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Short Article

Loss of Gata1 but Not Gata2 Converts Erythropoiesis to Myelopoiesis in Zebrafish Embryos

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Summary

The differentiation of hematopoietic progenitors into erythroid or myeloid cell lineages is thought to depend upon relative levels of the transcription factors *gata1* and *pu.1*. While loss-of-function analysis shows that *gata1* is necessary for terminal erythroid differentiation, no study has demonstrated that loss of *gata1* alters myeloid differentiation during ontogeny. Here we provide in vivo evidence that loss of Gata1, but not Gata2, transforms primitive blood precursors into myeloid cells, resulting in a massive expansion of granulocytic neutrophils and macrophages at the expense of red blood cells. In addition to this fate change, expression of many erythroid genes was found to be differentially dependent on Gata1 alone, on both Gata1 and Gata2, or independent of both Gata factors, suggesting that multiple pathways regulate erythroid gene expression. Our studies establish a transcriptional hierarchy of Gata factor dependence during hematopoiesis and demonstrate that *gata1* plays an integral role in directing myelo-erythroid lineage fate decisions during embryogenesis.

Introduction

The fate of common myelo-erythroid (CMPs) progenitors to become megakaryocyte-erythroid progenitors or myeloid progenitors is thought to be determined by *GATA1* and *PU.1* (reviewed in Graf, 2002). Overexpression of *GATA1*, a zinc finger transcription factor essential for erythroid differentiation, reprograms myeloid cells to undergo erythroid and megakaryocytic differentiation (Pevny et al., 1995; Iwasaki et al., 2003). Forced expression of *PU.1*, an Ets family transcription factor, represses erythropoiesis and promotes myeloid differentiation in erythroid cell lines (Yamada et al., 2001). PU.1 and GATA1 inhibit each other's function by physically interfering with the transcriptional control of their target genes, suggesting that the levels of each directly determines cell fate (Graf, 2002). Despite overexpression studies demonstrating the importance of GATA1 and PU.1 levels in controlling erythroid or myeloid cell fates, no genetic evidence has shown that loss of these factors alters myeloid versus erythroid lineage decisions during development.

In zebrafish, expression of hematopoietic genes such as *gata1* and *gata2* identifies the first primitive erythroid precursors at the 5-somite stage in the posterior mesoderm (reviewed in Davidson and Zon, 2004). After 10 somites, blood precursors express mature erythroid markers such as embryonic *globin*. By 24 hr postfertilization (hpf), these cells are encircled by endothelial cells of the trunk axial vein, and together form the intermediate cell mass (ICM), the teleost equivalent of the mammalian yolk sac blood island. After 24 hpf, the erythroblasts enter circulation, where they later mature into primitive erythrocytes. Zebrafish myelopoiesis is first detected by *pu.1* expression in the anterior mesoderm at the 6-somite stage (Lieschke et al., 2002). *pu.1* transcripts are also found in the ICM blood precursors between 10 and 18 somites, and at 20 somites maturing myeloid cells in the posterior ICM and anterior head region express *myeloperoxidase* (*mpo*), a granulocyte marker, and *l-plastin*, a macrophage marker (Bennett et al., 2001).

In this study, we examined the role of *gata1* and *gata2* in determining blood cell fate during zebrafish hematopoiesis. Despite an absence of erythroid cells in Gata1-deficient embryos (Lyons et al., 2002), hematopoietic cells present in the ICM of these embryos were found to differentiate into granulocytes and macrophages. By expression analysis, confocal microscopy, and cell histology, we show ICM blood precursors have converted into myeloid cells in the absence of Gata1. In contrast, loss of Gata2 did not shift differentiation of the ICM cells to myeloid lineages. Red blood cell genes were found to require either Gata1 or both Gata1 and Gata2 or were expressed in the absence of both Gata proteins, suggesting that other transcription factors regulate their erythroid expression. Furthermore, our results distinguish the functions of Gata1 and Gata2 and highlight the importance of Gata1 in promoting erythroid and suppressing myeloid cell fate decisions in zebrafish.

Results

Loss of Gata1 Results in Expanded Myelopoiesis

The mutual antagonism between *pu.1* and *gata1* and their coexpression in the ICM led us to ask if *gata1* regulates *pu.1* expression during zebrafish blood development. We designed two *gata1* morpholinos (MOs) and found them to cause anemia at 36 hpf, phenocopying the zebrafish *gata1* mutant, *vlad tepes* (*vlt*), while a 4-base pair mismatch MO did not (Lyons et al., 2002; Supplemental Table S1 at http://www.developmentalcell.

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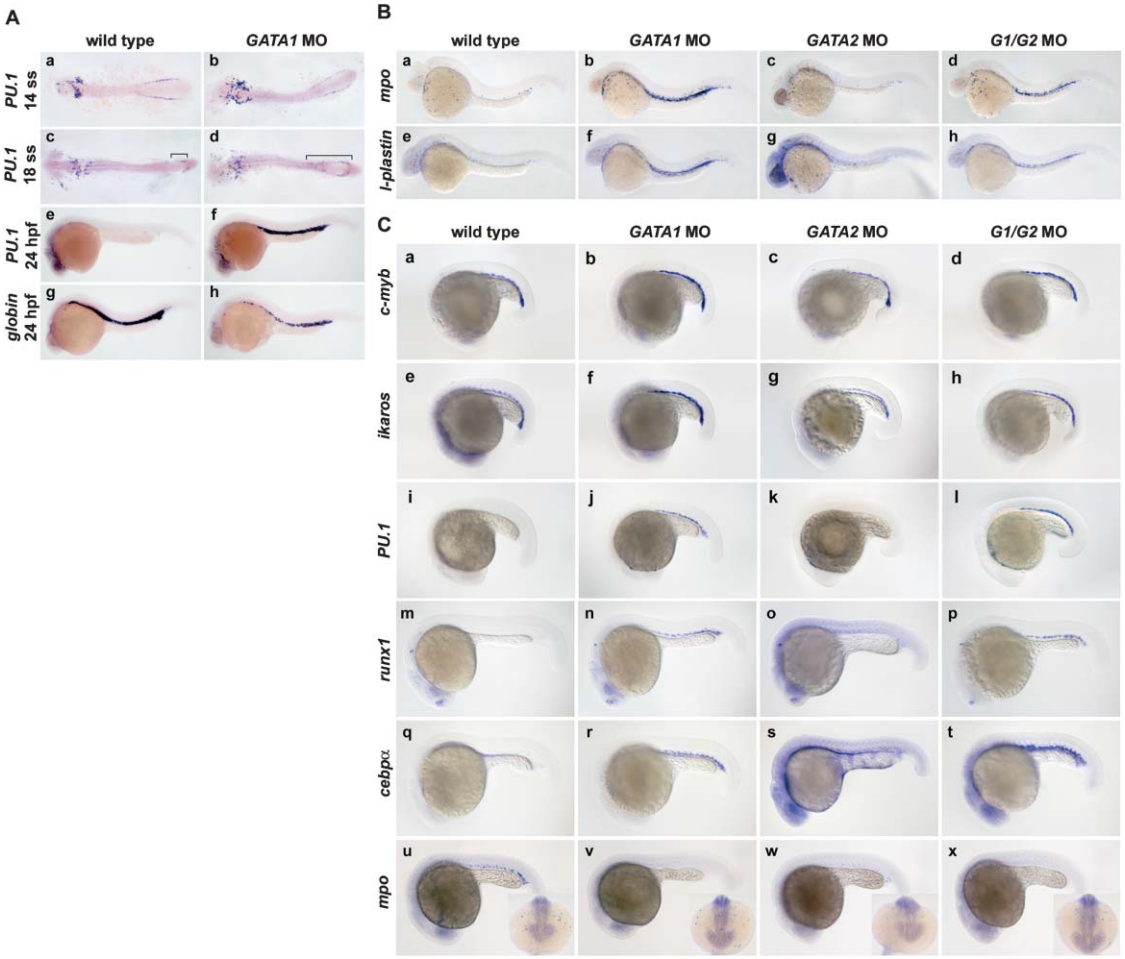

Figure 1. Loss of Gata1 Results in Expanded Myelopoiesis

(A) *pu.1* expression in control and *gata1* MO-injected embryos at 14 somites (a, b), 18 somites (c, d), and 24 hpf (e, f). Embryos (a–d) were flat-mounted with anterior (left) and posterior (right). *gata1* MO-injected embryos express *pu.1* at 18 somites (d, brackets) and 24 hpf (f) while wild-type embryos have downregulated *pu.1* transcripts (c, brackets, e) in the ICM precursors. *pu.1* is also expressed in anterior myeloid precursors (a–f). *gata1* MO-injected embryos have reduced expression of βe*1 globin* (h) in the ICM at 24 hpf compared to wild-types (g).

(B) The number of cells expressing *mpo* and *l-plastin* are increased in the vessels of *gata1* morphants (b, f, d, h) compared to wild-type embryos (a, e) and *gata2* morphants (c, g). *g1/g2* morphants (d, h) have decreased numbers of *mpo*- and *l-plastin*-expressing cells compared to the *gata1* morphants alone (b, f).

(C) Expression of *c-myb* (a–d) and *ikaros* (e–h) is maintained in the ICM cells of *gata1*, *gata2*, and *g1/g2* morphants at 20 somites. *pu.1* expression persists in the ICM of *gata1* (j) and *g1/g2* (l) morphants, but not *gata2* morphants (k) or wild-type embryos (i). Expression of *runx1* (m–p) and *cebp*α (q–t) persists in *gata1* (n, r) and *g1/g2* morphants (p, t) but not wild-type embryos (m, q) or *gata2* morphants (o, s) at 22 hpf. *cebp*α is expressed at low levels in some ICM cells and in gut endoderm (q–t). ICM cells expressing *mpo* at 22 hpf are reduced in *gata2* morphants (w) and lost in *gata1* (v) and *g1/g2* morphants (x) compared to wild-type embryos (u). Insets (u–x) are of cranial views of the same embryos showing normal *mpo*-expressing cells in wild-type (u) and *gata1* morphants (v) and reduced numbers of *mpo*-expressing cells in *gata2* (w) and *g1/g2* morphants (x).

com/cgi/content/full/8/1/109/DC1/). *pu.1* expression in the ICM and anterior blood region of *gata1* morphants resembled that of wild-type embryos at 5, 12, and 14 somites (Figure 1A, a and b; data not shown). Persistent expression of *pu.1* was found in the ICM precursors of *vlt* mutants and *gata1* morphants from 18 somites to 24 hpf (n = 47; 100%; Figure 1A, d and f), whereas in wild-type embryos, *pu.1* expression was downregulated in the ICM after 18 somites (Figure 1A, c and e). These data suggest that Gata1 is required to limit *pu.1* expression in ICM precursors after 18 somites.

Since forced expression of *PU.1* activates myeloid gene expression in vitro (Yamada et al., 2001), expression of the granulocyte marker, *mpo*, and the macrophage marker, *l-plastin*, were analyzed in Gata1-deficient embryos. Examination of *vlt* mutants and *gata1* morphants at 32 hpf and 4 dpf revealed a significant increase in the number of *mpo* (n = 36; 100%) and *l-plastin* (n = 37; 100%) positive cells (Figure 1B, b, d, f, and h; Supplemental Figure S1). These data suggest that ICM precursors have differentiated into macrophages and neutrophils in the absence of Gata1. Expression of the T cell marker, *rag1*, was normal in Gata1-deficient day 4 embryos, demonstrating that Gata1

exclusively regulates myelo-erythroid decisions in zebrafish (n = 16; 100%; data not shown).

Loss of Gata2 Does Not Cause Expanded Myelopoiesis

To determine if a myeloid expansion occurs in the absence of a related Gata family member also expressed in the ICM, a *gata2* morpholino and a 4-base pair mismatch control morpholino were designed (Supplemental Table S1). An increase in *mpo*- and *l-plastin*-expressing cells in *gata1* morphants at 32 hpf was not observed in *gata2* morphants (n = 30; 0%; Figure 1B, c and g), indicating the myeloid expansion is specific to loss of *gata1*. In mice, loss of *GATA2* results in modestly decreased numbers of erythroid and myeloid precursors that differentiate normally (Tsai and Orkin, 1997). Consistent with this, the number of *mpo*- and *l-plastin*-expressing myeloid cells at 32 hpf appeared slightly decreased in the *gata1/gata2* (*g1/g2*) double morphants (Figure 1B, d and h) compared to the *gata1* morphants alone (Figure 1B, b and f). These data suggest that *gata1* and *gata2* function distinctly to regulate hematopoietic differentiation and cell number, respectively.

Loss of Gata1 Does Not Affect Expression of Early Hematopoietic Genes

While the transcription factor genes *scl*, *lmo2*, and *gata2* are expressed in hematopoietic stem cells at 5 somites, transcripts of *c-myb*, *runx1*, and *ikaros* are found in both primitive erythroid and myeloid progenitors (Davidson and Zon, 2004). To determine if loss of Gata factors affects the expression of these genes, in situ hybridizations were performed with the *gata* morphants. There was no significant change in *gata2*, *scl*, and *lmo2* expression at 12 somites (data not shown) and *c-myb* (n = 41; 100%) and *ikaros* (n = 33; 100%; Figure 1C, a–h) expression at 20 somites in *gata1*, *gata2*, and *g1/g2* morphants, indicating that loss of Gata factors does not affect early blood formation.

In wild-type embryos, *runx1* is expressed early in anterior myeloid precursors and ICM cells until 18 somites. In Gata1-deficient embryos, *runx1* expression persisted in the ICM until 24 hpf (n = 37; 100%; Figure 1C, m–p), suggesting that ICM cells have retained progenitor characteristics. Increased expression of *CCAAT/enhancer binding protein* α (*cebp*α), a transcription factor found in myeloid progenitors essential for granulocytic maturation (Zhang et al., 1997), was also observed in Gata1-deficient embryos at 22 hpf (n = 24; 100%; Figure 1C, q–t). Examination of *mpo* expression prior to circulation revealed normal expression in the head region but a lack of *mpo*-expressing cells in the ICM of Gata1-deficient embryos at 24 hpf (n = 46; 100%; Figure 1C, u–x). While blood cells are specified in Gata1-deficient embryos, an absence of *mpo*-expressing cells at 24 hpf and persistent *runx1* expression suggests that the ICM cells are temporarily delayed in their differentiation. In contrast, *gata2* morphants never displayed persistent expression of *pu.1* (n = 15; 0%), *runx1* (n = 49; 0%), and *cebp*α (n = 15; 0%) after 18 somites, indicating that the myeloid expansion is specific to loss of Gata1.

Cell Morphology of Gata1-Deficient Cells

To examine Gata1-deficient ICM cell morphology, wild-type and Gata1-deficient ICM cells were isolated from *gata1-gfp* transgenic embryos. The *gata1-gfp* line used expresses *gfp* in the rostral myeloid cells, permitting both erythroid and myeloid populations to be isolated by flow cytometry (Supplemental Figure S2). GFP-sorted cells from 14 hpf wild-type and Gata1-deficient embryos morphologically resembled hematopoietic precursors (Figure 2A, a and b). GFP cells from wild-type 24 hpf embryos were round or polygonal in shape with coarse condensed chromatin and a dark blue cytoplasm, characteristics that define proerythroblasts (Figure 2A, c). In contrast, an increased number of cells from 24 hpf Gata1-deficient embryos resembled myeloblasts (wild-type: n = 146; 25% myeloblast-like; *gata1* MO: n = 56; 41% myeloblast-like; Figure 2A, d). At 48 hpf, most wild-type GFP-sorted cells resembled erythroblasts (Figure 2A, e), while many Gata1-deficient cells possessed features of promonocytes or maturing granulocytes (wild-type: n = 363; 13% myeloblast-like; *gata1* MO: n = 210; 43% myeloblast-like; Figure 2A, f, arrowheads). The increase in myeloid cells at 48 hpf provides further evidence that ICM cells differentiate into myelomonocytes in the absence of Gata1.

Erythroid Precursors Are Converted into Myeloid Precursors in the Absence of Gata1

As loss of *GATA1* in mice causes erythroid progenitors to undergo cell death (Pevny et al., 1995), apoptosis was examined in zebrafish *gata1* mutants by TUNEL staining. No appreciable increase in cell death was observed in *vlt* mutants (n = 6) between 12 somites and 24 hpf (Figure 2B, a and c; data not shown; N. Paffett-Lugassy, personal communication). Between 26 and 30 hpf, an approximate 2-fold increase in TUNEL-positive cells was observed in the ICM region of *vlt* mutants (n = 17) compared to wild-type and heterozygous siblings (n = 20; Figure 2B, b and d). This modest increase in cell death detected in *vlt* mutants was not significant enough to explain the loss of all ICM erythroid precursors.

To determine if ICM erythroid precursors are converting into myeloid cells, double in situ hybridizations for β*e1 globin* and *pu.1* expression were performed on wild-type and Gata1-deficient 22 hpf embryos and examined by confocal microscopy. In wild-type embryos, *globin* was expressed in ICM cells, while *pu.1* was predominantly expressed in the anterior blood region (data not shown; Figure 2C, a–c). Although *pu.1* can be expressed in a few cells in the posterior ICM at 22 hpf, wild-type cells were never found to coexpress *globin* and *pu.1* at this stage. In contrast, multiple ICM cells in the *gata1* morphants were found to coexpress *pu.1* and *globin*, suggesting that ICM cells were converting to the myeloid lineage (Figure 2C, d–f).

A Hierarchy of Gata-Dependent Erythroid Gene Expression

To understand the functional differences between *gata1* and *gata2* in hematopoietic regulation, expression of erythroid genes obtained from an in situ hybridization

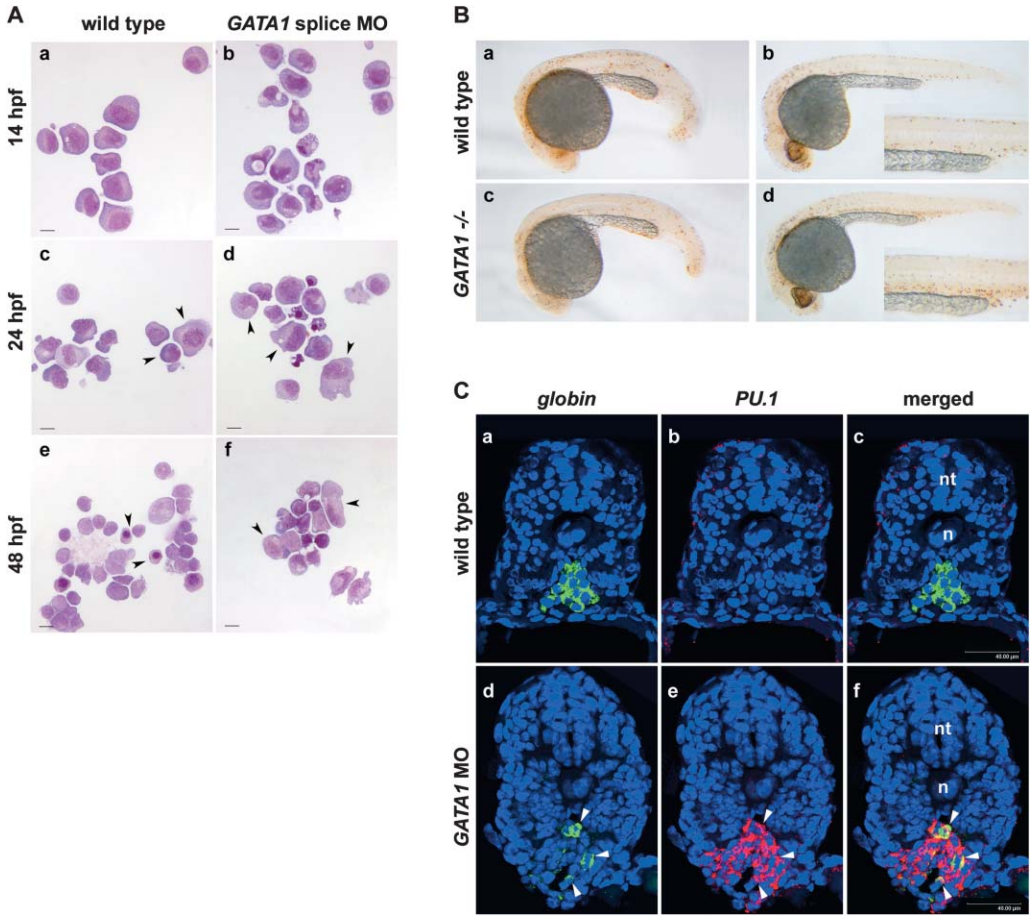

Figure 2. Erythroid Precursors Are Converted into Myeloid Precursors in the Absence of Gata1

(A) *gata1-gfp* cells from wild-type (a) and *gata1* splice MO-injected (b) embryos at 10 somites (14 hpf) resemble hematopoietic precursors. At 24 hpf, most wild-type *gata1-gfp* cells resemble proerythroblasts (c, arrowheads), but myeloid cells with indented nuclei are observed. Cells from *gata1* splice MO-injected embryos have myeloid features such as vacuoles and indented nuclei (d, arrowheads). Many erythrocytes and proerythroblasts are isolated from 48 hpf wild-type embryos (e, arrowheads) as well as a few myeloid cells. Cells from 48 hpf *gata1* splice MO-injected embryos have vacuoles and indented nuclei (f, arrowheads), suggesting that they are myelomonocytes (scale bars are 10 μm).

(B) Wild-type (a) and *vlt* mutant (c) embryos at 22 hpf have similar numbers of TUNEL-positive cells. *vlt* mutant embryos have an approximate 2-fold increase in apoptosis in their ICM region (d, inset) compared to their wild-type siblings (b, inset) at 28 hpf.

(C) Confocal imaging of transverse sections of DAPI-stained (blue) embryos that have undergone double in situ hybridization for βe*1 globin* (green) and *pu.1* (red). Wild-type embryos express βe*1 globin* and not *pu.1* in their ICM cells at 22 hpf (a–c). *gata1* MO-injected embryos express both βe*1 globin* and *pu.1* in their ICM cells at 22 hpf (d–f). Some cells coexpress (yellow) both genes (d–f, arrowheads; n, notochord; nt, neural tube; scale bar equals 40 μm).

screen was examined in *gata1*, *gata2*, and *g1/g2* MO-injected embryos (screen described in Supplemental Experimental Procedures). At 18 somites, *epsin* (involved in clathrin-mediated endocytosis) and *GTP binding-protein 1* (*gtpbp1*) were normally expressed in ICM cells; however, in Gata1-deficient embryos, their expression was absent (Figure 3A, a–d). Expression of *biliverdin reductase* (heme degradation), *SH3 domain binding protein 5 (SH3BP5)*, *HIF1α-like gene* (hypoxic response), and *5′nucleotidase type B* (cause of human hemolytic anemia; Bianchi et al., 2003) were absent in the ICM of Gata1-deficient embryos at 20–24 hpf (Figure 3A, e–h; data not shown). The absence of blood gene expression in Gata1-deficient embryos is consistent with mammalian studies indicating that *GATA1* regulates virtually all red blood cell genes (Orkin, 1992). Furthermore, these six genes were unaffected by loss of Gata2, demonstrating that their expression is specifically dependent on *gata1*.

ICM expression of another group of erythroid genes was reduced in Gata1-deficient embryos. A decrease in βe*1 globin* (n = 33; 100%), *alas2* (n = 27; 100%), *carbonic anhydrase* (n = 39; 100%), and *gata1* (n = 33; 100%) expression in *gata1* morphants and *vlt* mutants was observed (Figure 3B, b, f, j, and n; data not shown). Previous work in *vlt* and mouse *GATA1*-deficient cell lines also found multiple red cell-specific genes expressed in the absence of *gata1* (Weiss et al., 1994; Lyons et al., 2002). Similar to the reduction in myeloid cell number, *gata2* morphants had a subtle decrease in erythroid cell number as detected by *globin* (n = 16; 100%), *alas2* (n = 13; 100%), *carbonic anhydrase* (n =

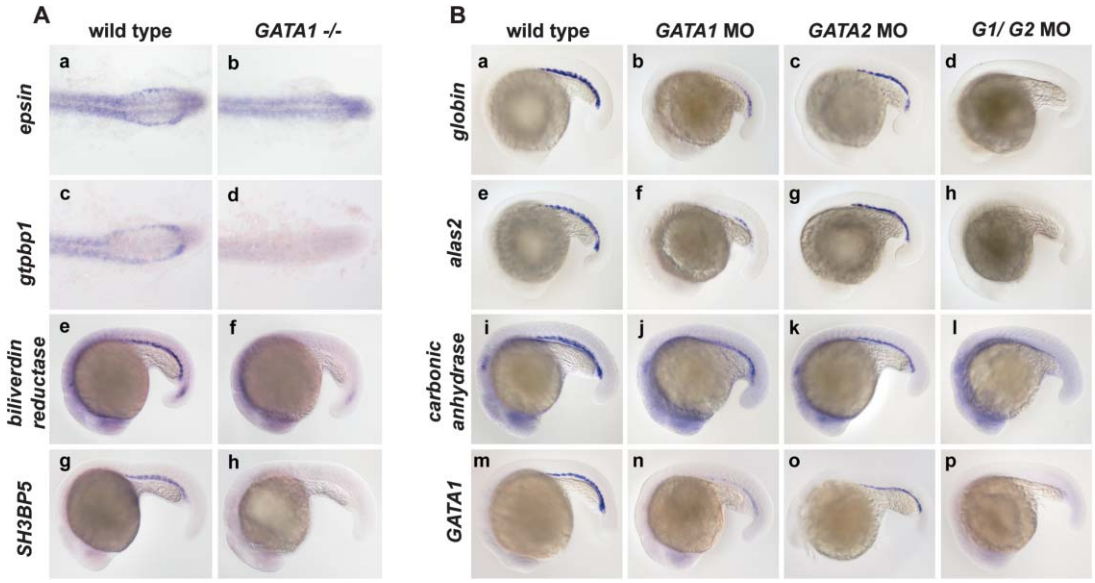

Figure 3. Erythroid Genes Are Differentially Regulated by Gata1 and Gata2

(A) Embryos (a–d) have been flat-mounted and photographed to show only the posterior tail region (anterior, left; posterior, right). Normal expression of *gdpbp1* and *epsin* (a, c) is lost in ICM erythroid precursors of *vlt* mutants at 18 hpf (b, d). Expression of *biliverdin reductase* and *SH3BP5* at 20 hpf (e) and 22 hpf (g), respectively, is absent in *vlt* mutant ICM cells (f, h).

(B) In situ hybridization for βe*1 globin* (a–d), *alas2* (e–h), *carbonic anhydrase* (i–l), and *gata1* (m–p) at 20 hpf. Compared to wild-type embryos (a, e, i, m), ICM expression of βe*1 globin*, *alas2*, *carbonic anhydrase*, and *gata1* was decreased in *gata1* morphants (b, f, j, n) and absent in *g1/g2* morphants (d, h, l, p). Decreased number of cells expressing βe*1 globin*, *alas2*, *carbonic anhydrase*, and *gata1* are observed in *gata2* morphants (c, g, k, o) compared to wild-types (a, e, i, m).

29; 100%), and *gata1* (n = 10; 100%) expression at 20 somites (Figure 3B, c, g, k, and o). In contrast, *globin* (n = 118; 91%), *alas2* (n = 16; 100%), *carbonic anhydrase* (n = 44; 100%), and *gata1* (n = 18; 100%) expression was lost in the *g1/g2* double morphants compared to wild-type embryos and *gata1* or *gata2* single morphants (Figure 3B, d, h, l, and p). This absence of expression may result from the additive decrease in erythroid gene expression, resulting from loss of Gata1 and Gata2. Alternatively, a decline in cell number caused by loss of Gata2 combined with reduced erythroid gene expression due to loss of Gata1 may lead to the absence of expression. Congruent with the former conclusion, suggesting Gata2 may regulate erythroid gene expression in the absence of Gata1, mouse studies have found that other GATA factors placed under *GATA1* transcriptional control can substitute for loss of *GATA1* during embryonic hematopoiesis (Takahashi et al., 2000). Nevertheless, our results suggest that erythroid genes differ in their requirements for Gata factors.

Gata-Independent Erythroid Gene Expression

To determine whether loss of both Gata factors ablated erythroid precursors, we utilized erythroid-specific genes identified from the in situ hybridization screen. The genes *KIAA0650*, *testhymin*, and *kelch repeat-containing protein* were never detected in the anterior myeloid cells and were found to be lost in the ICM of 18-somite stage *moonshine* (*mon*) embryos, which harbor a mutation in *TIF1*-γ and lack primitive erythrocytes (Ransom et al., 2004; data not shown). This suggests that these genes are specific to early ICM erythroid cells. *KIAA0650* (n = 45; 100%), *testhymin* (n = 45; 100%), and *kelch repeat-containing protein* (n = 54; 100%) were expressed in the ICM precursors of the *gata1*, *gata2*, and *g1/g2* double morphants at 12 somites (Figure 4A, e–o). We also found that *biklf*, a Kruppel-like transcription factor expressed in early erythroid precursors (Oates et al., 2001), was expressed normally in *gata1* (n = 21; 100%), *gata2* (n = 16; 100%), and *g1/g2* (n = 16; 100%) morphants at 12 somites (Figure 4A, a–d). The Gata-independent expression of *biklf*, *testhymin*, *KIAA0650*, and *kelch repeat-containing protein* suggests that other hematopoietic transcription factors induce their expression and that at least part of the erythroid program was initiated in *gata1* morphants. After 20 hpf, *gata1* and *g1/g2* morphants weakly expressed *biklf* (n = 87; 100%), *KIAA0650* (n = 20; 100%), and *testhymin* (n = 50; 100%) in the ICM compared to wild-types or *gata2* morphants (Figure 4B), suggesting that Gata1 is required to maintain wild-type expression levels of these genes. Additionally, their downregulation is concomitant with the conversion of the ICM cells to the myeloid lineage.

Discussion

Hierarchy of Regulation of Erythroid Gene Expression

As *gata1* is a major regulator of erythroid gene expression, we found that many erythroid genes were dependent on *gata1* for expression. A subset of erythroid genes had reduced, but not absent, expression in Gata1-deficient embryos. Similarly, the *GATA1* null mouse and

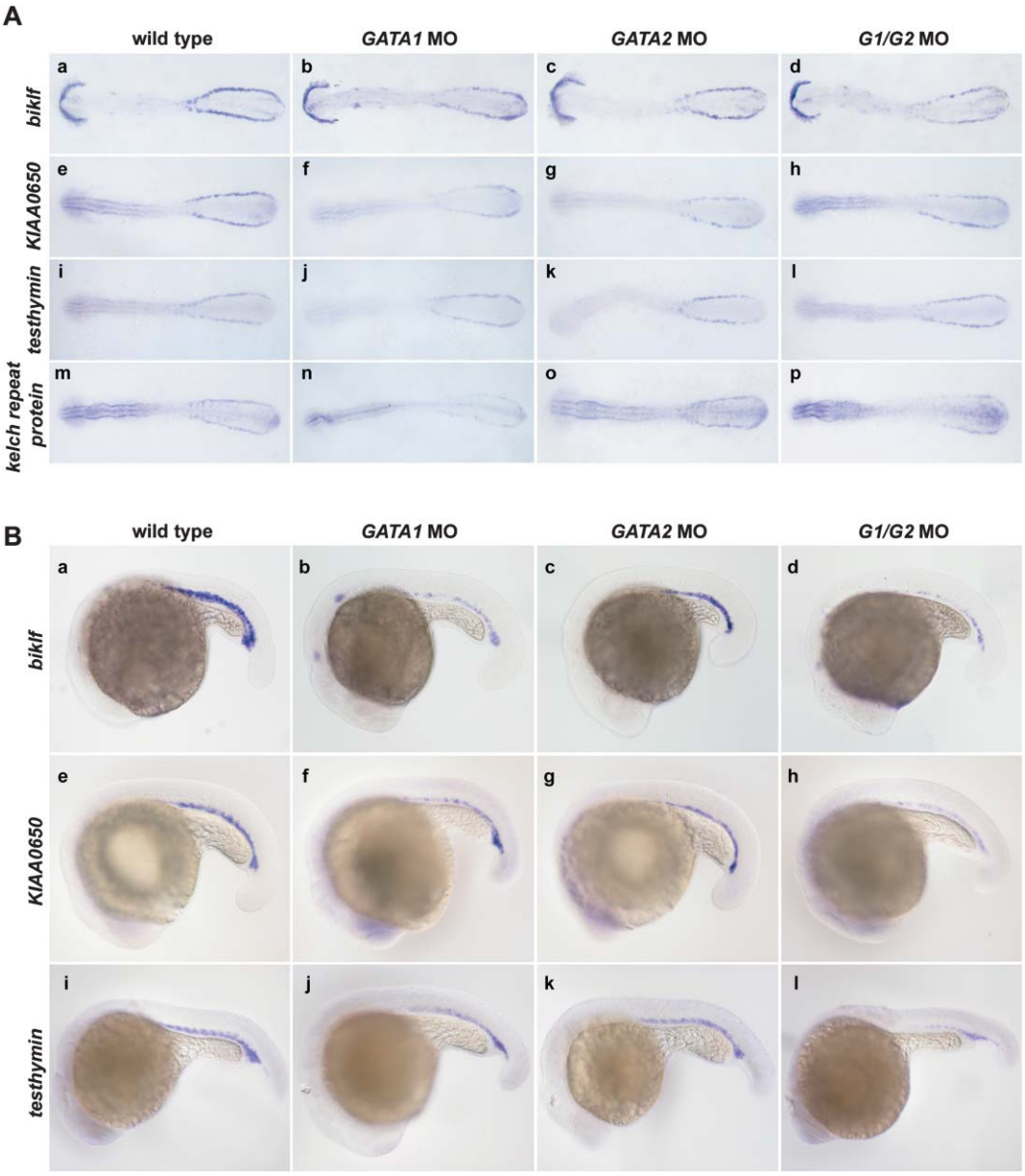

Figure 4. Expression of Gata-Independent Erythroid Genes

(A) Embryos were flat-mounted (anterior, left; posterior, right). Expression of *biklf* (a–d), *KIAA0650* (e–h), *testhymin* (i–l), and *kelch repeat-containing protein* (m–p) is maintained in ICM precursors of 12 somite *gata1*, *gata2*, and *g1/g2* morphants. Anterior expression of *biklf* stains the hatching gland (a–d).

(B) ICM expression of *biklf* (a–d) at 20 hpf and *KIAA0650* (e–h) and *testhymin* (i–l) at 22 hpf was decreased but present in *gata1* morphants (b, f, j) and *g1/g2* MO-injected embryos (d, h, l) compared to wild-type embryos (a, e, i). *gata2* morphants (c, g, k) had decreased numbers of ICM cells expressing *biklf*, *KIAA0650*, and *testhymin*.

GATA1-deficient cell lines exhibited residual expression of erythroid genes (Weiss et al., 1994; Pevny et al., 1995). Our data showing loss of expression of these genes in G1/G2-deficient embryos suggests that in the absence of Gata1, Gata2 regulates expression of a subset of erythroid genes in erythroid precursors. In support of this, zebrafish *g1/g2* morphants, unlike mouse *G1/G2* nulls in which yolk sac blood cells undergo apoptosis (Fujiwara et al., 2003), have primitive blood cells expressing *c-myb* and *ikaros* (common progenitors) and *biklf*, *kelch repeat-containing protein*, *testhymin*, and *KIAA0650* (erythroid progenitors). Although attempts to rescue erythropoiesis in Gata1-deficient embryos by *gata2* overexpression were technically confounded by *gata2* mRNA toxicity (data not shown), studies in mouse have demonstrated that *GATA2*, when placed under the control of the *GATA1* promoter, can substitute for *GATA1* during embryonic hematopoiesis (Takahashi et al., 2000). This is consistent with the hypothesis that *gata2* regulates some erythroid target genes in the absence of Gata1.

We discovered that the novel genes *kelch repeat-*

containing protein, *testhymin*, and *KIAA0650* were expressed independently of Gata factors. In contrast to genes like *c-myb* and *ikaros* that are found in G1/G2-deficient CMPs, these genes had erythroid-specific expression, suggesting that they exclusively mark the erythroid progenitor cell compartment. The early blood genes *biklf*, *scl*, and *lmo2* may regulate expression of these Gata-independent genes, and future studies will further elucidate erythroid gene regulation during hematopoiesis.

Gata1 but Not Gata2 Regulates Myelo-Erythroid Fate Decisions

Our findings demonstrate that *gata1* is necessary to promote erythroid cell fate and repress myeloid differentiation. Similar to *vlt*, GATA1-deficient mice lack mature red blood cells, and in vitro differentiation of *GATA1*$^{-}$ murine ES cells reveals an increase (>5-fold) in myeloid colonies (S. Orkin, personal communication). Despite these similarities, an increase in myeloid cells was not observed in *GATA1*$^{-}$ and *GATA1/GATA2*$^{-/-}$ mice and *GATA1*$^{-}$ cultured yolk sac cells (Fujiwara et al., 1996, 2003; Pevny et al., 1995), suggesting that the mouse yolk sac environment may not support this cell fate alteration. Loss of zebrafish *gata2* does not alter red blood cell maturation or myeloid differentiation, but does decrease blood cell number, consistent with decreased numbers of progenitors in *GATA2*-deficient murine embryonic stem cells (Tsai and Orkin, 1997).

Two proposed models may explain the dramatic shift toward myelopoiesis in Gata1-deficient embryos (Graf, 2002). In one model, the ICM contains both myeloid and erythroid progenitors; in the absence of Gata1, the erythroid progenitors undergo cell death while the myeloid progenitors expand and differentiate. In the second model, ICM CMPs differentiate into erythroid or myeloid cells, based on relative levels of *gata1* and *pu.1*. Our results support the latter model wherein the presence or absence of Gata1 determines the fate of ICM progenitors. In Gata1-deficient embryos, blood progenitors form normally as demonstrated by expression of *biklf*, *scl*, and *lmo2*. Without Gata1 antagonism, *pu.1* expression is not downregulated after 18 somites. This persistent *pu.1* expression is likely the instructive signal that guides ICM cells to activate a myeloid differentiation program (Rhodes et al., 2004 [this issue of *Developmental Cell*]). The decrease in cells expressing *biklf* and the novel erythroid-specific genes and the presence of ICM cells coexpressing *globin* and *pu.1* provides strong evidence for an ICM cell fate conversion when Gata1 is absent. Our study demonstrates that *gata1* has an important role in suppressing myeloid differentiation, presumably by antagonizing the effect on *pu.1*-initiated myelopoiesis. This work also provides genetic evidence that a hierarchy exists in the regulation of erythroid genes and that *gata1*, but not *gata2*, is critical for determining erythroid versus myeloid cell fates during embryonic development.

Experimental Procedures

Zebrafish Strains

Zebrafish breeding and staging were done as described (Westerfield, 1994). *vlt*^{m651} were genotyped (Lyons et al., 2002), and the *gata1-gfp* line (Long et al., 1997) and wild-type Tü were used.

In Situ Hybridizations and TUNEL

Antisense mRNA probes were made for *c-myb*, *biklf*, *l-plastin*, *mpo*, *pu.1*, *βe1 globin*, and *gata1* (Thompson et al., 1998; Bennett et al., 2001; Oates et al., 2001; Lieschke et al., 2002). Riboprobes of genes isolated from the in situ hybridization screen were described in Supplemental Experimental Procedures. Confocal imaging was done on paraffin-embedded 10 μm sections that underwent double in situ hybridization (Brent et al., 2003) with Tyramide Signal Amplification (Perkin Elmer, Molecular Probes). TUNEL was performed using the ApopTag Peroxidase In Situ Apoptosis detection kit (Chemicon).

Gene Knockdown by Morpholinos

Morpholino oligos were designed against the ATG of *gata1* (5′-CTG CAAGTGTAGTATTGAAGATGTC-3′), the first exon/intron boundary of *gata1* (5′-GTTTGGACTCACCTGGACTGTGTCT-3′), and the third exon/intron boundary of *gata2* (5′-CATCTACTCACCAGTCTGCGC TTTG-3′). Control morpholinos containing four base pair mismatches were also designed against the *gata1* splice MO (5′-GTTCGGACT CGCCTGTACTGTGTAT-3′) and the *gata2* splice MO (5′-CATCCAC TCACTAGTCTACGCTGTG-3′). Morpholinos were resuspended in nuclease-free water, and one nanoliter was injected at the 1- to 4-cell stage at the following concentrations: 1 mM *gata1* MO, 0.2 mM *gata2* splice MO, 0.4 mM *gata1* splice MO, 0.2 mM *gata2* splice mismatch MO, and 0.4 mM *gata1* splice mismatch MO.

Flow Cytometry and Histological Analysis

Approximately 200 to 400 uninjected or *gata1* splice MO-injected *gata1-gfp* transgenic embryos were collected (14 hpf, 24 hpf, and 48 hpf). Embryos were processed, isolated by GFP fluorescence using a FACSVantage flow cytometer (Beckton Dickinson), centrifuged, and stained with May-Grünwald and Giemsa solutions as described (Traver et al., 2003).

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