finding with Cosmo (29) identified WGATAA that we defined previously for GATA-1 occupancy in K562 cells (Fig. 3A) (12). A broader GATA consensus, [CG][AT]GATAA[GAC][GAC], resided at 8,176 (43.3%) of the GATA-2–occupied K562 cell peaks and 4,698 (35.4%) of the HUVEC peaks.

De novo motif finding using MEME (30) to pinpoint *cis*-elements at GATA-2–occupied sites in HUVEC revealed canonical motifs for the inflammatory regulator AP-1 (14) (Fig. 3B) and c-ETS (Fig. 3C), which has important functions in endothelial cells (31). AP-1 motifs (TGA[G/C]TCA) (32) resided in 45.4% of the HUVEC GATA-2 peaks (7,055 of 15,523 peaks). This was highly significant ($P < 0.01$) relative to 16.3% of GATA-2 peaks in K562 cells (3,451 of 21,167 peaks). Because 69% of GATA-2–occupied peaks overlapped with those occupied by the jun proto-oncogene (c-JUN) component of AP-1 (Fig. 3D and Dataset S3), 29% overlapped with FBJ murine osteosarcoma viral oncogene homolog (c-FOS) (Fig. 3E and Dataset S4), and only 1% (137) overlapped with peaks of c-Myc occupancy (23,525 total c-Myc peaks), this GATA-2–AP-1 link has broad importance. The location with respect to neighboring genes of sites containing both GATA-2 and c-JUN did not differ significantly from the location of the complete cohort of GATA-2 occupancy sites. Examples of AP-1 target genes in which GATA-2 overlapped with c-JUN and c-FOS include *IL-8* (33), *CXCL2* (34), and *CSF2* (35). GATA-2, c-JUN, and c-FOS overlapped at *RSPO3*, which is not known to be an AP-1 target (Fig. 3F). A high fraction of chromatin sites bound by both GATA-2 and c-Jun were enriched in H3K4me1 or H3K4me3 (76 and 83%, respectively).

We compared the frequency in which the c-ETS motif resided at GATA-2–occupied sites in HUVEC vs. K562 cells. In HUVEC,
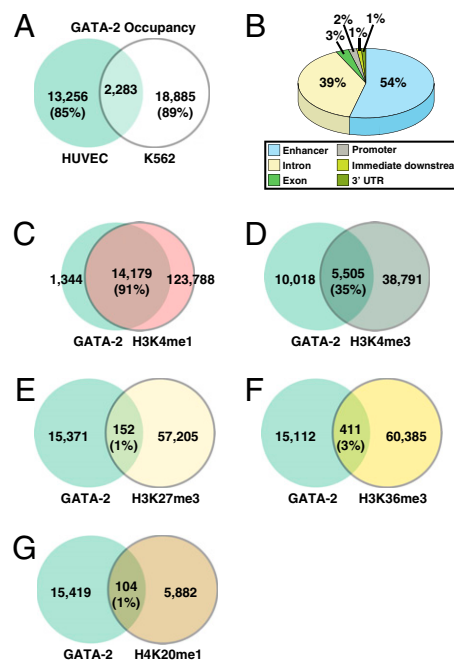

Fig. 2. Computational mining of ChIP-seq data. (A) Comparison of GATA-2–occupied peaks in HUVEC and K562 cells. (B) Locations of GATA-2–occupied peaks relative to nearest-neighbor genes determined with the Cis Element Annotation System (http://liulab.dfci.harvard.edu/CEAS/; http://ceas.cbi.pku.edu.cn/). (C–G) Comparison of GATA-2–occupied peaks and peaks of H3K4me1 (C), H3K4me3 (D), H3K27me3 (E), H3K36me3 (F), and H4K20me1 (G).

5,446 (35.1%) of the 15,523 GATA-2–occupied sites contained c-ETS motifs. In K562 cells, 2,737 (12.9%) of the 21,167 GATA-2–occupied sites contained c-ETS motifs. Based on a binomial test for proportions, the differential frequency of c-ETS motifs at GATA-2–occupied sites in HUVEC vs. K562 cells was highly significant ($P < 2.2e^{-16}$). Only 2,249 (14.5%) of the 15,523 GATA-2–occupied sites in HUVEC contained both c-ETS and AP-1 motifs. A GATA-2 peak was more likely to contain an AP-1 motif if it lacked a c-ETS motif ($P < 0.001$). Thus, it is likely that GATA-2 does not function frequently as a ternary complex with AP-1 and c-ETS in HUVEC.
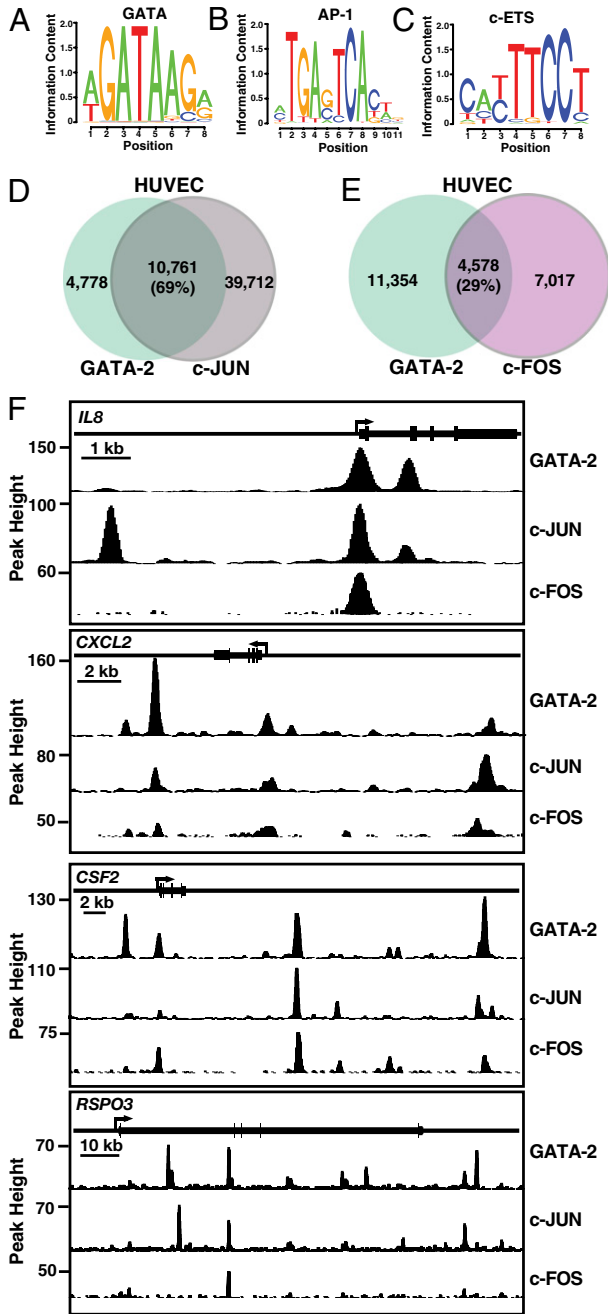
Direct GATA-2 Target Gene Ensemble in Endothelium. The frequent occurrence of AP-1 motifs at GATA-2–occupied sites and GATA-2 sharing of occupancy sites with a key component of AP-1 suggest that GATA-2 and AP-1 interact functionally. We used RNA interference to establish GATA-2 target genes in HUVEC. *GATA2* mRNA was much lower in HUVEC than in K562 cells (Fig. 4*A*) (12), but endogenous GATA-2 in HUVEC was detectable by Western blotting (Fig. 4*C*). *GATA2* siRNA significantly reduced *GATA2* mRNA and protein in HUVEC vs. control siRNA-transfected cells (Fig. 4 *B* and *C*) without overtly affecting HUVEC morphology. Expression profiling of two biological replicates was conducted, and genes dysregulated upon GATA-2 knockdown were analyzed further. The direct GATA-2 targets were identified by comparing the differential expression with GATA-2 occupancy determined by ChIP-seq. This analysis revealed 116 direct targets differentially expressed by twofold or more upon GATA-2 knockdown (Fig. 4*D*). Because knockdowns do not eliminate expression, the analysis underestimates the target gene ensemble.

The direct targets encode regulators of cell migration, including semaphorin 3A (36), and podocalyxin-like (37). GATA-2–activated direct targets encode proinflammatory mediators, including interleukins, the chemokine ligand CXCL2, and the
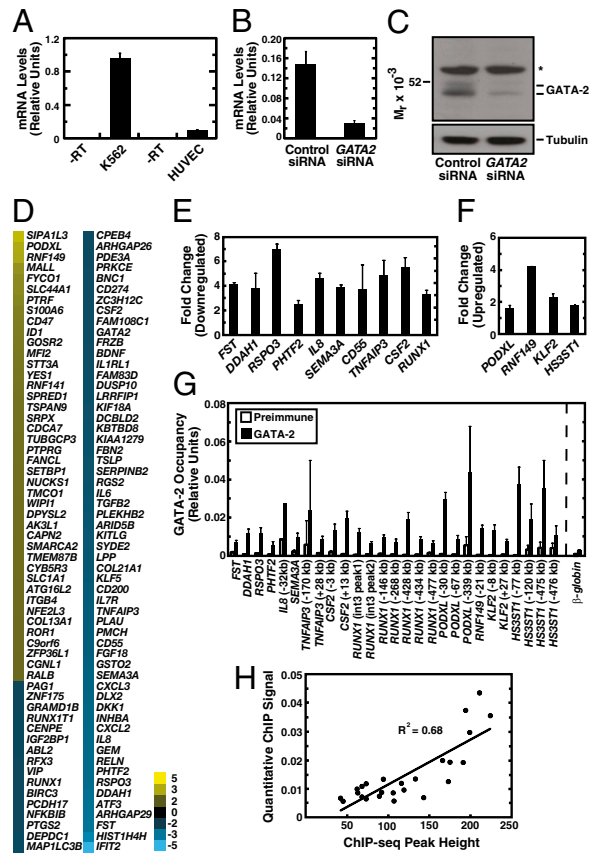

Fig. 3. Linking GATA-2 and AP-1 function. (*A–C*) Logos of overrepresented motifs from GATA-2–occupied ChIP-seq peaks: (*A*) GATA motif from constrained motif analysis and (*B*) AP-1 and (*C*) c-ETS motifs from de novo motif finding. (*D* and *E*) Comparison of GATA-2–occupied peaks and peaks of (*D*) c-JUN and (*E*) c-FOS occupancy in HUVEC. (*F*) Representative profiles demonstrating overlap among GATA-2, c-JUN, and c-FOS occupancy peaks.

Fig. 4. Direct GATA-2 target gene ensemble. (*A*) Comparison of GATA2 levels in HUVEC and K562 cells. (*B*) Quantitative RT-PCR analysis of *GATA2* transcript levels after treatment with nontargeting control siRNA or *GATA2* siRNA. (*C*) Western blotting of GATA-2 in HUVEC transfected with control or *GATA2* siRNA. Whole-cell samples were analyzed. *, nonspecific band. (*D*) Heat map depicting the mean fold change of expression at GATA-2–occupied loci resulting from *GATA2* knockdown (n = 2). (*E* and *F*) Quantitative RT-PCR validation of array results in GATA-2–activated (down-regulated with knockdown) (*E*) and repressed (up-regulated) genes (*F*). (*G*) Quantitative ChIP validation of GATA-2 occupancy at loci dysregulated by the knockdown. Data shown are mean ± SE; n = 3. (*H*) Correlation between height of ChIP-seq peak and quantitative ChIP signal.

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cytokine CSF2 (GM-CSF). GATA-2 directly repressed *KLF2*, the key mediator of shear stress signaling in endothelium that opposes inflammation. GATA-2 activated *RSPO3*, which controls placental vascular plexus development and regulates angiogenesis during embryogenesis (38). Secreted RSPO3 activates wingless-type/β-catenin signaling, which exerts a proinflammatory function (38). GATA-2 induced expression of activating transcription factor 3 (ATF3), a stress-regulated transcription factor that protects endothelial cells from TNF-α–induced cell death (39) and negatively regulates allergic airway inflammation (40).

A subset of GATA-2–activated and –repressed targets was subjected to additional validation by real-time RT-PCR (Fig. 4 *E* and *F*) and quantitative ChIP analysis using the human β-globin (*HBB*) promoter as a negative control (Fig. 4*G*). ChIP-seq peak height correlated ($R^2 = 0.68$) with the quantitative ChIP signal (Fig. 4*H*).

Our results indicate that GATA-2, c-JUN, and c-FOS commonly occupy the same chromatin sites containing GATA and AP-1 motifs. Taken together with the fact that AP-1 is a key regulator of inflammatory genes such as *IL-8* and *CSF2* that are GATA-2 targets (Fig. 4 *D* and *E*), this finding suggests the existence of a GATA factor–AP-1 regulatory network to control inflammatory genes. Ontological analysis of the direct GATA-2 target-gene ensemble revealed an enrichment of inflammatory genes, the majority of which are GATA-2–activated (Fig. 5*A*).

GATA-2 Requirement for Assembly of Functional AP-1 Complexes on Chromatin. In our loss-of-function analysis, HUVEC were cultured in medium containing FBS, EGF, and FGF2, which induce signaling that can activate AP-1 (13). Our results suggested that GATA-2 and AP-1 confer maximal AP-1 target gene transcription. We tested whether activity of presumptive AP-1 targets requires AP-1 in HUVEC. Transfection with dominant-negative c-FOS (A-FOS) (41, 42) significantly reduced *IL-8* ($P < 0.01$), *CSF2* ($P < 0.01$), and *IL-6* ($P < 0.01$) expression (Fig. 5*C*). We tested genes that emerged from our direct GATA-2 target screen that were not established AP-1 targets. Expression of *RSPO3* ($P < 0.01$), *CXCL1*, and *RUNX1* was reduced by A-FOS. c-JUN, and c-FOS knockdowns confirmed AP-1 regulation of select GATA-2 targets; *CSF2* and *CXCL1* expression were not affected significantly (Fig. 5 *B* and *C*). Thus, GATA-2 and AP-1 ensure maximal activity of a cohort of the target genes.

We considered potential mechanisms underlying the GATA-2–AP-1 cooperation (Fig. 6*A*). At target genes (Fig. 6*A*I), reduced GATA-2 occupancy might decrease AP-1 occupancy (Fig. 6*A*II), decrease AP-1 phosphorylation (Fig. 6*A*III), or induce AP-1–independent molecular alterations (Fig. 6*A*IV). To distinguish among these possibilities, occupancy of c-FOS, c-JUN, and c-JUN phosphorylated at serine 73 was analyzed in control siRNA- and *GATA2* siRNA-transfected cells. The analysis was conducted under conditions in which *GATA2* expression was greatly reduced (Fig. 6*B*). Knocking down GATA-2 significantly reduced phos-

phorylated c-JUN occupancy ($P < 0.05$), but not total c-JUN and c-FOS occupancy, at the *IL-8* promoter and distal sites at *RSPO3* and *ATF3* genes (Fig. 6*D*). Loss of chromatin-bound phosphorylated c-JUN was not associated with a change in total c-JUN (Fig. 6*C*).

To assess the specificity of the GATA-2–dependent phosphorylated c-JUN occupancy, occupancy was analyzed at the GATA-2–insensitive IL-1B and IL-17D genes (Fig. 6*D*). GATA-2 knockdown did not affect phosphorylated c-JUN occupancy at these genes. Occupancy of all factors was low at the *HBB* promoter and an unoccupied site 1 kb upstream of the *IL-8* promoter (Fig. 6*D*). The context-dependent GATA-2 requirement for phosphorylated c-JUN occupancy may explain why certain AP-1 target genes that are occupied by GATA-2 and AP-1 (Datasets S3 and S4) are insensitive to reducing GATA-2. In principle, GATA-2 may promote c-JUN phosphorylation indirectly or may promote phosphorylated c-JUN chromatin occupancy, and it has been reported that GATA-2 and c-JUN can interact (43). Coimmunoprecipitation analysis with HUVEC whole-cell lysates did not provide evidence for a stable complex containing GATA-2 and c-JUN.

We also tested whether c-JUN promotes GATA-2 chromatin occupancy, but knocking down c-JUN did not affect GATA-2 chromatin occupancy (Fig. S2).

To test whether GATA-2 facilitation of AP-1–mediated transcription has biological implications, we asked whether GATA-2 regulates secretion of the critical inflammatory mediator IL-8. GATA-2–knockdown HUVECs were treated with the proinflammatory factor TNF-α, which induced IL-8 secretion several fold as measured by ELISA (Fig. 6*E*). Knocking down GATA-2 significantly reduced IL-8 secretion ($P = 0.0015$), consistent with the mRNA expression data.

Linking GATA-2, AP-1, and Inflammatory Genes. The results described herein, representing the genome-wide analysis of a GATA factor in endothelium, provide a rigorous genetic framework for understanding GATA-2 function in vascular biology. GATA-2 controls expression of genes involved in establishing endothelial cell phenotypes and inflammation. Analysis of 116 GATA-2–occupied genes differentially regulated upon knocking down GATA-2 using Ingenuity Pathways Analysis (http://www.ingenuity.com/products/pathways_analysis.html) revealed a complex network (Fig. S3) in which factors were knitted together based on established protein–protein interactions and abilities to regulate expression of other factors. The network highlights a GATA-2 function in regulating genes mediating inflammation (Fig. 6*F*). GATA-2 targets also included genes encoding anti-inflammatory factors, including *TNFAIP3*, which is induced by proinflammatory factors and suppresses proinflammatory signaling (44, 45) and ATF3 (40), as described above. Certain AP-1 targets are regulated by the proinflammatory factor NF-κB (46). Scanning the GATA-2 peaks with the NF-κB position/weight matrix from the JASPAR database using the FIMO tool (47) revealed that only 9.1% of the
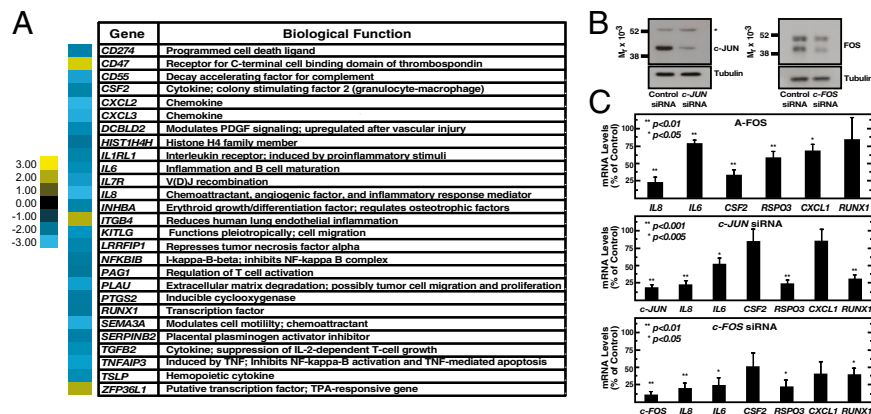

Fig. 5. GATA-2/AP-1–dependent regulation of inflammatory genes. (*A*) Genes involved in inflammation and their expression changes with GATA-2 knockdown. Blue, down-regulated with GATA-2 knockdown; Yellow, up-regulated with GATA-2 knockdown. (*B*) Western blotting of endogenous c-JUN and c-FOS in HUVEC transfected with c-JUN or c-FOS siRNA, respectively. Transfected cells were boiled in SDS-sample buffer, and whole-cell samples were analyzed by Western blotting. *, nonspecific band. (*C*) Real-time RT-PCR analysis of gene expression in HUVEC transfected with a dominant-negative AP-1 antagonist (A-FOS), c-JUN siRNA, or c-FOS siRNA. The expression of empty vector-transfected cells (A-FOS) or nontargeting control siRNA-transfected cells (c-JUN and c-FOS) was designated as 100%, and expression for each gene is represented as a percentage of control expression. Data shown are mean ± SE; $n ≥ 3$.

peaks match significantly ($P \leq 1e^{-4}$) to the matrix, a finding that is inconsistent with a frequent GATA-2–NF-κB association at HUVEC chromatin sites.

Because certain GATA-2 target genes with established roles in inflammation were co-occupied by GATA-2, c-JUN, and c-FOS, GATA-2 knockdown reduced phosphorylated c-JUN occupancy and IL-8 secretion, and AP-1 is a pivotal mediator of inflammation, it is attractive to propose that GATA-2–AP-1 cooperation is a common mode of orchestrating inflammatory processes. Vascular endothelial dysfunction involving inflammation underlies diseases including atherosclerosis, rheumatoid arthritis, and type II diabetes (15).

Given the biological importance of genes constituting the GATA-2 genetic network in HUVEC, why does the *Gata2*-knockout mouse appear to have morphologically normal vasculature, despite dying at E10.5 because of severe anemia (3)? Since multiple GATA factors are expressed in endothelium (48), a rigorous loss-of-function approach to ascertain GATA-2 function in the adult vasculature might require multigenic disruptions to reveal critical GATA factor-dependent pathways; alternatively, an important vascular activity of GATA-2 might not be apparent given the early embryonic lethality.

The direct regulation of inflammatory genes by GATA-2 has considerable biological and pathophysiological implications. It will be important to implement additional functional analyses and to determine whether endothelial cell subtypes differ in this mechanism. During the review of our manuscript, a genome-wide analysis of GATA-2 occupancy in human microvascular endothelial cells (HMVEC) was reported (49). Comparison of the HUVEC and HMVEC datasets will be informative.

Given the oncogenic activity of AP-1 subunits (50, 51), the role for GATA-3 in breast cancer (52), and links between GATA-2 dysregulation and leukemia (53), it will be important to determine whether GATA factors cooperate with AP-1 in diverse biological contexts and to what extent disruption of this mechanism underlies human pathologies. Because strong evidence implicates inflammation in a plethora of disease processes, including cancer, studies to evaluate GATA-2 function in endothelium in diverse disease models will be particularly instructive.

Materials and Methods

Cell Culture. HUVEC were maintained in Medium 200 (Cascade Biologics) supplemented with Low Serum Growth Supplement (Cascade Biologics) and 1% antibiotic/antimycotic (Invitrogen). Cells were passaged at ~70–80% confluence with 0.05% trypsin, and cells from passages four and six were used.

Antibodies. Rabbit anti–GATA-2 polyclonal antibody was described previously (54). Anti–c-FOS (SC-253) and anti–c-JUN (SC-45) were from Santa Cruz Biotech. Anti–p-c-JUN (Ser73) was from Cell Signaling Technology (#9164). Mouse monoclonal α-tubulin (#CP06) was from Calbiochem.

Transfection and RNA Interference. HUVEC were seeded 3–5 d before siRNA or plasmid transfection and grown to ~70% confluence. After trypsinization, ~10^6 cells per experiment were resuspended in HUVEC Nucleofector Solution (Lonza), and 0.45–0.60 nmol of siRNA, 5 μg A-FOS expression vector, or empty vector was transfected using the Nucleofector system (Lonza). siGENOME SMARTpool GATA2 siRNA, c-JUN siRNA, c-FOS siRNA, and control siRNA (SMARTpool #1) were from Dharmacon (Thermo Scientific). CMV-500-A-Fos and CMV-500 empty vector were provided by Charles Vinson (National Cancer Institute, Bethesda, MD). Cells were analyzed 24 or 40 h after siRNA or A-FOS transfection, respectively.

Generation of ChIP-Seq Data. HUVEC histone ChIP-seq data were generated at the Broad Institute and by the B. Bernstein group (Massachusetts General Hospital, Boston, MA). HUVEC c-JUN ChIP-seq data were generated by D. Raha and Mike Snyder (Stanford University, Stanford, CA). HUVEC c-Myc ChIP-seq data were generated by Vishy Iyer (University of Texas at Austin, TX). HUVEC GATA-2, HUVEC c-FOS, and K562 GATA-2 ChIP-seq data were generated by the P. Farnham group. These data were collected as part of the EN-CODE Consortium (http://www.genome.gov/10005107) and are available on the University of California, Santa Cruz Genome Browser.
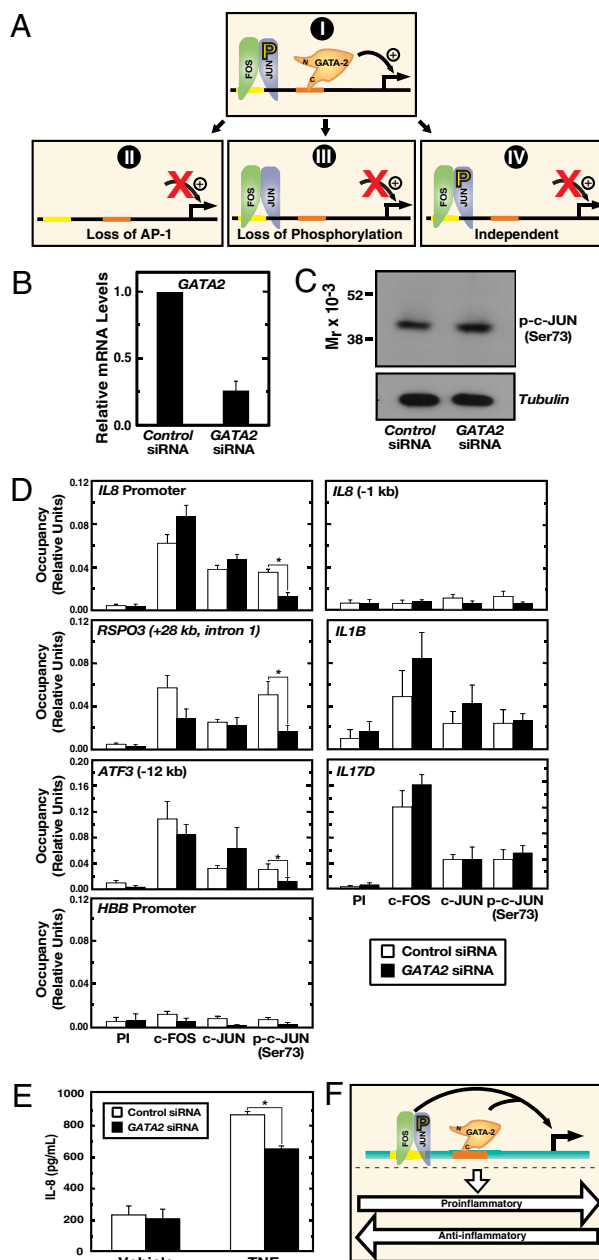

Fig. 6. GATA-2 requirement for assembly of functional AP-1 complexes on chromatin. (*A*) Models as described in *Results and Discussion*. (*B*) Real-time RT-PCR quantitation of *GATA2* mRNA. (*C*) Western blotting of c-JUN phosphorylated at serine 73 [p-c-JUN (Ser73)] in HUVEC transfected with control or *GATA2* siRNA. Whole-cell samples were analyzed by Western blotting. (*D*) Quantitative ChIP of preimmune control (PI), c-FOS, c-JUN, and p-c-JUN (Ser-73) occupancy at the *IL-8* promoter, *RSPO3*, and *ATF3*, AP-1 targets that are GATA-2–independent (*IL-1B*, *IL-17D*), and negative controls (*HBB* and a site 1 kb upstream of the *IL-8* promoter). Data shown are mean ± SE; *n* ≥ 3. (*E*) ELISA quantitation of IL-8 levels in medium from control siRNA- or GATA-2 siRNA-transfected cells treated with 10 ng/mL TNF-α or vehicle for 6 h. Data shown are mean ± SE; *n* = 6. (*F*) GATA-2–AP-1 coregulation of inflammatory genes in endothelium.

Quantitative ChIP Analysis. ChIP was conducted as described (55) using 1×10^8 HUVEC cells per condition for ChIP-seq, or 1×10^7 HUVEC cells per condition for quantitative ChIP.

ChIP-Seq Analysis. HUVEC were crosslinked for 10 min with 1% formaldehyde, snap frozen, and stored at −80 °C. ChIP was conducted as described (http://

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www.genomecenter.ucdavis.edu/farnham/pdf/FarnhamLabChIP%20Protocol.pdf). Chromatin from 10^8 cells was diluted with three volumes of immunoprecipitation (IP) dilution buffer and incubated at 4 °C overnight with 60 μL of rabbit anti–GATA-2 antibody. StaphA cells were blocked with BSA. StaphA and Staph-Seq cells (SIGMA) were used. Blocked StaphA/Staph-Seq cells (100 μL) were added to the antibody/chromatin mixture and rotated for 15 min at room temperature. StaphA/Staph-Seq cells were washed twice with dialysis buffer and four times with polyclonal IP wash buffer. After reversal of cross-links and RNase treatment, DNA was purified and analyzed as described with the Illumina GA2 platform (56). Short-sequence reads were aligned to the genome, and peaks were called using Sole-search (false-discovery rate, 0.0001; α value 0.001) with sequenced HUVEC input DNA as background (57). ChIP-seq data produced by the ENCODE Project Consortium were downloaded from the University of California, Santa Cruz browser and analyzed with Sole-search. Peak overlap was analyzed with Sole-search, allowing a distance of 200 bp between peaks.

Expression Profiling. Control siRNA- and *GATA2* siRNA-treated samples collected 24 h posttransfection were analyzed by hybridization to human 244k expression microarrays (Agilent Technologies). For each sample, 1.2 μg RNA was labeled with Cy-5 (control siRNA) or Cy-3 (*GATA2* siRNA), and labeled cRNAs were combined and hybridized. Data were collected with an Agilent scanner. Two biological replicates for control siRNA/GATA-2 siRNA pairs were each hybridized in duplicate, yielding similar results, and average signals between the four replicates were used to establish fold-change cutoffs.

PCR Primers. Primer sequences are indicated in Dataset S5.

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