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Report

Fli1 Acts at the Top of the Transcriptional Network Driving Blood and Endothelial Development

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Summary

Blood and endothelium arise in close association during development, possibly from a common precursor, the hemangioblast [1–4]. Genes essential for blood and endothelial development contain functional ETS binding sites, and binding and expression data implicate the transcription factor, friend leukaemia integration 1 (*Fli1*) [5–10]. However, loss-of-function phenotypes in mice, although suffering both blood and endothelial defects, have thus far precluded the conclusion that *Fli1* is essential for these two lineages [11, 12]. By using *Xenopus* and zebrafish embryos, we show that loss of *Fli1* function results in a substantial reduction or absence of hemangioblasts, revealing an absolute requirement. TUNEL assays show that the cells are eventually lost by apoptosis, but only after the regulatory circuit has been disrupted by loss of *Fli1*. In addition, a constitutively active form of *Fli1* is sufficient to induce expression of key hemangioblast genes, such as *Scl*/*Tal1*, *Lmo2*, *Gata2*, *Etsrp*, and *Flk1*. Epistasis assays show that *Fli1* expression is induced by *Bmp* signaling or *Cloche*, depending on the hemangioblast population, and in both cases *Fli1* acts upstream of *Gata2*, *Scl*, *Lmo2*, and *Etsrp*. Taken together, these results place *Fli1* at the top of the transcriptional regulatory hierarchy for hemangioblast specification in vertebrate embryos.

Results

Fli1 Is Required for Both Primitive and Definitive Hemangioblast Formation

In the early *Xenopus* embryo, we have described two populations of hemangioblasts that represent the precursors of the primitive and definitive blood and endothelial lineages [10]. The primitive hemangioblast population is located in the anterior of the neurula stage embryo (~16 hpf), just below the cement gland, whereas the definitive population is found in the dorsal lateral plate (DLP) of the tailbud stage embryo approximately 12 hr later. *Fli1* is one of the earliest markers to be expressed in both populations [10].

To knock down *Fli1* expression in *Xenopus* embryos, we made use of an antisense morpholino (MO) designed against the ATG region, and an optimal level of 40 ng per embryo was established by monitoring the downregulation of *Scl* expression in the anterior hemangioblast (Figure S1 available online). When targeted to the dorsal marginal zone (DMZ) at the 4/8 cell stage (the region giving rise to the primitive hemangioblast population [10]), the expression of many genes later expressed in erythroid, myeloid, or endothelial cells was downregulated (Figures 1A–1L, 1S, 1T, 1V, and 1W, arrows). Thus, expression of *Scl*, *Lmo2*, *Fli1*, *Flk1*, *Mpo*, *SpiB*, and *Runx1* were all severely reduced in *Fli1* MO-injected embryos (morphants) monitored at stage 17. This effect was specific to the hemangioblasts: neither *Runx1* expression in Rohon Beard cells of the neural plate nor *Lmo2* expression in the somites was affected (Figures S4A–S4D). The effect on *Gata2* expression, when *Fli1* MO was injected, was monitored by in situ hybridization on sections, because *Gata2* is expressed in all three germ layers at neurula stages [13]. *Gata2* expression in the *Fli1* morphants was reduced, albeit modestly, in the mesoderm of the anterior hemangioblast region (Figures 1I and 1L, arrows). The effect on *Scl* expression has been reported previously with the same MO [14], but here we show that all the hemangioblast genes tested were downregulated, consistent with a primary role for *Fli1* at the top of the genetic hierarchy leading to formation of the embryonic hemangioblast.

Because the effect of the *Fli1* MO on *Gata2* expression in the anterior hemangioblast was a subtle one, we turned to *Gata2* morphants to establish the hierarchy between *Gata2* and *Fli1*. A combination of MOs directed against the ATG regions of *Gata2A* and *B*, the two pseudo-alleles of *Xenopus laevis*, have previously been shown to block *globin* expression [15], and we titrated the MOs monitoring this activity to optimize the dose (Figure S2). When we injected these *Gata2* MOs into the DMZ at the 4-cell stage (the region containing the precursors of the anterior hemangioblast population), *Scl* and *Lmo2* expression at stage 16/17 was significantly reduced whereas *Fli1* expression remained unchanged (Figures 1M–1R). This, together with the reduction, albeit only slight, of *Gata2* expression in *Fli1* morphants, suggests that *Fli1* may be above *Gata2* in the regulatory hierarchy determining the development of the primitive hemangioblast population. The small effects of the *Fli1* and *Gata2* MOs on each other's expression compared to their effects on *Scl* and *Lmo2* expression are consistent with both *Fli1* and *Gata2* being upstream of *Scl* and *Lmo2*.

To further test the hierarchical relationship of *Fli1* with *Scl*, *Lmo2*, and *Gata2*, we tried to rescue the *Fli1* morphant phenotype by coinjection of mRNAs for *Scl*, *Lmo2*, and *Gata2*. As shown in Figures 1S–1X, the reduction of expression of *SpiB* and *Runx1* was partially rescued by coinjection of the three mRNAs, in a dose-dependent manner (data not shown), consistent with *Fli1* acting upstream of *Scl*, *Lmo2*, and *Gata2* in the anterior hemangioblast population.

In order to look at the adult hemangioblast, we targeted the *Fli1* MO to the ventral marginal zone (VMZ) at the 4/8 cell stage (the region giving rise to the DLP) [16]. We found that blood and endothelial gene expression in the adult DLP hemangioblast at tailbud stages was eliminated (Figures 2A–2L, black arrows). The expression of *Scl, Gata2, Lmo2, Flk1, Msr*, and *Tie2* (data not shown) were all downregulated. *Fli1* expression in

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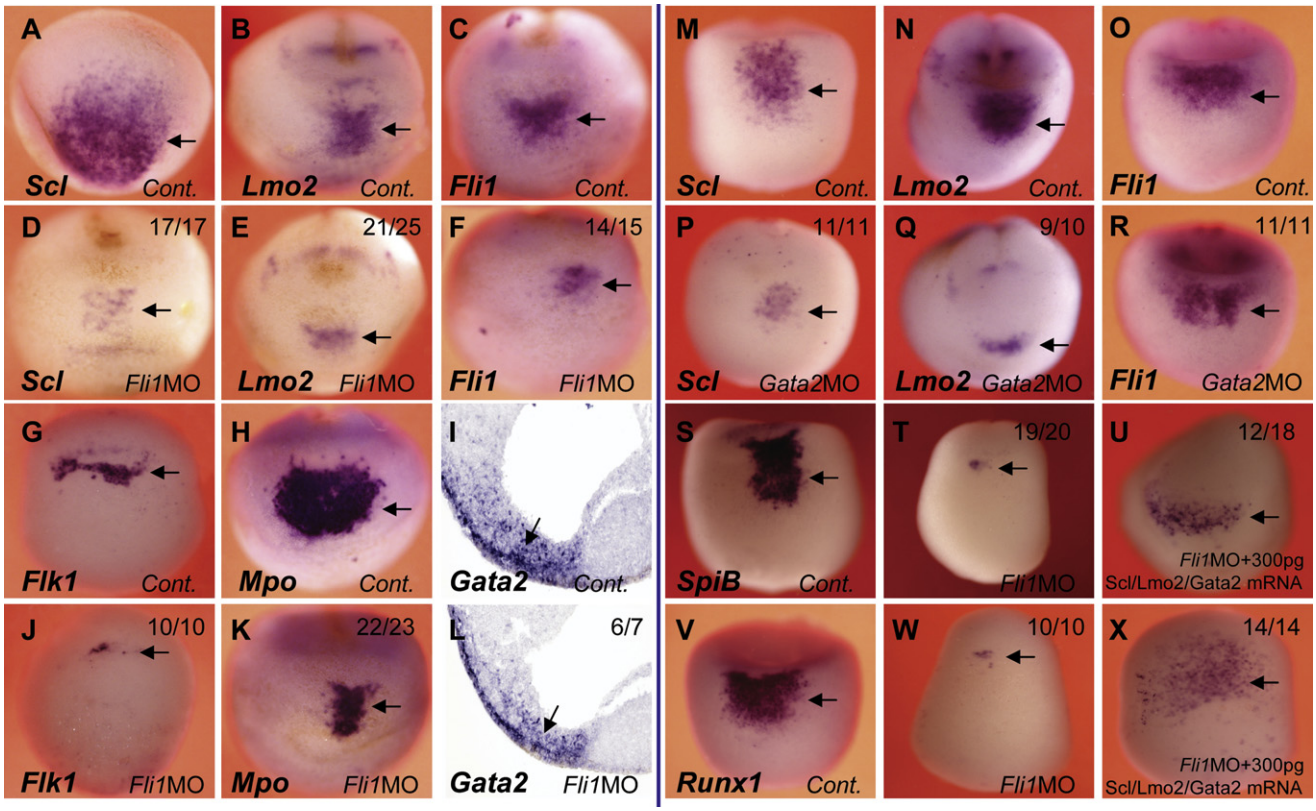

Figure 1. *Fli1* Is Required Upstream of *Gata2* for the Primitive Hemangioblast Program

(A–L) *Fli1* requirement for the primitive hemangioblast. *Xenopus* embryos injected with *Fli1* MO in the DMZ at the 4-cell stage were processed for in situ hybridization at stage 17, either as whole mounts (WISH) (A–H, J, K) or as sections (I, L).

(M–X) *Gata2* morphants and rescue experiments confirm that *Fli1* is at the top of the primitive hemangioblast hierarchy. *Gata2* MOs were injected into the DMZ at the 4-cell stage and embryos were processd for WISH at stages 16/17. Expression of *SpiB* (S–U) and *Runx1* (V–X) was partially rescued by coinjection of mRNA for *Scl*, *Lmo2*, and *Gata2*.

Arrows mark the primitive hemangioblast population. Proportion of embryos with phenotype shown indicated in top right corner.

the hemangioblast component of the DLP was also abolished (Figures 2A and 2B, cells outlined by red dotted line in Figure 2A). The remaining *Fli1* expression in the DLP region in *Fli1* morphants (Figure 2B, black dotted line) is in the pronephric duct (PND) precursors [10]. The reduced effect on the PND was confirmed with additional markers, *Pax2* and *Pax8*, and expression of all these markers plus *Runx1* and α*T4Globin* in the pVBI (also derived from the VMZ) was also decreased by this injection (Figures 2C–2L; Figure S3, red arrows). Overall, these results show that *Fli1* is required for primitive and definitive hemangioblast formation in *Xenopus* embryos.

To confirm the hierarchy of transcription factors involved in establishing the definitive hemangioblast program in the DLP, we targeted the *Gata2* MOs to the VMZ and monitored expression of *Fli1*, *Scl, Lmo2*, and *Msr* in the DLP soon after expression of these genes is first detectable. Whereas expression of *Scl*, *Lmo2*, and *Msr* was strongly downregulated in the *Gata2* morphants (Figures 2O–2T, arrows), the expression of *Fli1* remained unaffected (Figures 2M and 2N, arrows). These results suggest that *Fli1* is at the top of the transcriptional hierarchy programming the definitive hemangioblast.

Incorrectly Programmed Hemangioblasts in *Fli1* Morphants Undergo Apoptosis

We next asked whether the loss of expression of blood and endothelial markers in both embryonic and definitive hemangioblast populations was the result of respecification or loss of the cells. We monitored expression of *MyoD, Nkx2.5, Blimp1*, and *BMP4*, genes that are expressed in regions abutting the embryonic hemangioblast population, and found them unchanged in Fli1 morphants (Figures S4E–S4L), suggesting that respecification is not the cause of loss of hemangioblast gene expression. In contrast, TUNEL assays on Fli1 morphants at stage 17 (embryonic hemangioblasts) and stage 26 (adult hemangioblasts) revealed apoptosis in regions of the embryo where *Scl* expression was lost (Figures S5C, S5D, S5I, and S5J). Broader apoptosis in the DLP area at stage 26 may correspond to the partial loss of the PND (Figures S3E–S3H). Looking at times when *Scl* is first expressed (stage 14 for embryonic and stage 24 for adult hemangioblasts), we found loss of *Scl* expression in Fli1 morphants in the absence of apoptosis (Figures S5A, S5B, S5E, and S5H). Similar results were obtained for *Fli1* expression (data not shown). These results suggest that loss of the hemangioblast program leads to apoptosis rather than being a consequence of it.

Activated Fli1 Transactivates Early Hemangioblast Genes through *Scl*, *Etsrp*, and *Cloche*

Our experiments so far in *Xenopus* have established the hierarchical relationship between the transcription factors *Fli1*, *Gata2*, *Scl*, and *Lmo2*. We were interested to investigate this relationship in zebrafish and also to define the position of

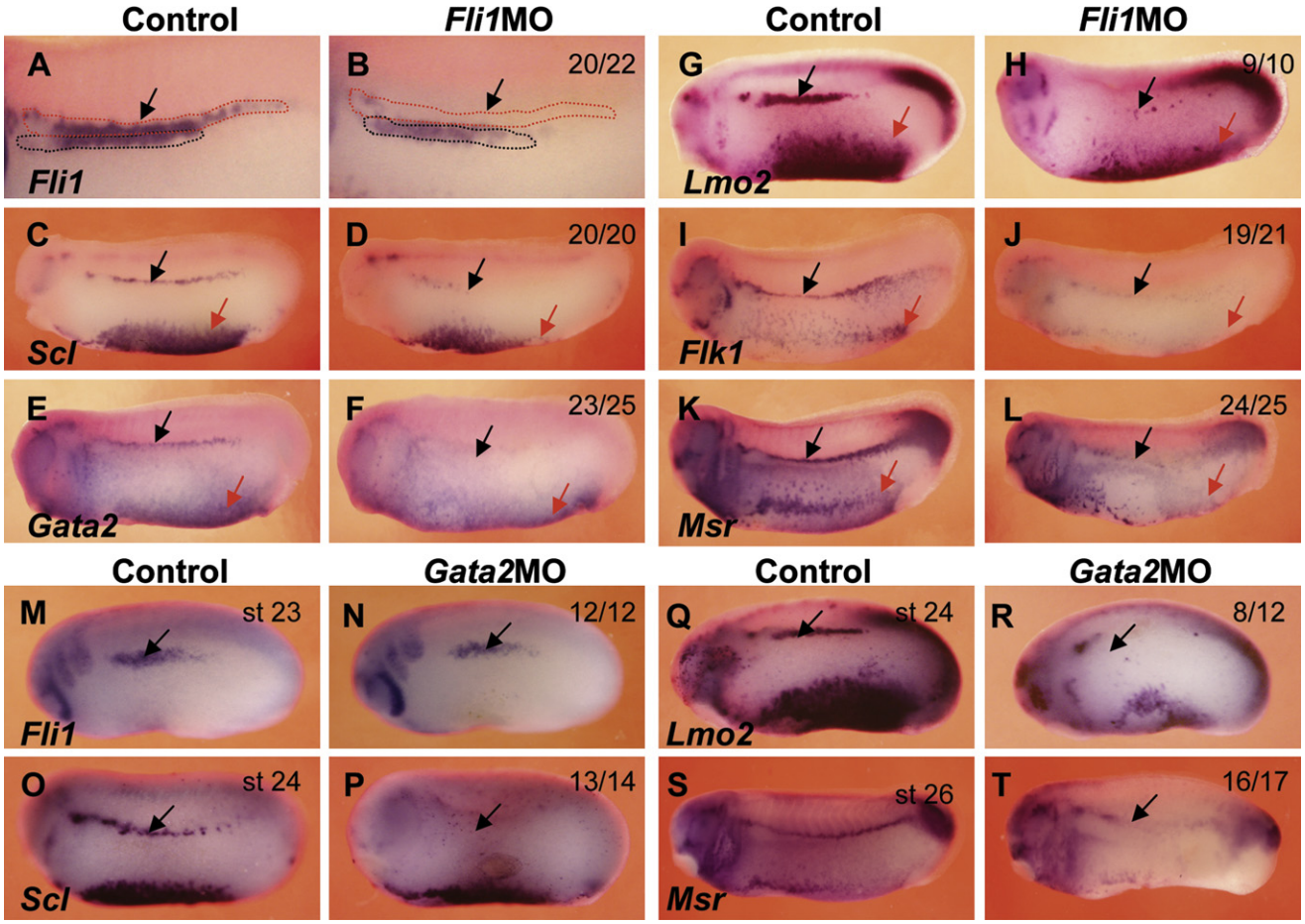

Figure 2. *Fli1* Is Required Upstream of *Gata2* for the Definitive Hemangioblast Program

(A–L) *Fli1* requirement for the definitive hemangioblast. *Xenopus* embryos injected with *Fli1* MO in the VMZ at the 4-cell stage were processed for WISH at stage 26. Note remaining DLP signal in (B) marks the pronephric duct (PND). Red outlines hemangioblasts, black outlines PND. Black arrows, DLP; red arrows, pVBI.

(M–T) *Gata2* morphants confirm that *Fli1* is at the top of the definitive hemangioblast hierarchy. Embryos injected with *Gata2* MOs in the VMZ at the 4-cell stage were processed for WISH for expression in the DLP (arrows) at stages shown.

Fli1 in relation to the important regulators of blood and endothelium, *Cloche* and *Etsrp*, that are specific to the zebrafish. We began by looking at loss-of-function embryos with an MO against the 5′ untranslated region (UTR) immediately upstream of the *Fli1* translation initiation codon. Although we were able to demonstrate activity of the MO by knocking down GFP expression from a transgene (data not shown), embryos injected with the MO were phenotypically normal and expression of blood and endothelial markers was unchanged (data not shown), raising the possibility of ETS factor redundancy [17]. Zebrafish have more ETS factors than other vertebrates (31 versus 24–29 for frog, mouse, and human), and, in particular, the *Fli1* locus has been duplicated giving rise to the *Fli1b* gene [18]. We and others have used combinations of MOs but thus far have been unable to significantly disrupt both blood and endothelium (data not shown) [19, 20].

We therefore turned to gain-of-function experiments. To functionally test the ability of Fli1 to transactivate early hemangioblast genes, we injected full-length *Fli1* RNA into zebrafish embryos but it had little effect on *Scl*, *Lmo2*, *Gata2*, or *Flk1* expression (Figure 3, compare [B], [E], [H], [N] with [A], [D], [G], [M]). The lack of effect is possibly because, like other ETS factors, Fli1 needs activating signals [21], or alternatively partners [5]. We therefore also tested a constitutively active form of Fli1, in which the transactivation domain of the viral VP16 protein was fused to full-length *Fli1* coding sequences. *Fli1*-VP16 RNA injection caused strong ectopic expression of *Scl*, *Lmo2*, *Gata2*, *Flk1*, and *Etsrp* as well as of *Fli1* itself (Figure 3, compare arrowed regions in [C], [F], [I], [L], [O], [T] with [A], [D], [G], [J], [M], [S]). In contrast, there was no expansion of expression of the blood marker *Gata1* (Figure 3, compare [R] with [P]). We therefore conclude that activated Fli1 protein can transactivate early hemangioblast genes but that the ectopic hemangioblast-like cells are unable to differentiate into red blood cells expressing *Gata1*. This might reflect an absence of the necessary blood differentiation signals in these ectopic regions of the embryo. Nevertheless, together with the observations that *Fli1* expression is initially unaffected in *Cloche* embryos (Figures S6A and S6B) [9], or by the loss of *Scl* or *Lmo2* [22, 23], these data are consistent with *Fli1* acting near the top of the hemangioblast genetic hierarchy.

To further establish the hierarchy between these key regulators, we coinjected embryos with *Fli1*-VP16 RNA and either *Scl* or *Etsrp* MOs. As already discussed, injection of *Fli1*-VP16 RNA alone leads to increased expression of *Flk1* (Figures 3O and 4B, arrows), whereas injection of *Scl* MO alone significantly

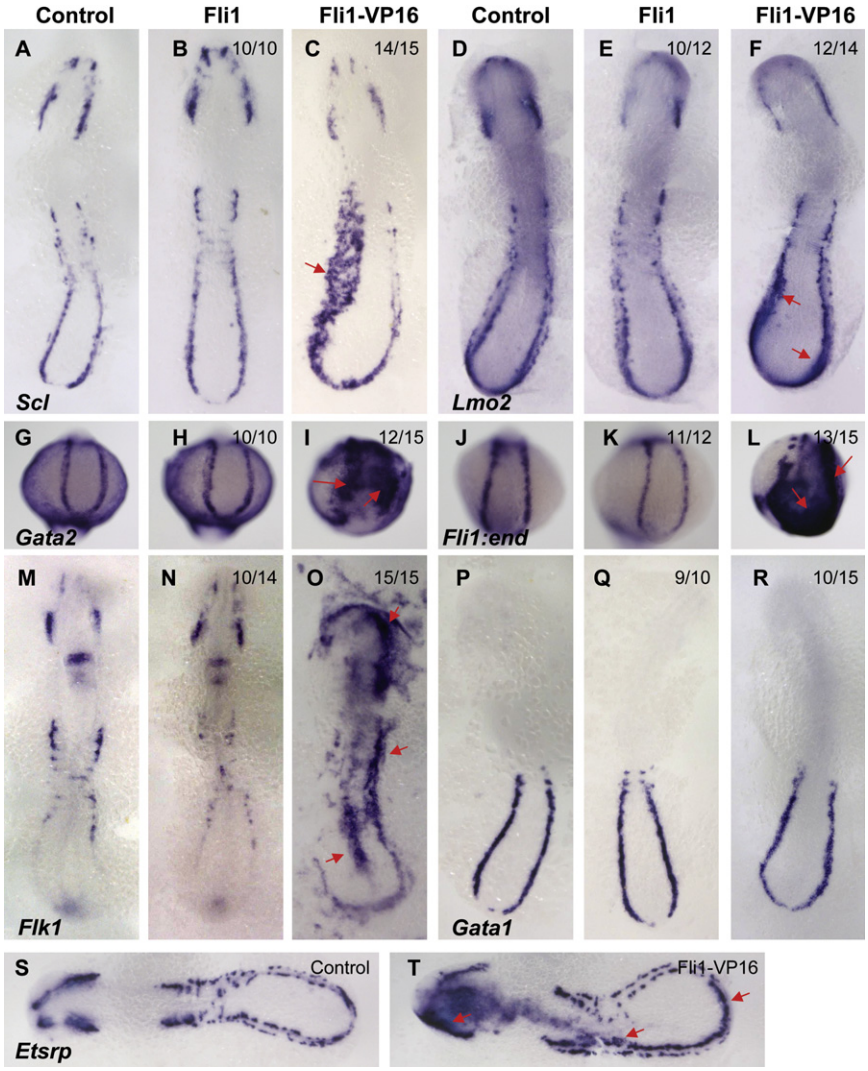

Figure 3. Upregulation of Blood and Endothelial Markers by *Fli1*-VP16

WISH of zebrafish 10 s stage embryos injected with *Fli1* or *Fli1*-VP16. In whole-mounted embryos, head to top, focusing on PLM (G–M). In flat-mounted embryos, head to top (A–F, M–R) or to left (S, T). Red arrows indicate expanded expression.

reduced *Flk1* expression (Figure 4C, arrows), consistent with previous data from us and others [23, 24]. Injection of *Fli1*-VP16 RNA together with *Scl* MO failed to rescue the loss of *Flk1* expression because of depletion of Scl protein, with the coinjected embryos showing the same phenotype as *Scl* MO injection alone (Figure 4D), suggesting that *Scl* is downstream of *Fli1* in hemangioblast formation. Similarly, injection of *Etsrp* MO greatly reduced endothelial cell numbers as reported previously (Figure 4E) [18]. In the coinjected embryos, *Fli1*-VP16 RNA failed to rescue the *Etsrp* MO phenotype (Figure 4F), indicating that *Etsrp* is also downstream of *Fli1*. Thus, *Fli1* transactivates at least one of its targets through *Scl* and *Etsrp*.

The *Cloche* gene has not yet been identified [25] and therefore we cannot determine whether *Cloche* expression is upregulated by *Fli1*-VP16. However, we can determine whether *Fli1* activity is dependent on *Cloche*. We therefore injected *Fli1*-VP16 RNA into $Clo^{-/-}$ embryos to determine whether hemangioblast gene expression could be rescued. When *Scl* expression was monitored at 10 somites, no rescue was seen, even though the *Fli1*-VP16 RNA was able to induce ectopic expression in wild-type embryos (Figures 4G–4J, arrows). Thus, the activity of *Fli1*-VP16 we see in wild-type embryos must be dependent on *Cloche*. We therefore conclude that *Fli1* is upstream or parallel to *Cloche* in the hemangioblast program. Taken together, these zebrafish data are consistent with *Fli1* acting at the top of the hemangioblast genetic hierarchy through, or together with, other key regulators, such as *Scl*, *Etsrp*, and *Cloche*.

Differential Control by Bone Morphogenetic Proteins of *Fli1* Expression in Adult and Embryonic Hemangioblasts

Inhibition of bone morphogenetic protein (Bmp) signaling in *Xenopus* embryos impairs the programming of both adult and embryonic hemangioblasts [10]. In the specific case of *Fli1*, though, although expression in the adult hemangioblast is dependent on Bmp signaling, it appears to be independent of it in the embryonic hemangioblast [10]. Bmp signaling is also required for hemangioblast induction in zebrafish but the response of *Fli1* has not been recorded [26]. To determine the situation in zebrafish, we made use of a heat-shock-inducible dominant-negative Bmp receptor transgenic line [26]. When we heat shocked this line at 30% epiboly (i.e., before gastrulation), the expression of *Fli1* as well as the other hemangioblast markers, *Scl* and *Gata2*, in the posterior lateral mesoderm (PLM) was substantially reduced (Figures S7A–S7F). We also checked *Fli1* expression in the *dino* mutant, a well-characterized mutant resulting in enhanced Bmp signaling. Consistently, the expression of *Fli1*, together with *Gata2* and *Scl*, was upregulated in the vicinity of the PLM (Figure S7G, arrows and data not shown). Cardiac precursor (*Nkx2.5*) and hindbrain (*Krox20*) marker expression acted as controls (Figure S7H).

The PLM in zebrafish gives rise to adult blood and endothelial cells and is therefore homologous to the DLP in frogs, in which *Fli1* expression is also regulated by Bmp signaling [10]. Furthermore, because the PLM in fish also gives rise to embryonic erythroid but not myeloid cells, it could be viewed as homologous to the posterior VBI precursors in *Xenopus* rather than the anterior hemangioblast [27]. Therefore, in order to determine if the zebrafish population homologous to the anterior hemangioblast in *Xenopus* behaves similarly with respect to the *Fli1* response to Bmp signaling, we monitored gene expression in the anterior lateral mesoderm (ALM) in heat-shocked embryos. We found that relatively normal numbers of *Fli1*-expressing cells were present at 6 somites (Figures S7I and S7J). We therefore conclude that the anterior hemangioblast populations in *Xenopus* and zebrafish that give rise to embryonic blood and endothelium express *Fli1* in

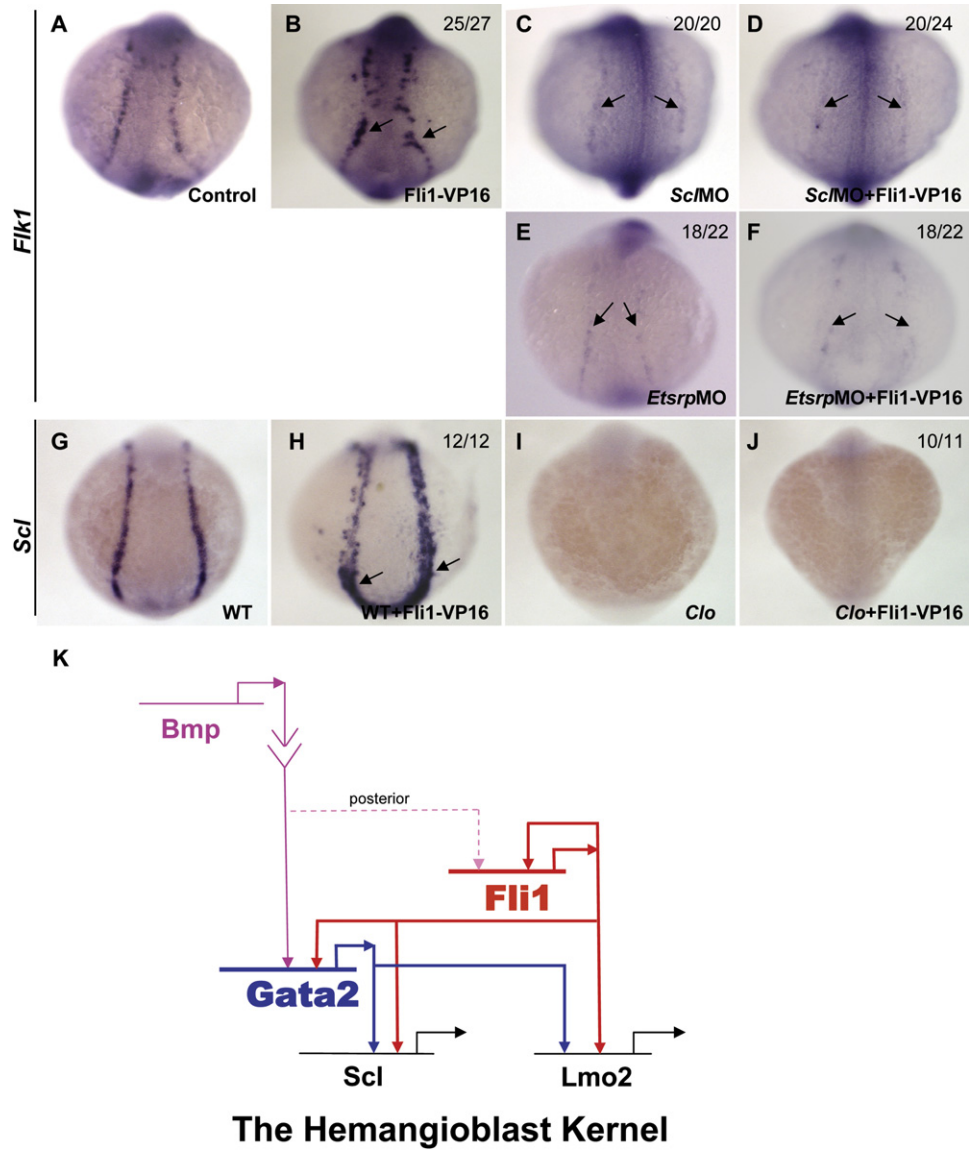

Figure 4. *Fli1* May Act in Parallel with *Cloche* but Upstream of *Scl* and *Etsrp*

(A–J) WISH on wild-type or *Cloche* mutant zebrafish embryos injected with combinations of *Fli1*-VP16 mRNA and/or *Scl*/*Etsrp* MOs. 10 s stage, dorsal view, head to top.

(K) A genetic regulatory network linking Bmp to *Fli1* at the top of the hemangioblast hierarchy, drawn according to Davidson et al. [29]. Solid lines, direct regulation; dashed lines, no evidence for direct or indirect. Evidence for links quoted in text, apart from *Gata2* as a direct Bmp target [32].

a Bmp-independent manner in contrast to the posterior hemangioblasts that give rise to adult blood.

Discussion

Most of the genes known to be essential for the early blood and endothelial programmes contain ETS binding sites [5, 28]. Several members of the ETS family are present in the precursors to blood and endothelium in the embryo, and of these, *Fli1* has emerged as a strong candidate based on its occupancy of these sites in putative hemangioblast-containing populations of cells [7]. However, the null phenotype in mice has been ambiguous [11, 12]. To clarify the early role of *Fli1* in blood and endothelial development, we took advantage of two organisms that have been studied extensively for the early development of these cells. In particular, the locations and gene-expression profiles of the cell populations giving rise to these two tissues are well documented. We find that, in the absence of *Fli1*, neither population is programmed to the hemangioblast fate and that eventually the cells are lost by apoptosis, confirming *Fli1* as a master regulator of blood and endothelial development.

In the building of genetic regulatory networks (GRNs), Davidson and his colleagues have suggested that three criteria need to be fulfilled to demonstrate regulatory connections between transcription factors [29]. First, the proposed target needs to respond appropriately when the proposed regulator's expression or activity is perturbed; second, the expression profiles of the genes need to fit the proposed relationship; and finally, there needs to be evidence that the regulator binds to its target. Pimanda et al. have drawn a core GRN, or kernel, for the hemangioblast, based on criteria 2 and 3 [7]. Here we

provide the perturbations necessary to test the validity of the proposed activities of *Fli1* and to a lesser extent *Gata2*, i.e., criterion 1. We show that *Fli1* fulfills all three criteria as a positive regulator of *Gata2*, *Scl*, *Lmo2*, and itself. Our data also show that *Gata2* fulfills all three criteria for *Scl* and *Lmo2*, but we find no evidence that it regulates *Fli1*, in contrast to the network of Pimanda et al. [7]. These conclusions have been built into a new hemangioblast kernel (Figure 4K). Such a core regulatory network may be conserved even beyond vertebrates because overexpression of the *Drosophila pointed* gene (the ortholog of the ETS family of proteins in vertebrates) drastically increased the number of circulating hemocytes in larvae [30].

Perturbations of *Scl* and *Lmo2* are consistent with the proposed locations of *Fli1* and *Gata2* in the hierarchy [22, 23]. Thus, loss of either has no effect on *Fli1* or *Gata2* expression, again in contrast to the network of Pimanda et al. [7]. Furthermore, *Scl* is required for *Fli1*-VP16 to drive *Flk1* expression (Figures 4A–4D), and loss of downstream *Runx1* and *SpiB* expression in *Fli1* morphants is rescued by a combination of *Scl*, *Lmo2*, and *Gata2* mRNAs (Figures 1S–1X). With respect to regulation of *Fli1* expression, we show that *Cloche* is required in the anterior hemangioblast (Figure S6), whereas *Bmp* is required in the posterior hemangioblast (Figure S7; see also [10]). Furthermore, although *Bmp* is not required to drive *Fli1* expression in the anterior hemangioblast, both *Bmp* and *Fli1* are required to drive *Scl*. In principle this could have indicated that *Fli1* is upstream of *Bmp*. However, at least for *Bmp4*, the dominant Bmp in early *Xenopus* embryos, this does not seem to be the case (Figures S4K and S4L). Thus, a feasible scenario in the anterior hemangioblast is that *Bmp* drives *Gata2* and *Gata2* plus *Fli1* drive *Scl* via the demonstrated ETS-Gata sites (Figure 4K) [5].

Careful temporal monitoring of hemangioblast marker expression and the onset of cell death in *Fli1* morphants demonstrated that disruption of the hemangioblast program occurred before the cells underwent apoptosis. Thus, *Fli1* is required for the induction of the hemangioblast program and also for cell survival, but whether *Fli1* is acting directly on the cell-death pathway cannot be determined from the available data. It is noteworthy, though, that *Fli1* has already been shown to display antiapoptotic activity in several cell types including avian erythroblasts, mouse fibroblasts, and lymphoid cells, possibly through the upregulation of Bcl2, an antiapoptotic protein [31]. So it is possible that the observed cell death is directly caused by the loss of *Fli1* rather than being a consequence of the misprogramming of the cells.

In conclusion, the data presented here finally confirm a position for *Fli1* at the top of the genetic cascade establishing the blood and endothelial programmes within cells of the mesoderm in the early embryo. We find some differences between the different populations with respect to the regulation of *Fli1* itself, but in each case we find that *Fli1* function is indispensable.

Accession Numbers

Tie2, Est clone, was deposited in the National Institute for Basic Biology (NIBB) (Japan) database with accession number ID XL064i22.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and seven figures and can be found with this article online at http://www.current-biology.com/cgi/content/full/18/16/1234/DC1/.

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References

1. Keller, G. (2001). The hemangioblast. In Stem Cell Biology, D.R. Marshak, R.L. Gardner, and D. Gottlieb, eds. (Cold Spring Harbor,NY: Cold Spring Harbor Laboratory Press), pp. 329–348.
2. Huber, T.L., Kouskoff, V., Fehling, H.J., Palis, J., and Keller, G. (2004). Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. Nature *432*, 625–630.
3. Ueno, H., and Weissman, I.L. (2006). Clonal analysis of mouse development reveals a polyclonal origin for yolk sac blood islands. Dev. Cell *11*, 519–533.
4. Vogeli, K.M., Jin, S.W., Martin, G.R., and Stainier, D.Y. (2006). A common progenitor for haematopoietic and endothelial lineages in the zebrafish gastrula. Nature *443*, 337–339.
5. Gottgens, B., Nastos, A., Kinston, S., Piltz, S., Delabesse, E.C., Stanley, M., Sanchez, M.J., Ciau-Uitz, A., Patient, R., and Green, A.R. (2002). Establishing the transcriptional programme for blood: The SCL stem cell enhancer is regulated by a multiprotein complex containing Ets and GATA factors. EMBO J. *21*, 3039–3050.
6. Landry, J.R., Kinston, S., Knezevic, K., Donaldson, I.J., Green, A.R., and Gottgens, B. (2005). Fli1, Elf1, and Ets1 regulate the proximal promoter of the LMO2 gene in endothelial cells. Blood *106*, 2680–2687.
7. Pimanda, J.E., Ottersbach, K., Knezevic, K., Kinston, S., Chan, W.Y., Wilson, N.K., Landry, J.R., Wood, A.D., Kolb-Kokocinski, A., Green, A.R., et al. (2007). Gata2, Fli1, and Scl form a recursively wired gene-regulatory circuit during early hematopoietic development. Proc. Natl. Acad. Sci. USA *104*, 17692–17697.
8. Gottgens, B., Broccardo, C., Sanchez, M.J., Deveaux, S., Murphy, G., Gothert, J.R., Kotsopoulou, E., Kinston, S., Delaney, L., Piltz, S., et al. (2004). The scl +18/19 stem cell enhancer is not required for hematopoiesis: identification of a 5′ bifunctional hematopoietic-endothelial enhancer bound by Fli-1 and Elf-1. Mol. Cell. Biol. *24*, 1870–1883.
9. Brown, L.A., Rodaway, A.R., Schilling, T.F., Jowett, T., Ingham, P.W., Patient, R.K., and Sharrocks, A.D. (2000). Insights into early vasculogenesis revealed by expression of the ETS-domain transcription factor Fli-1 in wild-type and mutant zebrafish embryos. Mech. Dev. *90*, 237–252.
10. Walmsley, M., Ciau-Uitz, A., and Patient, R. (2002). Adult and embryonic blood and endothelium derive from distinct precursor populations which are differentially programmed by BMP in *Xenopus*. Development *129*, 5683–5695.
11. Hart, A., Melet, F., Grossfeld, P., Chien, K., Jones, C., Tunnacliffe, A., Favier, R., and Bernstein, A. (2000). Fli-1 is required for murine vascular and megakaryocytic development and is hemizygously deleted in patients with thrombocytopenia. Immunity *13*, 167–177.
12. Spyropoulos, D.D., Pharr, P.N., Lavenburg, K.R., Jackers, P., Papas, T.S., Ogawa, M., and Watson, D.K. (2000). Hemorrhage, impaired hematopoiesis, and lethality in mouse embryos carrying a targeted disruption of the Fli1 transcription factor. Mol. Cell. Biol. *20*, 5643–5652.
13. Walmsley, M.E., Guille, M.J., Bertwistle, D., Smith, J.C., Pizzey, J.A., and Patient, R.K. (1994). Negative control of *Xenopus* GATA-2 by activin and noggin with eventual expression in precursors of the ventral blood islands. Development *120*, 2519–2529.

14. Inui, M., Fukui, A., Ito, Y., and Asashima, M. (2006). Xapelin and Xmsr are required for cardiovascular development in *Xenopus laevis*. Dev. Biol. *298*, 188–200.
15. Dalgin, G., Goldman, D.C., Donley, N., Ahmed, R., Eide, C.A., and Christian, J.L. (2007). GATA-2 functions downstream of BMPs and CaM KIV in ectodermal cells during primitive hematopoiesis. Dev. Biol. *310*, 454–469.
16. Ciau-Uitz, A., Walmsley, M., and Patient, R. (2000). Distinct origins of adult and embryonic blood in *Xenopus*. Cell *102*, 787–796.
17. Oikawa, T., and Yamada, T. (2003). Molecular biology of the Ets family of transcription factors. Gene *303*, 11–34.
18. Sumanas, S., and Lin, S. (2006). Ets1-related protein is a key regulator of vasculogenesis in zebrafish. PLoS Biol. *4*, e10.
19. Zhu, H., Traver, D., Davidson, A.J., Dibiase, A., Thisse, C., Thisse, B., Nimer, S., and Zon, L.I. (2005). Regulation of the lmo2 promoter during hematopoietic and vascular development in zebrafish. Dev. Biol. *281*, 256–269.
20. Pham, V.N., Lawson, N.D., Mugford, J.W., Dye, L., Castranova, D., Lo, B., and Weinstein, B.M. (2007). Combinatorial function of ETS transcription factors in the developing vasculature. Dev. Biol. *303*, 772–783.
21. Truong, A.H., and Ben-David, Y. (2000). The role of Fli-1 in normal cell function and malignant transformation. Oncogene *19*, 6482–6489.
22. Patterson, L.J., Gering, M., Eckfeldt, C.E., Green, A.R., Verfaillie, C.M., Ekker, S.C., and Patient, R. (2007). The transcription factors Scl and Lmo2 act together during development of the hemangioblast in zebrafish. Blood *109*, 2389–2398.
23. Patterson, L.J., Gering, M., and Patient, R. (2005). Scl is required for dorsal aorta as well as blood formation in zebrafish embryos. Blood *105*, 3502–3511.
24. Dooley, K.A., Davidson, A.J., and Zon, L.I. (2005). Zebrafish scl functions independently in hematopoietic and endothelial development. Dev. Biol. *277*, 522–536.
25. Stainier, D.Y., Weinstein, B.M., Detrich, H.W., 3rd, Zon, L.I., and Fishman, M.C. (1995). Cloche, an early acting zebrafish gene, is required by both the endothelial and hematopoietic lineages. Development *121*, 3141–3150.
26. Pyati, U.J., Webb, A.E., and Kimelman, D. (2005). Transgenic zebrafish reveal stage-specific roles for Bmp signaling in ventral and posterior mesoderm development. Development *132*, 2333–2343.
27. Detrich, H.W., Kieran, M.W., Chan, F.Y., Barone, L.M., Yee, K., Rundstadler, J.K., Pratt, S., Ransom, D., and Zon, L.I. (1995). Intraembryonic hematopoietic cell migration during vertebrate development. Proc. Natl. Acad. Sci. USA *92*, 10713–10717.
28. Swiers, G., Patient, R., and Loose, M. (2006). Genetic regulatory networks programming hematopoietic stem cells and erythroid lineage specification. Dev. Biol. *294*, 525–540.
29. Davidson, E.H., Rast, J.P., Oliveri, P., Ransick, A., Calestani, C., Yuh, C.H., Minokawa, T., Amore, G., Hinman, V., Arenas-Mena, C., et al. (2002). A provisional regulatory gene network for specification of endomesoderm in the sea urchin embryo. Dev. Biol. *246*, 162–190.
30. Zettervall, C.J., Anderl, I., Williams, M.J., Palmer, R., Kurucz, E., Ando, I., and Hultmark, D. (2004). A directed screen for genes involved in *Drosophila* blood cell activation. Proc. Natl. Acad. Sci. USA *101*, 14192–14197.
31. Pereira, R., Quang, C.T., Lesault, I., Dolznig, H., Beug, H., and Ghysdael, J. (1999). FLI-1 inhibits differentiation and induces proliferation of primary erythroblasts. Oncogene *18*, 1597–1608.
32. Friedle, H., and Knochel, W. (2002). Cooperative interaction of Xvent-2 and GATA-2 in the activation of the ventral homeobox gene Xvent-1B. J. Biol. Chem. *277*, 23872–23881.