BRG1 requirement for long-range interaction of a locus control region with a downstream promoter

Shin-Il Kim[a], Scott J. Bultman[b], Christine M. Kiefer[c], Ann Dean[c], and Emery H. Bresnick[a,1]

[a]Department of Pharmacology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53706; [b]Department of Genetics, University of North Carolina, Chapel Hill, NC 27599; and [c]Laboratory of Cellular and Developmental Biology, National Institutes of Diabetes and Digestive and Kidney Disorders, National Institutes of Health, Bethesda, MD 20892

Edited by Mark T. Groudine, Fred Hutchinson Cancer Research Center, Seattle, WA, and approved December 4, 2008 (received for review July 2, 2008)

The dynamic packaging of DNA into chromatin is a fundamental step in the control of diverse nuclear processes. Whereas certain transcription factors and chromosomal components promote the formation of higher-order chromatin loops, the co-regulator machinery mediating loop assembly and disassembly is unknown. Using mice bearing a hypomorphic allele of the BRG1 chromatin remodeler, we demonstrate that the *Brg1* mutation abrogated a cell type-specific loop between the β-globin locus control region and the downstream *βmajor* promoter, despite *trans*-acting factor occupancy at both sites. By contrast, distinct loops were insensitive to the *Brg1* mutation. Molecular analysis with a conditional allele of GATA-1, a key regulator of hematopoiesis, in a novel cell-based system provided additional evidence that BRG1 functions early in chromatin domain activation to mediate looping. Although the paradigm in which chromatin remodelers induce nucleosome structural transitions is well established, our results demonstrating an essential role of BRG1 in the genesis of specific chromatin loops expands the repertoire of their functions.

chromatin | erythroid | GATA-1 | globin | transcription

Integral to the developmental emergence of specialized cell types is the establishment of cell type-specific chromatin structures. Early studies developed important concepts regarding the impact of nucleosome positioning on protein-chromatin interactions (1), and more recently, ChIP technology (2) ushered in an explosive increase in information on the distribution of histone modifications and nucleosomes genome-wide (3). However, many questions remain unanswered regarding how higher-order chromatin structures are established and regulated.

Nucleosomal filaments assemble into 30-nm fibers, which fold into higher-order loops (4). Chromosome conformation capture (3C) (5) studies have provided evidence for looping in response to *trans*-acting factor binding to chromatin (6–10). Key regulators of erythropoiesis—GATA-1 (11, 12), erythroid Krüppel-like factor (EKLF) (13), and the GATA-1-coregulator friend of GATA-1 (FOG-1) (14)—induce looping at the β-globin locus, in which the proximity of the locus control region (LCR) relative to a distant promoter increases (15, 16). The E-protein-interacting factor NL1/Ldb1 also occupies the LCR and promotes looping (17). However, the role of chromatin modifying and remodeling co-regulators in looping is largely unexplored.

Histone acetylation counteracts higher-order folding of chromatin templates in vitro (18), and broad acetylation characterizes active chromatin domains (19, 20). Thus, it seems likely that histone acetylases and deacetylases are components of the looping machinery. As methylation of histone H3 at lysine 9 serves as a ligand that mediates heterochromatin protein 1 binding during heterochromatin assembly (21–23), the relevant methyltransferases might control looping. Although chromatin remodeling complexes, such as switch/sucrose nonfermentable (SWI/SNF), induce nucleosome structural transitions and alter nucleosome positioning (24, 25), their role in looping is unknown.

Chromatin modifying and remodeling co-regulators have broad biological roles, and therefore genetic perturbations of the respective genes in mice often yield early embryonic lethality. The generation of conditional knockouts or hypomorphic alleles represents a powerful strategy for conducting mechanistic analyses. A mouse strain was isolated containing an ethyl-nitrosourea-induced hypomorphic *Brg1* mutation (26). Although this mutation resides within the ATPase domain, ATPase activity appears to be unaltered. $Brg1^{null/ENU1}$ mice (*Brg1*-mutant) are anemic and die by embryonic day 14.5. β-globin transcription is severely reduced in *Brg1*-mutant fetal livers, even though factors occupy the LCR and the promoter (27). BRG1 is required for maximal RNA polymerase II (Pol II) and serine 5-phosphorylated Pol II (Ser-5-Pol II) occupancy at the promoter. Herein, we demonstrate that GATA-1 recruits BRG1 to a promoter within the β-globin locus more rapidly than other co-regulators and at the commencement of looping. As *Brg1*-mutant cells lacked the β-globin locus loop, whereas additional loops were insensitive to the *Brg1* mutation, these results establish a selective role for a chromatin remodeling enzyme in looping.

Results

Progressive Assembly of a Cell Type-Specific Chromatin Loop. GATA-1 induces a chromatin loop at the β-globin locus, increasing proximity of the LCR and the distant *βmajor* promoter (15, 27). As GATA-1 occupies the LCR before the promoter (27, 28), LCR occupancy might suffice to promote looping (model 1; Fig. 1*A*). By contrast, concomitant LCR and promoter occupancy might be required for looping (model 2). Alternatively, GATA-1 occupancy at the LCR might instigate looping, with subsequent GATA-1 occupancy at the promoter establishing and/or stabilizing the loop (model 3).

To distinguish among these models, we systematically monitored the kinetics of looping and other steps in the activation mechanism. In GATA-1-null cells stably expressing an estrogen receptor ligand binding domain fusion to GATA-1 (ER-GATA-1) (29, 30), ER-GATA-1 activation induces looping at the β-globin locus (15, 27). Culturing G1E-ER-GATA-1 cells at 25 °C abolishes looping (27). At 25 °C, ER-GATA-1 occupies the LCR, but ER-GATA-1 and additional factors are undetectable at the promoter (27). We developed a system in which the LCR complex assembles at 25 °C before looping, and then the culture temperature is changed to 37 °C (Fig. 1*B*), allowing for analysis of steps before, during, and after looping. Subsequent to the temperature transition, *βmajor* primary and mRNA transcripts were maximally induced by 14 and 24 h, respectively (Fig. 1*C*). ER-GATA-1 occupied the LCR DNaseI hypersensitive site HS2 at time 0 (47% of maximum), and occupancy was maximal by 30 min to 3 h (Fig. 1*D*). Considerably

Author contributions: S.-I.K. and E.H.B. designed research; S.-I.K., S.J.B., and C.M.K. performed research; S.J.B. contributed new reagents/analytic tools; S.-I.K., A.D., and E.H.B. analyzed data; and S.-I.K. and E.H.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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

This article contains supporting information online at www.pnas.org/cgi/content/full/0806420106/DCSupplemental.

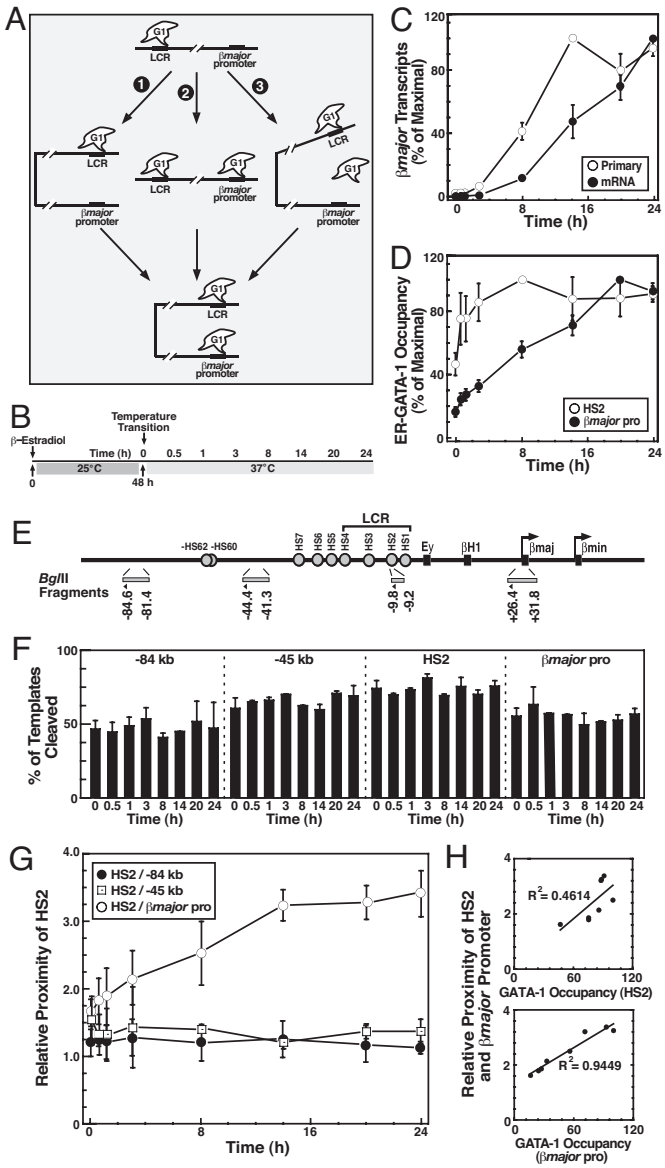

Fig. 1. Progressive assembly of a cell-specific chromatin loop. (*A*) Models of GATA-1-mediated chromatin loop assembly. *Model 1*, GATA-1 occupancy at the LCR induces looping; *Model 2*, simultaneous GATA-1 occupancy at the LCR and the *βmajor* promoter induces looping. *Model 3*, GATA-1 occupancy at the LCR precedes occupancy at the *βmajor* promoter and concomitant looping. (*B*) G1E-ER-GATA-1 cells were treated with β-estradiol at 25 °C for 48 h, and the culture temperature was then increased to 37 °C. At various times thereafter (in h), cells were harvested and analyzed. (*C*) ER-GATA-1-mediated activation of *βmajor* transcription. Real-time RT-PCR was used to analyze *βmajor* primary transcripts and mRNA in G1E-ER-GATA-1 cells under conditions indicated in *B*. Values were normalized by *Gapdh* mRNA (mean ± SE, three independent experiments). (*D*) Quantitative ChIP analysis of ER-GATA-1 occupancy at HS2 and the *βmajor* promoter in G1E-ER-GATA-1 cells under conditions indicated in *B* (mean ± SE, four independent experiments). (*E*) Murine β-globin locus organization. HSs are depicted as filled circles, and embryonic (*Ey* and *βH1*) and adult (*βmaj* and *βmin*) globin genes are depicted as boxes. The diagram depicts the 3C strategy. BglII fragments and primers are depicted as shaded rectangles and triangles, respectively. (*F*) Quantitation of BglII cleavage efficiencies at the indicated sites using real-time PCR. (*G*) 3C analysis of the proximity of a BglII fragment containing the LCR (HS2) relative to fragments containing the −84 and −45 kb regions lacking known regulatory elements or the *βmajor* promoter in G1E-ER-GATA-1 cells under conditions indicated in *B* (mean ± SE, three independent experiments). (*H*) Linear regression analyses of GATA-1 occupancy at the LCR versus looping (*Top*) and GATA-1 occupancy at the *βmajor* promoter versus looping (*Bottom*).

less ER-GATA-1 occupied the promoter at time 0 (16% of maximum), which peaked at 20 h (Fig. 1*D*).

Chromosome conformation capture analysis was conducted to measure the relative proximity of the LCR (HS2) to the *βmajor* promoter. As a control, the relative proximity of regions far upstream of the LCR (−84 kb and −45 kb) to HS2 was assessed (Fig. 1*E*). Under conditions in which ER-GATA-1 activation did not affect BglII cleavage of chromatin at the −84 kb, −45 kb, HS2, and *βmajor* promoter sites (Fig. 1*F*), ligation of HS2 to the *βmajor* promoter increased as a function of ER-GATA-1 activation (Fig. 1*G*). Ligation of the −84 kb and −45 kb sites to HS2 were unchanged. Quantitative comparison of the kinetics of looping with ER-GATA-1 occupancy at the LCR and promoter revealed a tight correlation between looping and promoter occupancy ($R^2 = 0.95$; Fig. 1*H*) and also with primary transcript generation (Fig. 1*C*). These results indicate that either ER-GATA-1 co-occupies the LCR and promoter before looping (model 2), or ER-GATA-1 occupies the LCR, followed by concomitant looping and ER-GATA-1 occupancy at the promoter (model 3).

Rapid Mobilization of the Chromatin Remodeler BRG1 at GATA-1 Target Sites. GATA-1 interacts with multiple co-regulators (31), including FOG-1 (14, 32), CREB-binding protein (CBP)/p300 (33), MED1 (34), and BRG1 (27, 28), and all except MED1 have been shown to occupy the LCR (27, 28, 35, 36). We tested whether ER-GATA-1 occupancy at the LCR and promoter is coupled to co-regulator recruitment at these sites. Co-regulators occupied the LCR maximally by 3 to 8 h [Fig. 2 *A–D* and supporting information (SI) Fig. S3]. FOG-1, CBP, and MED1 occupied the promoter maximally by 20 h (Fig. 2 *E–G* and Fig. S3), consistent with slow ER-GATA-1 occupancy (Fig. 1*D*). BRG1 occupied the promoter maximally by 3 h (Fig. 2*H* and Fig. S3), before major increases in other co-regulators (Fig. 2 *E–G*) and before substantial ER-GATA-1 occupancy at the promoter (Fig. 1*D*). Rapid BRG1 occupancy at the promoter, which was maximal when looping had increased only slightly (Fig. 1*G*), was confirmed with a distinct BRG1 antibody (data not shown). No BRG1 occupancy was detected at the inactive *necdin* promoter (Fig. 2*I*). Thus, BRG1 occupies the promoter before maximal looping, and this is one of the earliest, if not the earliest, GATA-1-dependent step at the promoter or any other GATA-1-regulated promoter studied. Moreover, ER-GATA-1 recruits BRG1 at *Alas2*, a distinct GATA-1 target gene (Fig. S1).

Whereas BRG1 is recruited to chromatin by many factors (37), its role as a co-regulator for EKLF (38), a *trans*-acting factor that activates the β-like globin genes (13), has been highlighted. EKLF binds BRG1, and BRG1 mediates EKLF-dependent transcriptional activation in vitro (38). As EKLF functions at the *βmajor* promoter (28), ER-GATA-1 might rapidly mobilize EKLF and therefore BRG1 at the promoter. However, the kinetics of EKLF occupancy at the promoter were slow (Fig. 2*K* and Fig. S3), resembling ER-GATA-1 (Fig. 1*D*), FOG-1 (Fig. 2*E*), CBP (Fig. 2*F*), and MED1 (Fig. 2*G*), but not BRG1 (Fig. 2*H*). The lack of concomitant EKLF and BRG1 occupancy at the promoter (Fig. 2*L*) indicates that EKLF does not mediate rapid BRG1 recruitment and reinforces our previous analysis demonstrating non-correlative EKLF and BRG1 chromatin occupancy (28). Furthermore, the p45 subunit of nuclear factor erythroid-2 (p45/NF-E2), which also associates with BRG1 (39), occupied the promoter with identical kinetics to ER-GATA-1 (data not shown).

Expanding the Repertoire of Chromatin Remodeler Functions: Selective Control of Chromatin Looping in Vivo. Given the rapidity in which ER-GATA-1 recruits BRG1 to the *βmajor* promoter relative to other co-regulators (Fig. 2*H*), we reasoned that BRG1 might function uniquely in an early activation step. Previously, we used *Brg1*-mutant mice to analyze the role of BRG1 in assembly of the

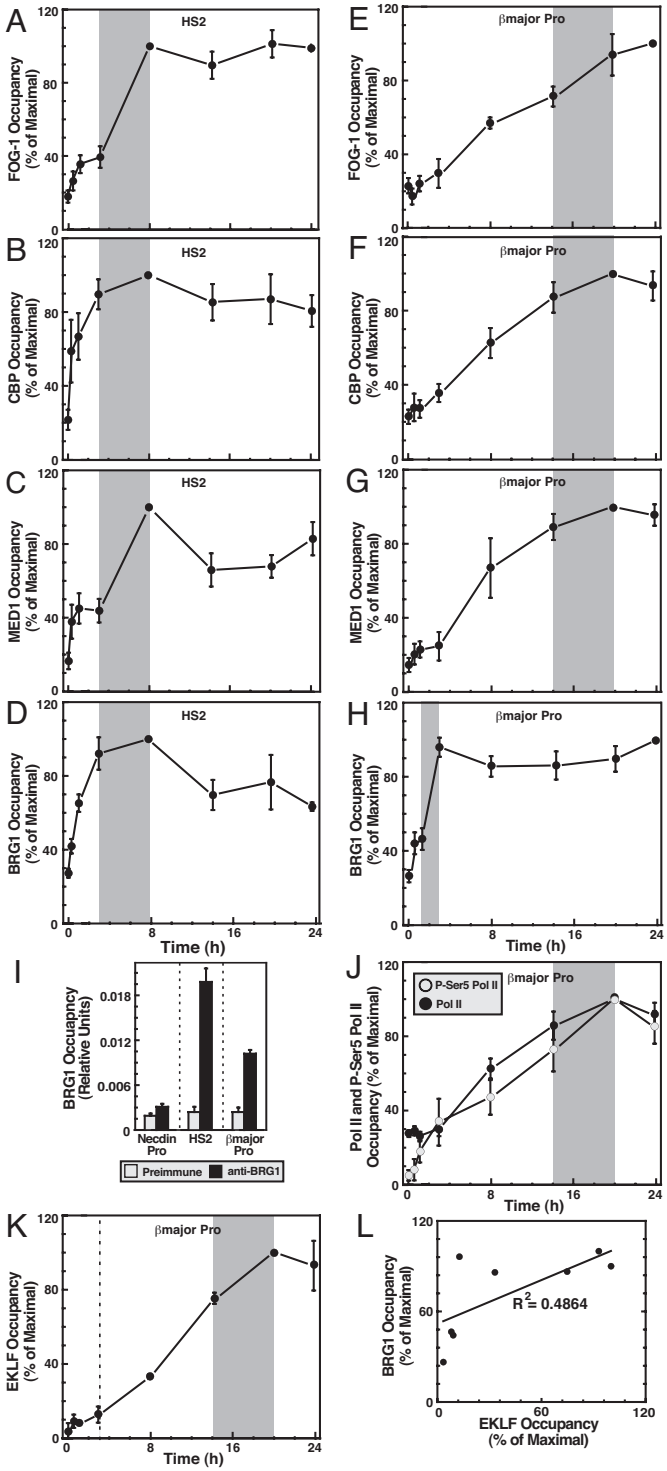

Fig. 2. GATA-1 rapidly mobilizes BRG1 at the promoter. (*A–D*) Co-regulator-LCR interactions. Quantitative ChIP was used to measure FOG-1 (*A*), CBP (*B*), MED1 (*C*), and BRG1 (*D*) occupancy at the LCR (HS2) in G1E-ER-GATA-1 cells under conditions indicated in Fig. 1*B*. (*E–K*) Factor-promoter interactions. Quantitative ChIP was used to measure FOG-1 (*E*), CBP (*F*), MED1 (*G*), BRG1 (*H*), Pol II and Ser-5-Pol II (*J*), and EKLF (*K*) occupancy at the *βmajor* promoter in G1E-ER-GATA-1 cells under conditions indicated in Fig. 1*B*. Quantitative ChIP analysis (in relative units) of BRG1 occupancy at the LCR (HS2), *βmajor* promoter, and necdin promoter (negative control) in G1E-ER-GATA-1 cells under conditions of Fig. 1*B* (*I*) (mean ± SE, three or four independent experiments). The shaded area indicates the range of times in which maximal factor occupancy is achieved. (*L*) Linear regression analysis of EKLF versus BRG1 occupancy at the *βmajor* promoter.

promoter complex (27). Although GATA-1 and p45/NF-E2—both of which are implicated in *βmajor* activation (31)—occupy the promoter normally in the mutant mice, Pol II and Ser-5-Pol II occupancy are significantly reduced (27). Of note, GATA-1 and p45/NF-E2 occupy the promoter in erythroid cells from mice lacking the LCR (15, 40). Thus, *Brg1*- and LCR-mutant mice share certain molecular hallmarks.

As ER-GATA-1 recruits BRG1 rapidly to the promoter (Fig. 2), BRG1-dependent chromatin remodeling might be important for establishing the chromatin loop. Looping and recruitment of maximal levels of Pol II and Ser-5-Pol II to the promoter are impaired when G1E-ER-GATA-1 cells are cultured at 25 °C (27). Reductions of 50% and 70% in Pol II and Ser-5-Pol II, respectively, correlate with dramatically reduced *βmajor* transcription (27, 40). To determine whether BRG1 influences looping, 3C analysis was conducted with WT and *Brg1*-mutant E12.5 fetal livers. We also analyzed a deproteinized BAC containing the murine β-globin locus as well as un-induced and induced G1E-ER-GATA-1 cells. The relative proximities of BglII fragments (Fig. 3*A*) from the BAC were equivalent (Fig. 3*B*) and remarkably resembled un-induced G1E-ER-GATA-1 cells (Fig. 3*C*). ER-GATA-1 activation increased the relative proximity of the LCR and the *βmajor* promoter (Fig. 3*C*). The patterns obtained with WT fetal livers (Fig. 3*D*) and induced G1E-ER-GATA-1 cells (Fig. 3*C*) were indistinguishable. The results with *Brg1*-mutant fetal livers (Fig. 3*D*) and un-induced G1E-ER-GATA-1 cells were indistinguishable (Fig. 3*C*). The BglII cleavage efficiencies were comparable in un-induced versus induced G1E-ER-GATA-1 cells, and also in WT versus *Brg1*-mutant fetal livers (Fig. 3*E*). Thus, BRG1 resembles GATA-1 in mediating establishment and/or maintenance of the loop.

β-globin locus looping also requires FOG-1 (15), LDB1 (17), and EKLF (16). Thus, BRG1 might be required for expression of genes encoding these factors, indirectly influencing looping. By contrast to BRG1-dependent *βmajor* and α-globin expression (S.J.B., unpublished work), GATA-1, LDB1, and FOG-1 mRNA levels are unaltered in *Brg1*-mutant fetal liver ($P = 0.95$, $P = 0.93$, and $P = 0.99$, respectively; Fig. 4*A*). Additional evidence that impaired FOG-1 function does not underlie the looping defect is based on normal GATA-1 occupancy at the *βmajor* promoter in *Brg1*-mutant cells (27), despite the FOG-1 requirement for GATA-1 occupancy at the promoter (41, 42). EKLF mRNA is slightly, but insignificantly, reduced ($P = 0.12$; Fig. 4*A*), and EKLF occupancy at HS2 and *βmajor* promoter is indistinguishable in WT and BRG1 mutant fetal liver cells at embryonic day 12.5 (Fig. 4*B*). These results indicate that GATA-1, FOG-1, LDB1, and EKLF deficiencies do not underlie the looping defect. The normal expression of several erythroid genes suggests that a differentiation blockade does not underlie the looping defect. Finally, the co-immunoprecipitation of ER-GATA-1 and endogenous BRG1 (Fig. 4*C*) further supports a mechanism in which ER-GATA-1-mediated BRG1 recruitment is important for BRG1-dependent looping.

ER-GATA-1 induces a loop at *c-Kit*, which correlates with repression (43), whereas it represses *Gata2* (44) without disrupting a *Gata2* loop (45). *c-Kit* and *Gata2* mRNAs are expressed in WT and *Brg1*-mutant fetal livers, with expression being ≈2-fold higher in *Brg1*-mutant fetal liver (Fig. 4*A*). To address whether the *c-Kit* loop is BRG1-dependent, we conducted 3C analysis with WT and *Brg1*-mutant fetal livers, measuring the relative proximity of the +5 and +58 kb fragments that constitute the established loop (43). The proximity of the +5 and +58 kb fragments was indistinguishable in WT and *Brg1*-mutant fetal livers (Fig. 4*D*). A *Gata2* loop that exists in transcriptionally active and inactive states (45) was also unchanged (Fig. 4*E*). BRG1 is therefore not required for establishing or maintaining chromatin loops globally.

Pol II resides at the β-globin LCR and has been proposed to undergo long-range transfer to the promoter via looping (46). As the LCR is a site of intergenic transcription (47–50),

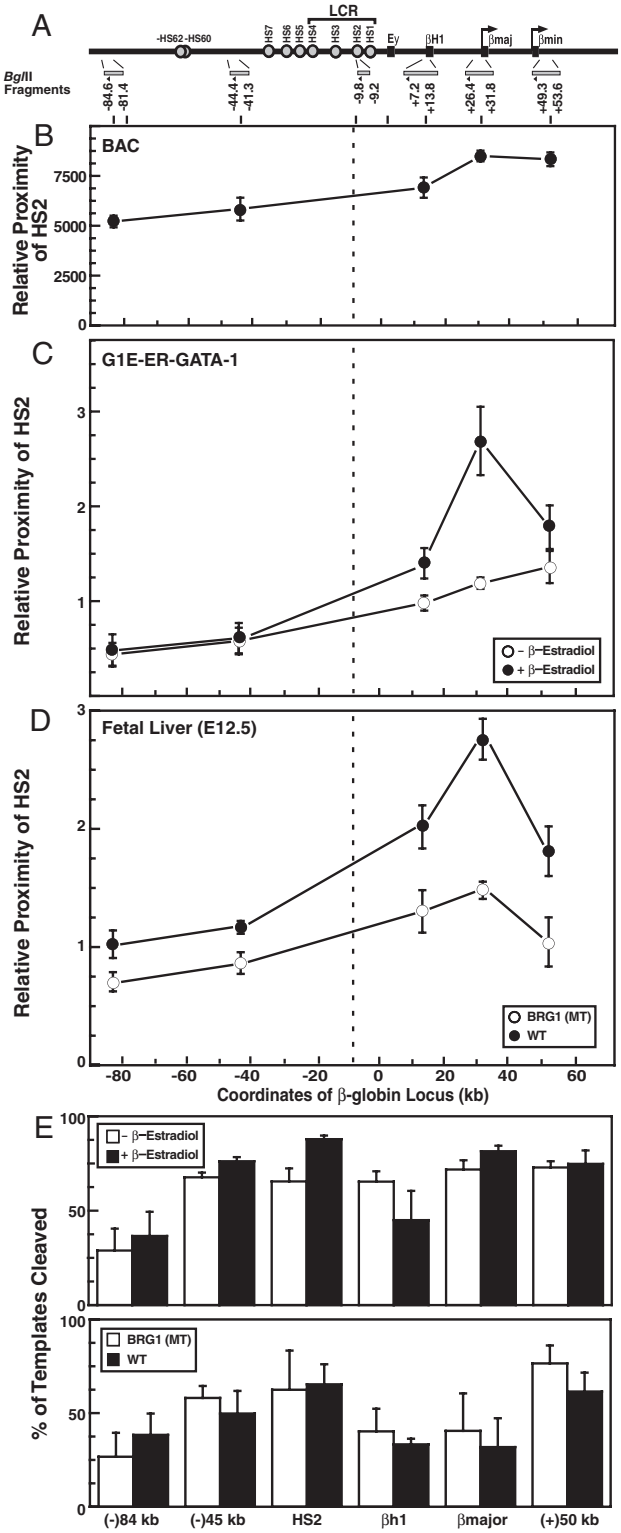

Fig. 3. BRG1 requirement for chromatin looping. (*A*) 3C strategy: BglII fragments and primers are depicted as shaded rectangles and triangles, respectively. (*B–D*) 3C analysis of higher-order structure. The proximity of a BglII fragment containing the LCR (HS2) was measured relative to fragments lacking known regulatory regions (−84 kb, −45 kb, and 3′ of *βminor*), as well as the βh1 and *βmajor* promoters, using the following samples: deproteinized BAC DNA (*B*), untreated or β-estradiol-treated G1E-ER-GATA-1 cells (*C*), and E12.5 fetal liver cells from WT or BRG1 mutant (*MT*) mice (*D*) (mean ± SE, three independent experiments). The vertical dotted line denotes HS2. (*E*) Real-time PCR quantification of BglII cleavage efficiencies. Untreated or β-estradiol-treated G1E-ER-GATA-1 cells (*Top*); WT or BRG1 MT fetal liver cells (*Bottom*).

LCR-associated Pol II might also generate functional transcripts and/or alter chromatin structure in a transcription-dependent manner. However, blocking Pol II elongation has little to no effect on the β-globin locus histone modification pattern (50). GATA-1 increases Pol II occupancy at the LCR, although Pol II occupies the LCR in GATA-1-null cells (30). The GATA-1-dependent increase in LCR-associated Pol II might elevate intergenic transcription as a step in looping. We tested whether GATA-1 regulates intergenic transcripts at the β-globin locus and whether the region between the LCR and the promoter gives rise to transcripts. Few if any transcripts between the LCR and the promoter were detected in un-induced and induced G1E-ER-GATA-1 cells (Fig. S2). Both GATA-1-independent and GATA-1-induced transcripts were detected at the LCR. ER-GATA-1 induced transcripts at HS1 and HS4 with kinetics consistent with LCR complex assembly (Fig. 2 *A–D*), representing GATA factor-regulated intergenic transcription.

Discussion

In principle, chromatin remodelers could control looping via repositioning nucleosomes, modifying nucleosome structure, and/or enhancing factor access to chromatin (25, 51). However, such roles have not been described. We demonstrate herein that a cell type-specific activator occupies a LCR before a distant promoter while rapidly mobilizing a chromatin remodeler at the promoter (Fig. 4*F*). Despite the capacity of GATA-1 to bind multiple co-regulators, BRG1 is mobilized at the promoter more rapidly than other co-regulators in response to GATA-1 occupancy at the LCR. Given that BRG1 occupies the β-globin locus (28, 36), is recruited to chromatin sites by GATA-1, interacts with GATA-1 (Fig. 4*C*), and is rapidly attracted by GATA-1 to the promoter (Fig. 2), it is likely that BRG1 functions directly to establish the chromatin loop. However, one cannot rule out the possibility that BRG1 facilitates chromatin occupancy by unidentified factor(s) and/or functions in a multi-protein complex with other pro-looping factors to induce looping.

In *Brg1*-mutant fetal livers, the loop is undetectable (Fig. 3*D*) despite GATA-1 and p45/NF-E2 occupancy at the *βmajor* promoter (27). Thus, even when *trans*-acting factors co-occupy distal and proximal sites, a chromatin remodeler can be essential to establish and/or maintain loops. As BRG1 is also required for maximal Pol II occupancy at the promoter, it is attractive to propose that looping is required to achieve maximal Pol II levels at the promoter. Whether the BRG1 requirement for looping involves a canonical mechanism to aid unidentified factors in accessing the locus or a novel mechanism is unclear, but it is attractive to propose that remodeling enzymes are key components of the machinery that regulates higher-order chromatin transitions. Whereas purified SWI/SNF can induce higher-order DNA and poly-nucleosomal structures in vitro (52), our results establish a link between chromatin remodelers and chromatin looping in vivo. As certain loops are BRG1-independent, the collapse of the LCR-promoter interaction does not reflect global changes in higher-order structures throughout the nuclear milieu.

With regard to how BRG1 mediates looping, it is instructive to consider the GATA-3-regulated T_H2 cytokine locus (53). GATA factors have unique and overlapping functions (54–56) and can elicit opposite transcriptional responses through common chromatin sites (56). GATA-3, STAT6, and BRG1 occupy the repressed T_H2 locus, in which an LCR associates with the promoters in a "poised" configuration (53). Special AT-rich sequence binding protein 1 (SATB1), a broadly expressed factor that binds the chromatin remodeling components human ACF1 and human SNF2H (57), is required for assembly of a higher-order structure, Pol II and c-Maf occupancy, and transcriptional activation (53). Without SATB1, GATA-3 is insufficient to induce the higher-order

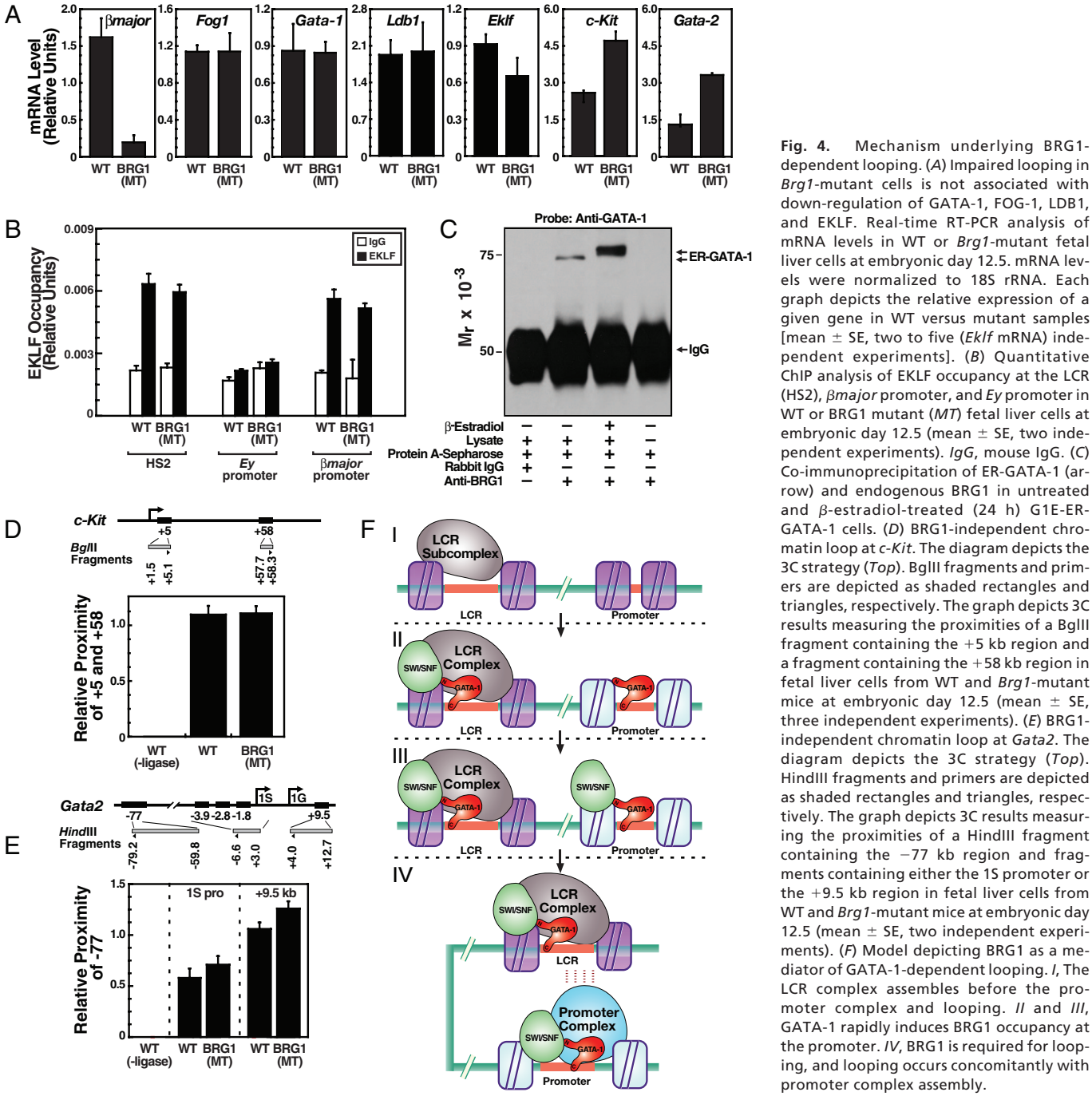

Fig. 4. Mechanism underlying BRG1-dependent looping. (*A*) Impaired looping in *Brg1*-mutant cells is not associated with down-regulation of GATA-1, FOG-1, LDB1, and EKLF. Real-time RT-PCR analysis of mRNA levels in WT or *Brg1*-mutant fetal liver cells at embryonic day 12.5. mRNA levels were normalized to 18S rRNA. Each graph depicts the relative expression of a given gene in WT versus mutant samples [mean ± SE, two to five (*Eklf* mRNA) independent experiments]. (*B*) Quantitative ChIP analysis of EKLF occupancy at the LCR (HS2), *βmajor* promoter, and *Ey* promoter in WT or BRG1 mutant (*MT*) fetal liver cells at embryonic day 12.5 (mean ± SE, two independent experiments). *IgG*, mouse IgG. (*C*) Co-immunoprecipitation of ER-GATA-1 (arrow) and endogenous BRG1 in untreated and β-estradiol-treated (24 h) G1E-ER-GATA-1 cells. (*D*) BRG1-independent chromatin loop at *c-Kit*. The diagram depicts the 3C strategy (*Top*). BglII fragments and primers are depicted as shaded rectangles and triangles, respectively. The graph depicts 3C results measuring the proximities of a BglII fragment containing the +5 kb region and a fragment containing the +58 kb region in fetal liver cells from WT and *Brg1*-mutant mice at embryonic day 12.5 (mean ± SE, three independent experiments). (*E*) BRG1-independent chromatin loop at *Gata2*. The diagram depicts the 3C strategy (*Top*). HindIII fragments and primers are depicted as shaded rectangles and triangles, respectively. The graph depicts 3C results measuring the proximities of a HindIII fragment containing the −77 kb region and fragments containing either the 1S promoter or the +9.5 kb region in fetal liver cells from WT and *Brg1*-mutant mice at embryonic day 12.5 (mean ± SE, two independent experiments). (*F*) Model depicting BRG1 as a mediator of GATA-1-dependent looping. *I*, The LCR complex assembles before the promoter complex and looping. *II* and *III*, GATA-1 rapidly induces BRG1 occupancy at the promoter. *IV*, BRG1 is required for looping, and looping occurs concomitantly with promoter complex assembly.

structure. Additional BRG1 occupies the T_H2 locus upon activation (53), but whether it controls higher-order folding is unclear, and if SATB1 resembles BRG1 in controlling looping in a locus-specific manner is unknown. It will be informative to use conditional mutations and hypomorphic alleles to determine if other SWI/SNF components (and also distinct chromatin remodelers) are critical for the genesis of loops, if BRG1 is uniquely endowed with this activity, and how the underlying mechanisms relate to that of SATB1.

Materials and Methods

Cell Culture. G1E cells expressing ER-GATA-1 were cultured in Iscove modified Dulbecco medium (Gibco/BRL) containing 2% penicillin-streptomycin (Gibco/BRL), 2 U/mL erythropoietin, 120 nM monothioglycerol (Sigma), 0.6% conditioned medium from a Kit ligand-producing CHO cell line, 15% FBS (Gemini Bioproducts), and 1 μg/mL puromycin (Sigma).

Quantitative ChIP Assay. Real-time PCR-based quantitative ChIP analysis was conducted as described (58) and in *SI Materials and Methods*.

Quantitative Real-Time RT-PCR. RNA analysis was conducted as described in *SI Materials and Methods*.

Protein Analysis. Protein analysis was conducted as described in *SI Materials and Methods*.

Chromosome Conformation Capture Assay. 3C analysis was conducted as described (15, 45). A 190-kb BAC (RP23–370E12) clone containing sequences from −100 to +92 kb of the murine β-globin locus was used to assess primer

efficiencies using different primer sets. The BAC clone was a gift from M. Groudine (Fred Hutchinson Cancer Research Center, Seattle, WA). *Gata2* (RP23–196G1) and *c-Kit* (RP23–274L11) BAC clones were from Invitrogen. G1E-ER-GATA-1 cells were induced with β-estradiol for 24 h, cells were harvested, and analyzed. Single-cell suspensions from fetal livers of WT and BRG1-mutant embryos at embryonic day 12.5 were also analyzed. 3C products were normalized to a control interaction at *Ercc3* (59). Band intensities were quantified with ImageJ v1.38 software. 3C primer sequences are available upon request.

ACKNOWLEDGMENTS. This work was funded by National Institutes of Health Grant DK50107 (to E.H.B.), an AHA Predoctoral Fellowship (S.I.K.), and the Intramural Program of the National Institute of Diabetes and Digestive and Kidney Diseases (C.M.K. and A.D.).

1. Hager GL, *et al.* (1993) Influence of chromatin structure on the binding of transcription factors to DNA. *Cold Spring Harb Symp Quant Biol* 58:63–71.
2. Orlando V, Strutt H, Paro R (1997) Analysis of chromatin structure by in vivo formaldehyde cross-linking. *Methods* 11:205–214.
3. Bernstein BE, Meissner A, Lander ES (2007) The mammalian epigenome. *Cell* 128:669–681.
4. Felsenfeld G, Groudine M (2003) Controlling the double helix. *Nature* 421:448–453.
5. Dekker J, Rippe K, Dekker M, Kleckner N (2002) Capturing chromosome conformation. *Science* 295:1306–1311.
6. Tolhuis B, *et al.* (2002) Looping and interaction between hypersensitive sites in the active beta-globin locus. *Mol Cell* 10:1453–1475.
7. Spilianakis CG, Flavell RA (2004) Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. *Nat Immunol* 5:1017–1027.
8. Eivazova ER, Aune TM (2004) Dynamic alterations in the conformation of the Ifng gene region during T helper cell differentiation. *Proc Natl Acad Sci USA* 101:251–256.
9. Vernimmen D, *et al.* (2007) Long-range chromosomal interactions regulate the timing of the transition between poised and active gene expression. *EMBO J* 26:2041–2051.
10. Wang Q, Carroll JS, Brown M (2005) Spatial and temporal recruitment of androgen receptor and its coactivators involves chromosomal looping and polymerase tracking. *Mol Cell* 19:631–642.
11. 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.
12. Evans T, Felsenfeld G (1989) The erythroid-specific transcription factor Eryf1: a new finger protein. *Cell* 58:877–885.
13. Miller IJ, Bieker JJ (1993) A novel, erythroid cell-specific murine transcription factor that binds to the CACCC element and is related to the Kruppel family of nuclear proteins. *Mol Cell Biol* 13:2776–2786.
14. Tsang AP, *et al.* (1997) FOG, a multitype zinc finger protein as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation. *Cell* 90:109–119.
15. 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.
16. Drissen R, *et al.* (2004) The active spatial organization of the beta-globin locus requires the transcription factor EKLF. *Genes Dev* 18:2485–2490.
17. Song SH, Hou C, Dean A (2007) A positive role for NL1/Ldb1 in long-range beta-globin locus control region function. *Mol Cell* 28:810–822.
18. Tse C, Sera T, Wolffe AP, Hansen JC (1998) Disruption of higher-order folding by core histone acetylation dramatically enhances transcription of nucleosomal arrays by RNA polymerase III. *Mol Cell Biol* 18:4629–4638.
19. Forsberg EC, *et al.* (2000) Developmentally dynamic histone acetylation pattern of a tissue-specific chromatin domain. *Proc Natl Acad Sci USA* 97:14494–14499.
20. Bulger M, *et al.* (2003) A complex chromatin landscape revealed by patterns of nuclease sensitivity and histone modification within the mouse beta-globin locus. *Mol Cell Biol* 23:5234–5244.
21. Bannister AJ, *et al.* (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* 410:120–124.
22. Jacobs SA, *et al.* (2001) Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. *EMBO J* 20:5232–5241.
23. Lachner M, *et al.* (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* 410:116–120.
24. Peterson CL, Dingwall A, Scott MP (1994) Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. *Proc Natl Acad Sci USA* 91:2905–2908.
25. Saha A, Wittmeyer J, Cairns BR (2006) Chromatin remodeling: the industrial revolution of DNA around histones. *Nat Rev Mol Cell Biol* 7:437–447.
26. Bultman SJ, Gebuhr TC, Magnuson T (2005) A Brg1 mutation that uncouples ATPase activity from chromatin remodeling reveals an essential role for SWI/SNF complexes in beta-globin expression and erythroid development. *Genes Dev* 19:2849–2861.
27. Kim S-I, *et al.* (2007) Dissecting molecular steps in chromatin domain activation during hematopoietic differentiation. *Mol Cell Biol* 27:4551–4565.
28. 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.
29. Gregory T, *et al.* (1999) GATA-1 and erythropoietin cooperate to promoter erythroid cell survival by regulating bcl-xl expression. *Blood* 94:87–96.
30. Johnson KD, *et al.* (2002) Cooperative activities of hematopoietic regulators recruit RNA polymerase II to a tissue-specific chromatin domain. *Proc Natl Acad Sci USA* 99:11760–11765.
31. Kim S-I, Bresnick EH (2007) Transcriptional control of erythropoiesis: emerging mechanisms and principles. *Oncogene* 26:6777–6794.
32. 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.
33. Blobel GA, *et al.* (1998) CREB-binding protein cooperates with transcription factor GATA-1 and is required for erythroid differentiation. *Proc Natl Acad Sci USA* 95:2061–2066.
34. Stumpf M, *et al.* (2006) The mediator complex functions as a coactivator for GATA-1 in erythropoiesis via subunit Med1/TRAP220. *Proc Natl Acad Sci USA* 103:18504–18509.
35. Letting DL, Rakowski C, Weiss MJ, Blobel GA (2003) Formation of a tissue-specific histone acetylation pattern by the hematopoietic transcription factor GATA-1. *Mol Cell Biol* 23:1334–1340.
36. Mahajan MC, *et al.* (2005) Heterogeneous nuclear ribonucleoprotein C1/C2, MeCP1, and SWI/SNF form a chromatin remodeling complex at the beta-globin locus control region. *Proc Natl Acad Sci USA* 102:15012–15017.
37. de la Serna IL, Ohkawa Y, Imbalzano AN (2006) Chromatin remodelling in mammalian differentiation: lessons from ATP-dependent remodellers. *Nat Rev Genet* 7:461–473.
38. Zhang W, Kadam S, Emerson BM, Bieker JJ (2001) Site-specific acetylation by p300 or CREB binding protein regulates erythroid Kruppel-like factor transcriptional activity via its interaction with the SWI-SNF complex. *Mol Cell Biol* 21:2413–2422.
39. Brand M, *et al.* (2003) Dynamic changes in transcription factor complexes during erythroid differentiation revealed by quantitative proteomics. *Nat Struct Mol Biol* 11:73–80.
40. Sawado T, Halow J, Bender MA, Groudine M (2003) The beta-globin locus control region (LCR) functions primarily by enhancing the transition from transcription initiation to elongation. *Genes Dev* 17:1009–1118.
41. Pal S, *et al.* (2004) Coregulator-dependent facilitation of chromatin occupancy by GATA-1. *Proc Natl Acad Sci USA* 101:980–985.
42. Letting DL, *et al.* (2004) Context-dependent regulation of GATA-1 by friend of GATA-1. *Proc Natl Acad Sci USA* 101:476–481.
43. Jing H, *et al.* (2008) Exchange of GATA factors mediates transitions in looped chromatin organization at a developmentally regulated gene locus. *Mol Cell* 29:232–242.
44. 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.
45. Grass JA, *et al.* (2006) Distinct functions of dispersed GATA factor complexes at an endogenous gene locus. *Mol Cell Biol* 26:7056–7067.
46. Johnson KD, Christensen HM, Zhao B, Bresnick EH (2001) Distinct mechanisms control RNA polymerase II recruitment to a tissue-specific locus control region and a downstream promoter. *Mol Cell* 8:465–471.
47. Kong S, Bohl D, Li C, Tuan D (1997) Transcription of the HS2 enhancer toward a cis-linked gene is independent of the orientation, position, and distance of the enhancer relative to the gene. *Mol Cell Biol* 17:3955–3965.
48. Kim A, Zhao H, Ifrim I, Dean A (2007) Beta-globin intergenic transcription and histone acetylation dependent on an enhancer. *Mol Cell Biol* 27:2980–2986.
49. Gribnau J, *et al.* (2000) Intergenic transcription and developmental remodeling of chromatin subdomains in the human beta-globin locus. *Mol Cell* 5:377–386.
50. Johnson KD, *et al.* (2003) Highly restricted localization of RNA polymerase II to the hypersensitive site cores of a tissue-specific locus control region. *Mol Cell Biol* 23:6468–6493.
51. Cote J, Quinn J, Workman JL, Peterson CL (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF. *Science* 265:53–60.
52. Bazett-Jones DP, *et al.* (1999) The SWI/SNF complex creates loop domains in DNA and polynucleosomal arrays and can disrupt DNA-histone contacts within these domains. *Mol Cell Biol* 19:1470–1478.
53. Cai S, Lee CC, Kohwi-Shigematsu T (2006) SATB1 packages densely looped, transcriptionally active chromatin for coordinated expression of cytokine genes. *Nat Genet* 38:1278–1288.
54. Molkentin JD (2000) The zinc finger-containing transcription factors GATA-4, −5, and −6. Ubiquitously expressed regulators of tissue-specific gene expression. *J Biol Chem* 275:38949–38952.
55. Cantor AB, Orkin SH (2002) Transcriptional regulation of erythropoiesis: an affair involving multiple partners. *Oncogene* 21:3368–3376.
56. Bresnick EH, Martowicz ML, Pal S, Johnson KD (2005) Developmental control via GATA factor interplay at chromatin domains. *J Cell Physiol* 205:1–9.
57. Yasui D, *et al.* (2002) SATB1 targets chromatin remodeling to regulate genes over long distances. *Nature* 419:641–645.
58. Im H, *et al.* (2004) Measurement of protein-DNA interactions in vivo by chromatin immunoprecipitation. *Methods Mol Biol* 284:129–146.
59. Palstra RJ, *et al.* (2003) The beta-globin nuclear compartment in development and erythroid differentiation. *Nat Genet* 35:190–194.