The chromatin-remodeling enzyme BRG1 plays an essential role in primitive erythropoiesis and vascular development

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ATP-dependent chromatin-remodeling complexes contribute to the proper temporal and spatial patterns of gene expression in mammalian embryos and therefore play important roles in a number of developmental processes. SWI/SNF-like chromatin-remodeling complexes use one of two different ATPases as their catalytic subunit: brahma (BRM, also known as SMARCA2) and brahma-related gene 1 (BRG1, also known as SMARCA4). We have conditionally deleted a floxed Brg1 allele with a Tie2-Cre transgene, which is expressed in developing hematopoietic and endothelial cells. Brg1fl/fl-Tie2-Cre+ embryos die at midgestation from anemia, as mutant primitive erythrocytes fail to transcribe embryonic α- and β-globins, and subsequently undergo apoptosis. Additionally, vascular remodeling of the extraembryonic yolk sac is abnormal in Brg1fl/fl-Tie2-Cre+ embryos. Importantly, Brm deficiency does not exacerbate the erythropoietic or vascular abnormalities found in Brg1fl/fl-Tie2-Cre+ embryos, implying that Brg1-containing SWI/SNF-like complexes, rather than Brm-containing complexes, play a crucial role in primitive erythropoiesis and in early vascular development.

KEY WORDS: SWI/SNF, Brg1, Tie2-Cre, Erythropoiesis, β-globin, Vascular remodeling, Angiogenesis

INTRODUCTION

Developmental processes require changes in gene expression to achieve cellular differentiation. Eukaryotes use chromatin-modifying factors to aid in the regulation of gene expression because large nuclear factors necessary for transcription cannot access DNA when it is tightly bound to histones in nucleosomes. Two main classes of chromatin-modifying factors achieve changes in chromatin structure and organization at individual genes. One class covalently modifies histone proteins to achieve a heritable epigenetic mark instructing further genetic regulation. The second class uses energy derived from ATP hydrolysis to alter the conformation or position of nucleosomes, thereby transiently making gene promoters accessible or inaccessible to large nuclear factors. Both classes of chromatin-modifying factors are necessary to achieve proper temporal and spatial patterns of gene expression in the embryo, and therefore play important roles in a number of developmental processes (de la Serna et al., 2006; Margueron et al., 2005).

The mammalian SWI/SNF-related chromatin-remodeling complexes comprise one major family of ATP-dependent chromatin-modifying factors. These large, multi-protein complexes use one of two different ATPases as their catalytic subunit: brahma (BRM, also known as SMARCA2) and brahma-related gene 1 (BRG1, also known as SMARCA4). The significance of SWI/SNF-related complexes in mammalian development is particularly well demonstrated by the phenotypes associated with mice carrying mutations in Brg1. Brg1+/- embryos die at the peri-implantation stage of development, and conditional alleles have been used to demonstrate the role of Brg1 in T-cell development, limb morphogenesis, skin development, gliogenesis and zygotic genome activation (Bultman et al., 2000; Bultman et al., 2006; Gebuhr et al., 2003; Indra et al., 2005; Matsumoto et al., 2006). By contrast, Brm+/- mice develop normally, although adult mutants are 15% heavier than their control littermates, possibly because of increased cellular proliferation (Reyes et al., 1998). As BRG1 is significantly upregulated in Brm+/- mice, it has been hypothesized that BRG1 can functionally compensate for the loss of BRM during development (Reyes et al., 1998).

We previously isolated and characterized an N-ethyl-N-nitrosourea (ENU)-induced point mutation in Brg1 (Brg1ENU1) that changes a single amino-acid residue (E1083G) in a highly conserved region of the catalytic ATPase domain (Bultman et al., 2005). The mutant protein is stable, assembles into SWI/SNF-related complexes, and exhibits normal ATPase activity, but has diminished nucleosome-remodeling capability. Brg1ENU1 embryos fail to transcribe adult β-globin genes, thereby indicating that BRG1 plays an important role in chromatin remodeling of the β-globin locus during definitive erythropoiesis. However, because hypomorphic Brg1enu1/Brg1ENU1 embryos express embryonic β-globin genes and undergo normal primitive erythropoiesis, it has been unclear whether BRG1 and SWI/SNF-related complexes are involved in this earlier hematopoietic process. Using a conditional null allele, we now report that BRG1 and SWI/SNF-related complexes are involved in this earlier hematopoietic process. Using a conditional null allele, we now report that BRG1, but not BRM, is indeed recruited to the β-globin locus control region in primitive erythrocytes, and is required for the transcription of embryonic β-globin genes and for erythroblast survival. We also demonstrate for the first time that BRG1 is required for embryonic α-globin expression, although expression of adult α-globins does not depend upon BRG1-induced remodeling in primitive erythrocytes. Finally, BRG1 appears to play an important role in vascular development that is separable from its role in primitive erythropoiesis.

MATERIALS AND METHODS

Mice

The Brg1-floxed mice (Gebuhr et al., 2003), the Brm+/- mice (Reyes et al., 1998), the Tie2-Cre transgenic mice (Koni et al., 2001) and the ROSA26R mice (Soriano, 1999) have been described. All mice were maintained on a mixed genetic background at the University of North Carolina, Chapel Hill Animal Facility. Brg1-floxed mice were genotyped by Southern blot analysis using a 449 bp probe against the 5’ portion of intron 14 and genomic DNA digested with BamHI. The PCR primers used to generate the Southern probe

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were as follows: forward, 5'-TGGCATCTCATTTGTGTGGT-3'; and reverse, 5'-ACAGCCTCGTGGTAGGGATG-3'. The Southern blot yields a 7.7 kb floxed allele, a 6.0 kb wild-type allele and a 5.5 kb excised allele. 

Brg I-floxed embryos were genotyped by PCR using the following primers: forward, 5'-GTCTATTTTGTCTATGCCG-3'; and reverse, 5'-GCCTTTGCTCCTAAGTGAAG-3'. These primers flank the 3' LoxP site, and yield a 387 bp floxed allele and a 230 bp wild-type allele. The PCR was performed at an annealing temperature of 51°C. Brm+/+ mice and embryos were genotyped by PCR using the following primers for the 197 bp wild-type allele: forward, 5'-ATATCAGGAGAGGCCACAA-3'; and reverse, 5'-TGGACATTGTGGAGGAGG-3'. The 600 bp targeted allele was amplified using the same forward primer and the following reverse primer: 5'-CTATGCCTCTTATCGCTCCTC-3'. The PCR was performed at an annealing temperature of 55°C. Tie2-Cre transgenic mice and embryos were genotyped using a gene-specific forward primer (5'-GGGAAGTCGC-AAAGTTGTGAGTT-3') and a Cre-specific reverse primer (5'-TGAGACCATGCTGTCCTC-3') that amplifies a 400 bp product. Control primers amplifying a 324 bp product from the IIL2 gene were used as a template control: forward, 5'-CTAGGCCCAAGATTTGAGATCT-3'; and reverse, 5'-GTAGGTTGAAATCTTACATCCTC-3'. The PCR was performed at an annealing temperature of 51°C. ROSA26R mice were genotyped as described (Soriano, 1999). All histological sections were scraped from paraffin or OCT (Tissue-Tek) into DEXPAT Reagent (TaKaRa) before genotyping.

Histology

Embryos and yolk sacs were dissected from maternal tissue, immersion-fixed in 4% paraformaldehyde overnight, dehydrated, embedded in paraffin, sectioned (10 μm), and stained with hematoxylin and eosin. For cryosections, embryos were dissected, fixed, and passed through 10% sucrose (10 minutes), 15% sucrose (10 minutes), 20% sucrose (1 hour) and 20% sucrose/OCT (overnight), and then embedded in OCT on dry ice before sectioning (10 μm) and mounting on Superfrost/Plus microscope slides (Fisher). Electron microscopy was performed as described (Schwarz et al., 2002) on embryonic day 10.5 (E10.5) yolk sacs from two mutant and two control littermate embryos with visible heart beats.

Staining

Whole-mount immunostaining for platelet/endothelial cell adhesion molecule 1 (PECAM1) was performed as described using a rat anti-mouse PECAM1 monoclonal antibody (BD Pharmingen) (Schlaeger et al., 1995). Whole-mount β-galactosidase staining was performed as described (Schlaeger et al., 1995), and stained embryos were subsequently cryosectioned and counterstained with Nuclear Fast Red. Transferase-mediated deoxyuridine triphosphate (dUTP) nick-end labeling (TUNEL) staining was performed on paraffin-embedded tissues using the In Situ Cell Death Detection Kit, Fluorescein (Roche), according to the manufacturer’s instructions. Benzidine staining was performed on cryosectioned embryos and yolk sacs. After a brief submersion in PBS, slides were incubated in methanol (15 seconds), 1% benzidine (Sigma-Aldrich) in methanol (5 minutes), 2.5% hydrogen peroxide in 70% ethanol (2.5 minutes), and washed in water (2.5 minutes).

In situ hybridization

Gene-specific antisense probes to the murine εy, βH1, ζ and αI/2 globins have been described (Kingsley et al., 2006). A 382 bp antisense probe for murine Band3 was amplified from E8.5 yolk sac cDNA using the following primers: forward, 5'-AGGAGCTAACCACCTCCTGTTG-3'; and reverse, 5'-TCTGATTCCTGTAGTAGGAAGC-3'. A 425 bp antisense probe for murine Alas2 was amplified from E8.5 yolk sac cDNA using the following primers: forward, 5'-CCATCTGAGGACGTGATGAGA-3'; and reverse, 5'-CATAGATGTCGTGCTTGGAG-3'. In vitro transcription was performed to generate digoxigenin-labeled RNA probes. Cryosectioned embryos were subjected to a 10-minute proteinase K (2.5 μg/ml) pretreatment before in situ hybridization. Prehybridization and hybridization incubations were carried out at 60°C in a mixture of 50% formamide, 5×SSC, 2% blocking reagent (Roche), 0.1% Triton-X100, 0.5% CHAPS, 50 μg/ml yeast tRNA, 5 mM EDTA and 100 μg/ml heparin. After overnight hybridization, slides were washed and incubated with anti-digoxigenin-AP Fab fragments (Roche) overnight at 4°C. After further washing, slides were incubated with NBT/BCIP at room temperature for several hours, or at 4°C for up to three days.

Chromatin immunoprecipitation (ChIP)

To obtain primitive erythrocytes, approximately 40 wild-type E9.5-E10.5 embryos were separated from their placentae by severing the umbilical vessels. The embryos with their attached yolk sacs were immediately placed in minimal essential media containing 2% fetal bovine serum (JRH Biosciences) and were rocked on a Nutator mixer for approximately 30 minutes at room temperature while allowing the hearts to pump the majority of circulating blood out of the embryos and yolk sacs. Embryos and yolk sacs were removed from the media, and the remaining blood cells were counted on a hemocytometer. Typically 20×10^6 to 50×10^6 blood cells were collected. The fresh cells were used in ChIP assays as previously described (Bultman et al., 2005), with some modifications: 7×10^6 cells were used for each ChIP or mock reaction; 0.6% formaldehyde was used to cross-link the protein-protein and protein-DNA interactions; and chromatin was sonicated with eleven 10-second pulses at 10% maximum power on a Branson Digital Sonifier. The anti-BRG1 antibody J1 (a gift from G. Crabtree and W. Wang, Stanford University) and the anti-acetyl-histone 3 antibody (Santa Cruz, 06-599) were used for immunoprecipitations. The β-globin locus control region DNAseq hypersensitive site 3 (HS3) primers that were used for amplification of the ChIP products and controls were as follows: forward, 5'-AGGCTCTTCTAAGGGACGTGAGA-3'; and reverse, 5'-AGACTACCCCTTGGACTGAA-3'. The 158 bp product was amplified at an annealing temperature of 55°C for 35 cycles. The amplification product was visualized by ethidium bromide staining of 2% agarose gel. A 425 bp antisense probe for Tie2 was used as a control and was amplified at an annealing temperature of 55°C for 35 cycles. The amplification product was visualized by ethidium bromide staining of 2% agarose gel. A 425 bp antisense probe for Tie2 was used as a control and was amplified at an annealing temperature of 55°C for 35 cycles. The amplification product was visualized by ethidium bromide staining of 2% agarose gel.

RESULTS

**Brg1**/hiTie2-Cre+/- embryos die during development

We initially analyzed mice carrying a conditionally floxed allele of **Brg1** in combination with a Tie2-Cre recombinase transgene, which is expressed in developing hematopoietic and endothelial cells (Koni et al., 2001). We recovered no live **Brg1**/hiTie2-Cre+/- offspring from matings between **Brg1**/hi and **Brg1**/hiTie2-Cre+/- mice (Table 1), indicating that expression of **Brg1** on Tie2+ cells is critical for embryonic development. These data also indicate that BRG1 does not compensate for loss of **Brg1** on Tie2+ cells, despite the observation that BRG1 and BRM can play partially redundant roles in vitro (Chiba et al., 1994; Phelan et al., 1999; Strobeck et al., 2002).
Histological sections of Cre+/0 Brm–/–;Brg1fl/fl in endothelial cell; VE, visceral endoderm. Scale bars in E,F: 5 cells and blood cell fragments. EB, embryonic blood cell; EC, developmental importance of numbers. Together, our genetic data clearly illustrate the BRG1 in blood and vascular development from our observation that ablation of BRG1 in developing endothelium and hematopoietic cells comes

Table 1. Brg1fl/+;Tie2-Cre+/0 embryos do not die during development

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<th>Genotype</th>
<th>Live progeny (expected progeny)</th>
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<td>Brm+/--;Brg1fl/+;Tie2-Cre+/0</td>
<td>24 (18)</td>
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<tr>
<td>Brm+/--;Brg1fl/+;Tie2-Cre+/0</td>
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Brm+/--;Brg1fl/+ females were mated with Brm+/--;Brg1fl/+;Tie2-Cre+/0 males, and live progeny from 12 litters were genotyped and scored at weaning. Only one live Brm+/--;Brg1fl/+;Tie2-Cre+/0 mouse was recovered (x13, P<0.001).

Table 2. Brm+/--;Brg1fl/+;Tie2-Cre+/0 embryos do not die during development

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Brg1fl/+;Tie2-Cre+/0 blood cells undergo apoptosis

In order to determine the time of death of Brg1fl/+;Tie2-Cre+/0 embryos, we performed dissections on E8.5-E12.5 embryos. We chose midgestation for our initial analysis as many mutants with hematopoietic or vascular defects die at this developmental stage (Coultas et al., 2005; Orkin and Zon, 1997). We determined that Brg1fl/+;Tie2-Cre+/0 embryos die at E10.5-E11.0, based on the absence of a visible heartbeat and the onset of necrosis. By E9.5, mutant embryos are visibly paler than their control littermates, and mutant yolk sacs lack the large, blood-filled vitelline vessels that are readily visible in control yolk sacs. Importantly, no exacerbation of the timing or severity of this gross mutant phenotype is detected on a Brm–/– background (11 Brm–/–;Brg1fl/+;Tie2-Cre+/0 embryos were assayed at E9.5-E10.5), further demonstrating the importance of Brg1 over Brm in hematopoietic and/or vascular development.

The extreme pallor of mutant embryos at E9.5 cannot be explained by hemorrhage. Upon gross dissection, no embryonic blood is visibly pooled in the extravascular exocoelomic, amniotic or pericardial cavities of mutant embryos. Likewise, histological analysis of control and mutant embryos from 19 litters (E8.5-E10.5), embedded intact within the maternal uterus so as not to disrupt any sites of pooled embryonic blood, failed to reveal any sign of hemorrhage.

The extraembryonic yolk sac is the initial site of hematopoiesis and produces large, nucleated blood cells that begin to circulate between the yolk sac and embryo at E8.25 (McGrath et al., 2003). Such blood cells are abundant in histological sections of E8.5 Brm–/–;Brg1fl/+;Tie2-Cre+/0 yolk sacs, demonstrating that hematopoiesis is unimpaired (Fig. 1B). However, by light and electron microscopy, a dramatic scarcity of blood cells is found in mutant embryos and yolk sacs at E9.5 and E10.5 (Fig. 1D). Furthermore, many of those blood cells that remain are fragmented and show abnormal membrane or nuclear morphology (Fig. 1F). In order to determine whether embryonic blood cells undergo apoptosis between E8.5 and E9.5, we performed TUNEL staining on sections of E9.5 mutant and control littermate embryos and yolk sacs. Blood cells from mutant embryos show more evidence of TUNEL staining than those from control embryos (13.6±0.75% versus 0.4±0.2%, mean±s.e.m.), indicating that blood from Brg1fl/+;Tie2-Cre+/0 embryos is subject to aberrant apoptosis (Fig. 2).

To determine whether the blood cell death detected in mutant embryos is cell autonomous, we crossed Brg1fl/+;Tie2-Cre+/0 embryos onto the ROSA26R mouse line (Soriano, 1999). ROSA26R mice express β-galactosidase upon Cre-mediated deletion of a ‘STOP’ signal; therefore, cells that express Tie2-Cre turn blue upon X-gal staining in the presence of this reporter line. At E8.5, both control and mutant embryos display blue endothelium and comparable amounts of blue blood cells in their yolk sac vasculature (Fig. 3A.B.E). These data indicate that the majority of embryonic blood cells express Tie2-Cre at E8.5 and that Brg1 is not required for
production of these cells. At E9.5, both control and mutant embryos continue to display blue endothelium, but whereas control embryos reveal a preponderance of circulating blue blood cells (86±3.9%), mutants (which contain fewer blood cells overall) display a minority of blue blood cells (33.5±11.5%; Fig. 3C-E). Additionally, many of the blue blood cells in mutants are fragmented or display abnormal morphology (Fig. 3D, inset), whereas the non-blue blood cells retain normal morphology. This indicates that blood cells undergoing Brgly hemoglobin transcription. Because of the mixed population of wild-type and mutant blood cells in Brgly embryos at E9.5 (see Fig. 3D), we could not analyze the expression of α-globin and β-globin genes by RT-PCR. Instead, we performed in situ hybridization on circulating blood cells to detect hemoglobin production and/or accumulation is defective in Brgly primitive erythrocytes. Gobin tetramers encoded by the α- and β-globin gene loci are critical components of hemoglobin. The globin loci are multi-gene clusters: in mice there are three functional α-globins (α1, α2 and α3) and four functional β-globins (β1, β2, β3 and β4). Whereas primitive erythroblasts express all of the globin genes, definitive erythrocytes express only the adult globins (α1, α2, β1 and β2). Therefore a transition occurs during development (E10.5-E13.5) in which embryonic globin expression diminishes and adult globin expression escalates.

Brg1fl/fl:Tie2-Cre+/0 blood cells have defective embryonic globin transcription

The majority of circulating blood cells at E9.5 are large, nucleated erythroblasts, which are generated in yolk sac blood islands between E7.25-E9.0 (Palis et al., 1999; Wong et al., 1986). Once formed, these ‘primitive’ erythroblasts continue to divide for several days before entering the blood stream, undergoing maturation, and eventually enucleating (Kingsley et al., 2004). ‘Definitive’ erythroblasts, which originate in the yolk sac, placenta and aorta/gonad/mesonephros region, colonize the fetal liver where they expand and differentiate before entering the blood stream as mature, enucleated erythrocytes at E11.5-E12.5 (reviewed by McGrath and Palis, 2005; Mikkola et al., 2005). Definitive erythrocytes are the predominant cell type in the embryonic circulation after E12.5.

As they mature, both primitive and definitive erythroblasts accumulate hemoglobin, the oxygen-carrying component of red blood cells. In order to assess the production of hemoglobin in Brgly:Tie2-Cre+/0 embryonic blood cells, we stained histological sections of control and mutant E9.5 embryos with benzidine (see Fig. S1 in the supplementary material). Although blood cells in control embryos exhibit strong staining, many blood cells in mutant embryos show little or no benzidine staining. These data indicate that hemoglobin production and/or accumulation is defective in Brgly:Tie2-Cre+/0 primitive erythrocytes.

Brg1fl/fl:Tie2-Cre+/0 embryos undergo apoptosis at E9.5. (A-D) TUNEL staining on histological sections of E9.5 littermate control Brgly (A) and mutant Brgly:Tie2-Cre+/0 (B) embryos, and their corresponding control (C) and mutant (D) yolk sacs. The images were merged from separate DAPI (blue) and TUNEL (green) acquisitions. The arrow in A points to the lumen of an embryonic heart (outlined in white), filled with embryonic blood cells. The inset in B focuses on TUNEL-positive blood cells found within one of the paired dorsal aortae (arrows and outlined in white) of the mutant embryo. No TUNEL-positive blood cells are detected in the control yolk sac vessel (C, outlined in white), but TUNEL-positive blood cells are evident in the mutant yolk sac vessel (D, outlined in white; shown at higher magnification in the inset in D). Scale bars: 100 μm. (E) Mean percentages of TUNEL-positive blood cells from multiple serial sections of two control and two mutant embryos stained in three independent experiments. A total of 596 and 362 blood cells were counted from control and mutant sections, respectively. Errors were calculated as s.e.m.

Brg1fl/fl:Tie2-Cre+/0 blood cells have defective embryonic globin transcription
Kingsley et al., 2004). Using an anti-BRG1 antibody, we were able to demonstrate BRG1 binding to the DNAse I hypersensitive site 3 (HS3) of the β-globin LCR, where it is recruited in definitive erythrocytes as well (Fig. 4J). These ChIP data indicate that the defects we detect in embryonic β-globin expression by in situ hybridization directly result from loss of BRG1-induced chromatin remodeling at the β-globin LCR in mutant primitive erythrocytes. Furthermore, we were able to detect BRG1 binding at the promoter of the embryonic α-globin ζ, whereas BRG1 does not bind the promoter of the adult α-globins αl/2 (Fig. 4I). These ChIP results for the α-globin genes are consistent with our in situ hybridization results, which indicate BRG1 involvement in ζ but not αl/2 expression at E9.5. Therefore, we provide the first evidence that BRG1 is important for embryonic α-globin transcription in primitive erythrocytes. Overall, the reduction in blood cells expressing embryonic globins, and the reduction in blood cells expressing Tie2-Cre by reporter analysis in E9.5 mutant embryos (see Fig. 3D,E), lead us to hypothesize that primitive erythrocytes having undergone excision of Brgl are unable to support normal levels of embryonic globin transcription, resulting in their destruction through apoptosis.

**Vascular remodeling is abnormal in Brglfl/fl:Tie2-Cre+/-yolk sacs**

Because Tie2-Cre is expressed in developing endothelium as well as in hematopoietic cells, we analyzed the vasculature of Brglfl/0:Tie2-Cre+/- embryos by immunostaining embryos and yolk sacs with an antibody against the endothelial cell marker PECAM1. Yolk sac vascular development consists of two major steps: vasculogenesis, the process by which new blood vessels arise and form a primitive vascular plexus; and angiogenesis, the process by which the homogenous plexus undergoes vascular remodeling resulting in a hierarchical vascular tree comprising large and small vessels (Sato and Loughna, 2002). By E8.5, the time at which the primitive vascular plexus is formed, Tie2-Cre-mediated excision occurs efficiently in yolk sac endothelium (Fig. 3A,B). Yet Brglfl/0:Tie2-Cre+/- yolk sac vasculature is indistinguishable from that seen in control yolk sacs at this time (Fig. 3A,B), demonstrating that the vascular plexus formation/maintenance stages of vasculogenesis proceed normally in mutant yolk sacs. By E9.5, however, Brglfl/0:Tie2-Cre+/- yolk sacs demonstrate an aborted vascular remodeling process (Fig. 5D,E). Whereas their littermate controls successfully undergo angiogenesis, resulting in yolk sacs with interconnected large and small vessels, mutant yolk sacs progress beyond the plexus stage of vascular development but fail to establish a mature vascular tree. Instead, the mutant yolk sac vasculature consists of small and medium-sized vessels that occasionally fail to interconnect. Many dead-end or tapering vascular termini are visible in the mutant yolk sacs, indicating that vascular sprouting or pruning fails to progress normally (Fig. 5E). Dysregulated cardiac function and blood flow are unlikely causes of these vascular abnormalities because heart beat rates are normal and pericardial edema is rarely detected in Brglfl/0:Tie2-Cre+/- embryos at E9.5 when the yolk sac vascular abnormalities are readily apparent. Interestingly, PECAM1 immunostaining of the vasculature within the mutant embryo proper appears normal and comparable to that seen in control littermates or stage-matched embryos at E9.5 (data not shown). Therefore, the vascular abnormalities we observe on the Brglfl/0:Tie2-Cre+/- background are specific to the yolk sac. Finally, although BRM expression in the yolk sac is restricted to vascular endothelium (Dauvillier et al., 2001), we did not see any exacerbation of the Brglfl/0:Tie2-Cre+/- vascular phenotype on a Brm-/- background. Therefore BRM does not appear to play a functionally redundant role with BRG1 during early vascular development.
DISCUSSION

Brg1 is one of two alternative catalytic subunits found in mammalian SWI/SNF-like chromatin-remodeling complexes, which are important transcriptional regulators during embryonic development. We provide the first evidence of a role for Brg1 in embryonic β-globin expression and in primitive erythropoiesis in vivo. The phenotype of our conditional null mutants is more severe than that of hypomorphic Brg1null/ENU1 embryos (Bultman et al., 2005). Erythroblasts in Brg1null/ENU1 embryos contain a stable BRG1 protein that assembles into SWI/SNF-like complexes and retains normal ATPase activity. However, although the mutant BRG1ENU1 protein is effectively recruited to the β-globin LCR, as demonstrated by ChIP assays, the LCR does not establish DNAse I hypersensitive sites characteristic of open chromatin. Because of this closed chromatin configuration at the LCR, adult β-globin transcription is reduced in Brg1null/ENU1 embryos, and definitive erythroblasts undergo developmental arrest, leading to embryonic lethality by E14.5. By contrast, Brg1fl/fl:Tie2-Cre+/0 erythroblasts contain little or no Brg1 protein, and fail to transcribe embryonic β-globins during primitive erythropoiesis, resulting in embryonic lethality by E11.0. Together, these hypomorphic and conditional null mutations demonstrate the requirement for Brg1-mediated chromatin remodeling during both embryonic and adult β-globin transcription.
Like Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+/-</sup> mutants, mice targeted for deletion of the Krüppel-like factor 2 (Klf2) have a significant reduction of embryonic β-globin gene expression and an upregulation of apoptosis in primitive erythrocytes (Basu et al., 2005). However, unlike Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+/-</sup> mutants, which die at E10.5–E11.0, Klf2<sup>-/-</sup> embryos persist until E12.5–E14.0, when they succumb to heart failure (Lee et al., 2006). Notably, Klf2<sup>-/-</sup> is dispensable for adult β-globin gene expression in definitive erythrocytes (Basu et al., 2005). Perhaps Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+/-</sup> mutants die at E10.5 from anemia whereas Klf2<sup>-/-</sup> embryos do not become anemic, because Brg<sup>1</sup> is necessary for transcription of both embryonic and adult β-globins whereas Klf2<sup>-/-</sup> is only critical for embryonic β-globin transcription. We hypothesize that embryonic β-globins are required for the survival of primitive erythrocytes, but that definitive erythrocytes, which express adult β-globins, can compensate for loss of primitive erythrocytes at the primitive/definitive transition. This hypothesis is substantiated by the observation that mice depleted of both the embryonic β-globins survive development whereas embryos depleted of all of the embryonic and adult β-globins, as a result of targeted deletion of the β-globin locus control region, die early in embryogenesis (Bender et al., 2000; Hu et al., 2007).

In addition to deficits in embryonic β-globin transcription, we also observe a reduction in embryonic α-globin (ζ) transcription in Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+/-</sup> erythroblasts, although adult α-globin transcription occurs normally in these cells (Fig. 4). Furthermore, we demonstrate through ChIP assays that BRG1 binds the promoter of ζ but not the promoter of the adult α-globins. These data indicate that BRG1 facilitates α-globin expression at the individual gene promoters rather than at the major regulatory element (MRE), which is a long-distance upstream regulatory element comparable to the β-globin LCR. In this regard, the regulation of α-globin and β-globin expression by SWI/SNF-mediated chromatin remodeling appear to be distinct in primitive erythrocytes. β-Thalassemia occurs when a reduced synthesis of β-globin chains leads to an excessive accumulation of insoluble α-globin chains in red blood cells. The resulting blood cells have morphological abnormalities (reminiscent of those seen in Fig. 1F and Fig. 3D), and are vulnerable to mechanical injury and death. Although embryonic α-globin expression is compromised in Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+</sup> erythroblasts, the adult α-globins are expressed normally and should be able to serve the survival needs of an embryo when β-globin chains are available (Leder et al., 1997). However, because of defective β-globin transcription in our mutant blood cells, the adult α-globins have no binding partners and presumably accumulate detrimentally. Therefore, we provide the first demonstration that loss of Brg<sup>1</sup> in primitive erythroblasts leads to severe β-thalassemia and lethality.

Because SWI/SNF might be expected to mediate transcription of multiple genes during erythropoiesis, we assessed expression of two other markers of erythroid development: Alas2, an important enzyme in heme biosynthesis; and Band3 (Slc4a1), an integral membrane protein on the surface of erythrocytes that provides cellular mechanical stability. By in situ hybridization, we found normal expression of Alas2 but deficient expression of Band3 in Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+</sup> erythroblasts (see Fig. S2 in the supplementary material), indicating that BRG1-mediated chromatin remodeling may be important for Band3 transcription. Because Band3<sup>-/-</sup> mice survive development (Peters et al., 1996; Southgate et al., 1996), we do not believe that aberrant Band3 expression contributes to the lethality we observe in Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+</sup> embryos. Nevertheless, in combination with the globin expression data presented here, these expression analyses provide interesting evidence for the specificity of SWI/SNF activity. Altogether, we demonstrate changes in the expression of embryonic globins and Band3, but no changes in the expression of adult α-globins or Alas2 in our mutant erythroblasts. These data support a growing body of evidence that SWI/SNF complexes control a limited number of targets and are not simply indiscriminate transcriptional regulators (reviewed by Kwon and Wagner, 2007). As such, these and other chromatin remodeling complexes have the capacity to regulate a wide variety of nuanced developmental processes.

Finally, we believe Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+</sup> embryonic lethality is due to anemia resulting from failure of hemoglobin synthesis and subsequent apoptosis of red blood cells, because other mutants with defective primitive erythropoiesis, such as Gata1, Gata2 and Rbmn2 mutants, all die from anemia at the same time in development as Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+</sup> embryos (E10-11.5) (Fujiwara et al., 1996; Tsai et al., 1994; Warren et al., 1994). Importantly, these mutants with defects in primitive erythropoiesis do not harbor secondary vascular defects. Therefore, we suspect that the vascular phenotypes we observe in Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+</sup> mutant yolk sacs are separable from the mutant blood phenotype. We plan to verify this hypothesis in the future by rescuing expression of Brg<sup>1</sup> in developing erythrocytes and determining whether the vascular abnormalities in Brg<sup>1</sup>/<sup>fl/fl</sup>:Tie2-Cre<sup>+</sup> yolk sacs are still apparent at E9.5.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/135/3/493/DC1

References


