Distinct Roles for SCL in Erythroid Specification and Maturation in Zebrafish*

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The stem cell leukemia (SCL) transcription factor is essential for vertebrate hematopoiesis. Using the powerful zebrafish model for embryonic analysis, we compared the effects of either reducing or ablating Scl using morpholino-modified antisense RNAs. Ablation of Scl resulted in the loss of primitive and definitive hematopoiesis, consistent with its essential role in these processes. Interestingly, in embryos with severely reduced Scl levels, erythroid progenitors expressing gata1 and embryonic globin developed. Erythroid maturation was deficient in these Scl hypomorphs, supporting that Scl was required both for the erythroid specification and for the maturation steps, with maturation requiring higher Scl levels than specification. Although all hematopoietic functions were rescued by wild-type Scl mRNA, an Scl DNA binding mutant rescued primitive and definitive hematopoiesis but did not rescue primitive erythroid maturation. Together, we showed that there is a distinct Scl hypomorphic phenotype and demonstrated that distinct functions are required for the roles of Scl in the specification and differentiation of primitive and definitive hematopoietic lineages. Our results revealed that Scl participates in multiple processes requiring different levels and functions. Further, we identified an Scl hypomorphic phenotype distinct from the null state.

Hematopoiesis occurs through progressive commitment of hematopoietic stem cells toward differentiated lineages (1, 2). Hematopoietic development can be directly observed and manipulated using the zebrafish model, Danio rerio (3). Zebrafish have become a powerful vertebrate model since embryos are externally fertilized and are optically clear, making them easily accessible (4, 5).

Vertebrate embryonic blood formation is marked by two successive stages of blood development, termed primitive and definitive hematopoiesis (6). In zebrafish, the primitive wave of hematopoiesis results in differentiation of erythrocytes (7) and the myeloid lineages, including both macrophage/monocytes (8) and granulocytes (9, 10). The myeloid and erythroid lineages develop from two distinct anatomical locations during primitive hematopoiesis. The posterior lateral mesoderm (PLM),2 which forms the intermediate cell mass (ICM) (7), just ventral to the notochord, is the site of erythroid development. The anterior lateral mesoderm (ALM), located rostrally in the head region, gives rise to the monocyte/macrophages and granulocytes (8–11). Definitive hematopoiesis is thought to begin in cells in the ventral wall of the aorta by ~30–36 hpf. By 4 dpf, the site of hematopoiesis has shifted to the kidney, where differentiation of hematopoietic lineages occurs throughout adult life (6).

The stem cell leukemia (SCL) gene, first identified to be involved in the common t(1:14) translocation seen in childhood T-cell acute lymphoblastic leukemia (12, 13), is required for specification of mesoderm to hematopoietic stem cells (13–15) and is required for both primitive and definitive hematopoiesis (16–19). Furthermore, it plays a role in vasculogenesis and is thought to operate at the level of the proposed upstream precursor to both hematopoietic and vascular cells, the hemangioblast (20, 21).

scl is expressed in the hematopoietic cells of both the ALM and the PLM in zebrafish (20, 22). In this study, we explored the role of scl in zebrafish hematopoiesis using morpholino-modified antisense oligonucleotide (MO) knockdown of Scl. Using this approach, we were able to selectively either reduce or completely ablate functional Scl. Upon complete ablation of Scl, we showed here that Scl is required for primitive and definitive hematopoiesis, consistent with two other recent studies (23, 24).

In embryos in which functional Scl is severely reduced, but not completely absent, a distinct hypomorphic phenotype was observed. Scl-reduced embryos lacked primitive myeloid development and definitive hematopoiesis. Interestingly, primitive erythroid specification remained intact, as demonstrated by the presence of gata1- and globin-expressing erythroid progenitors. These progenitors, however, were unable to form mature erythrocytes. Thus, low levels of Scl were sufficient for primitive erythroid specification but not maturation. In Scl-ablated embryos, primitive erythroid specification and myeloid differentiation could be rescued with either wild-type Scl or a DNA binding mutant of Scl, whereas primitive erythroid maturation could only be rescued with the wild-type form. These studies revealed a distinct Scl hypomorphic phenotype and demonstrated differential effects of Scl reduction on hematopoietic specification and development of the myeloid and erythroid developmental programs in primitive hematopoiesis. Further, these findings demonstrated distinct roles for zebrafish Scl in primitive erythroid specification and erythroid maturation. The ability of an Scl DNA binding mutant to rescue early erythroid development, but not maturation, suggested that Scl may directly bind DNA at target genes involved in erythroid maturation, whereas it may exist in a multiprotein complex in which other factors bind DNA during erythroid specification. These results demonstrated that transcription factors such as Scl can play distinct roles in regulation of different genes based on the other members of the transcriptional complex. The zebrafish
offers a rapid in vivo system for analysis of transcription factors in development.

**EXPERIMENTAL PROCEDURES**

**Zebrafish Maintenance and Breeding**—Zebrafish were maintained and bred essentially as described in *The Zebrafish Book* (25). Experiments were performed using a standard wild-type strain (EK) and a *gata1* promoter-driven DsRed transgenic line (26).

**MO Microinjection**—Embryos used for microinjection were obtained from natural spawning of fish as indicated. Morpholinos were created by Gene Tools, LLC (Philomath, OR). The genomic structure of *scl* was determined based on BLAST comparisons between the genomic sequence in bacterial artificial chromosome clone dz129I22 and the cDNA (GenBank™ accession numbers AF038873 and BC068324). The sequences that masked the *scl* splice donor site of exon 2 and exon 3 were chosen through the Gene Tools oligonucleotide design service, according to the manufacturer’s guidelines. The MOs used in the study were the following: exon 2 splice donor (ex 2), MO 5'-H11032-AAAGCGGCGTTACCTGTTAATAGTG-3'; exon 2 mismatch (mis 2), MO 5'-H11032-AAAACCGGCcTTAgCTcTTAATAcTG-3' (lowercase letters indicate the base changes from the exon 2 splice donor MO); exon 3 splice donor (ex 3), MO 5'-TAAAATGCTCTTACCATCGTTGATT-3'; exon 3 mismatch (mis 3), 5'-TAAAATcCTgTTAgCATCcTTcATT-3' (lowercase letters indicate the base changes from the exon 3 splice donor MO). MOs were injected at doses that resulted in minimal morphologic deformities. The quantities of MO injected were 1 mM for ex 2 and mis 2 MO and 1.5 mM for ex 3 and mis 3 MO. For all results, injections were repeated in at least three independent experiments with 20–30 embryos for each sample.

**Whole-mount in Situ Hybridization**—Whole-mount in situ hybridization was performed as described previously (27). Antisense RNAs for in situ hybridization were transcribed from plasmid DNAs linearized before the coding sequence and transcribed using SP6, T3, or T7 RNA polymerases as appropriate. The zebrafish cDNAs used were as follows: *scl* (20, 22), *gata2* (7, 28), *lmo2* (29), *c-myb* (29), *lys C* (30), *mpo* (9), *l-pl* (8), *c/ebp* (27), *pu.1* (9, 10), *dra* (8), *band 3* (31), *b-spectrin* (32), *gata1* (7), embryonic α-globin (33), *ikaros* (34), and *pax2.1* (35). Some images of embryos were taken in two focal planes and merged in Adobe Photoshop to allow simultaneous sharp focus of yolk sac and tail structures in a single image. All results were obtained in at least 80% of embryos in two independent experiments with over 30 embryos total for all in situ results.

**RNA Isolation**—RNA was collected at the 16-somite stage for reverse transcriptase-PCR (RT-PCR). Approximately 20–30 injected embryos were collected, and RNA was then isolated using TRIzol (Invitrogen) according to manufacturer’s instructions for RNA isolation.

**RT-PCR**—Two micrograms of isolated RNAs were used in 20-ml reaction volumes to synthesize cDNA using SuperScript II (Promega) according to the manufacturer’s instructions. Reactions without SuperScript II were performed in parallel as negative controls. Primers used for amplification of RNA products were as follows: forward primer, P284 5'-ATCGCGCCGAAGGATGATG3' (targeted to a sequence in...
exon 2); reverse primer, P208 5’-CACCGTTCACATTCTGCTG-3’
(targeted to a sequence of exon 4).

PCR conditions used were denaturation for 30 s at 94 °C, annealing
for 30 s at 58 °C, and then extension for 1.0 min 72 °C for 30 cycles
followed by a single 5-min extension at 72 °C after the last cycle. Prod-
ucts were subcloned by TA cloning (Invitrogen) and sequenced, with at
least four subclones sequenced.

mRNA Rescue—Capped mRNA was synthesized using an mMessage
kit (Ambion). scl cDNA was linearized with SsPl followed by transcrip-
tion with T3 or SP6 polymerase. Rescue experiments were performed by
injecting MOs with and without 100 pg of scl mRNA. Injections with
eGFP mRNA were performed in parallel with rescue injections as neg-
ative controls. The DNA binding mutant, RER-
scl, was synthesized
using a Stratagene QuikChange site-directed mutagenesis kit according
to manufacturer’s instructions with the following primers: 5’-CACGA-
ACAGTGCCGCGGCTGCGACAGCAGAATGTGAACGGT-
G-3’ and 5’-CACCGTTCACATTCTGCTGTCGCCAGGCCG-
CACTGTTCGTG-3’.
RESULTS

Normal Scl Expression Is Reduced or Ablated by scl MOs—To examine the consequences of Scl knockdown, we designed two MO oligonucleotides directed to the zebrafish scl sequence. The MOs were designed to inhibit splicing by masking the splice donor sites for exons 2 (ex 2 MO) and exon 3 (ex 3 MO). To determine the efficacy of each of the two splicing MOs separately or together, ex 2, ex 3, or ex 2/ex 3 MOs were injected into single cell embryos followed by collection of RNA at 17 or 18 somites (17 hpf) and RT-PCR to analyze splice products. Amplification of uninjected, mis 2, or mis 3 embryo cDNAs with exon 2 (P284) and exon 4 (P208) primers demonstrated a single PCR product of ~650 bp, the expected size of the fragment amplified from correctly spliced scl mRNA (Fig. 1A). RT-PCR with ex 2, ex 3, or ex 2/ex 3 MO RNAs revealed only aberrant splice products (Fig. 1A).

Sequencing of each variant revealed that all resulted in use of cryptic splice sites outside the region masked by the MOs. One product was amplified from ex 3 MO-injected embryos (Fig. 1, A–C), which predicted a protein product missing the entire basic helix-loop-helix domain (bHLH), known to be required for Scl DNA binding and function in vivo (36). In multiple amplifications, either a single larger product (variant b) or a dominant larger product and a minor smaller product, variant c, were amplified from exon 2 MO-injected embryos (Fig. 1A). The larger product predicted a truncated protein lacking the bHLH domain, whereas the smaller product predicted a substitution of amino acid Gln-121 → His and deletion of 27 amino acids (position 122–148) followed by an intact bHLH domain (Fig. 1, B and C). Although the protein predicted by variant c would result in a functional product, variant c was absent in some PCR amplifications and was a minor product in others, suggesting that it might account for a small amount of functional protein in vivo. RT-PCR from ex 2/ex 3 MO-injected embryos demonstrated a single product, variant d, resulting from cryptic splicing at exon 2 (Fig. 1B, at a unique cryptic splice site, close to variant b) and exon 3 (Fig. 1B, the same site used for variant a), resulting in a truncated predicted protein lacking the bHLH domain (Fig. 1, A–C).

Since the mechanism of splicing MO-directed inactivation is based on inhibition of proper mRNA splicing, the total levels of mRNA are not necessarily affected by MOs. However, in many cases, it appeared that targeted transcripts are absent or present due to nonsense-mediated mRNA decay or autoregulation of the gene. To examine scl mRNA expression patterns, we examined scl MO-injected embryos by RNA in situ hybridization. scl expression appeared mildly reduced in exon 3 MO-injected embryos (Fig. 2) and severely reduced in ex 2 MO-injected embryos (Fig. 2), whereas expression of scl in ex 2/ex 3 MO-injected embryos was undetectable. Based on the sequencing analysis of splice products, the mRNA present in ex 3 MO-injected embryos should encode non-functional protein, whereas a small amount of the residual mRNA from ex 2 may have functional product (variant c). However, the total scl mRNA levels from ex 2-injected embryos was low. These results showed that the combined ex 2 and ex 3 MO injections resulted in stronger knockdown of scl mRNA than either MO alone. However, each single MO resulted in significant reduction in functional Scl.

Scl MO-injected Embryos Lack Both Primitive and Definitive Hematopoiesis—Embryos injected with ex 2, ex 3, or ex 2/ex 3 MOs were assessed for blood development by light microscopy. In all scl MO-injected embryos, 80–100% of injected embryos lacked any circulating erythrocytes and did not have visible hemoglobinized blood pooled in the embryo, whereas less than 5% of control (mis) MO-injected embryos lacked circulating erythrocytes at 26–30 hpf (Fig. 3A).
(Fig. 5D), which both mark granulocytes\(^3\), were absent in scl MO-injected embryos, but not in mis MO-injected control embryos. Thus, both the granulocyte and the macrophage myeloid subsets were absent in either the single MO or combination scl MO-injected embryos.

To examine early myeloid lineage development, embryos injected with each of the scl MOs or the mis MOs were analyzed by RNA in situ hybridization to follow the expression of genes that are activated early in myelopoiesis. pu.1 encodes a hematopoietic transcriptional activator required for the differentiation of myeloid lineage cells and B-lymphocytes. pu.1 is also expressed in early erythroid progenitors but is negatively regulated upon differentiation to mature erythrocytes (37). RNA in situ hybridization of single or double scl MO-injected embryos at the 16-somite stage (17 hpf) revealed the absence of pu.1 expression in the ALM (Fig. 5B). Expression of pu.1 in the PLM was lost in the ex 2/ex 3 MO-injected embryos but was variably retained in the single MO-injected embryos (data not shown).

To examine myeloid development upstream of pu.1, draculin (dra) expression was assessed. dra encodes a 12-zinc finger protein that displays a hematopoietic expression pattern in zebrafish embryos (8) with hematopoietic expression beginning at three somites (11 hpf) in the ALM and PLM. Although the function of dra has not yet been explored, expression of dra occurs at approximately the same time as scl and precedes pu.1 (8). It is therefore presumed to occur upstream of pu.1, in the precursors of the myeloid lineage in the ALM and in the precursors of the erythroid lineage in the PLM. dra expression was absent from the ALM in all scl MO-injected embryos (Fig. 5A). PLM expression of dra was absent in the ex 2/ex 3 embryos but was largely preserved in all the single MO-injected embryos (Fig. 6A). The loss of gene expression in the ALM and PLM was specific to hematopoietic markers. Examination of other mesodermal, non-hematopoietic genes, including the central nervous system and kidney marker, pax2.1 (Figs. 5E and 6E) and the cardiac marker, gata5 (data not shown), showed no change in their expression in embryos injected with ex 2/ex 3 MOs. In summary, development of the myeloid lineage in the ALM was lost in all scl MO-injected embryos. The block to development in the ALM occurred upstream of dra and pu.1. In contrast, expression of dra and pu.1 in the PLM/ICM region of the embryo was less affected in single MO-injected embryos than in the combined ex 2/ex 3 MO embryos, suggesting differential sensitivity to dosage of Scl.

**Figure 5. scl morphants lack primitive myeloid development.** A, B, and E, anterior views of 14-somite embryos showing the ALM. C and D, lateral views with head to the left of 28-hpf embryos. Whole-mount RNA in situ hybridizations with dra (A), pu.1 (B), lys C (C), l-pl (D), and pax2.1 (E). Control embryos were injected with either mis MO or a combination of mis MOs. Identical results were obtained with all control injections. Ex 2 or ex 3 MO-injected embryos demonstrated the same findings, shown in the middle panel. A mix of ex 2/ex 3 MO-injected embryos is shown in the right panel.

\(^3\) S. E. Lyons, manuscript submitted.

**Primitive Erythroid Specification, but Not Erythroid Maturation, Was Retained in Single scl MO-injected Embryos—**The expression of dra and pu.1 in the ICM/PLM was unexpected and suggested that hematopoietic development in the PLM, the site of erythropoiesis, was relatively preserved in embryos injected with either ex 2 or ex 3 scl MOs separately. To further examine which step(s) in erythroid development might be affected, the expression of several erythroid-specific genes was...
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FIGURE 6. Single scl MO-injected embryos retain early erythroid development. A–E, posterior views of 14-somite embryos showing PLM (A, B, C, and D) and lateral views with head to the left of 28-hpf embryos (D). Whole-mount RNA in situ hybridizations were performed on embryos injected with mis MOs (control), ex 2, or ex 3 MOs or the combination of ex 2/ex 3 MOs. Identical results were obtained with ex 2 and ex 3 MOs injected separately. A, dra; B, gata1; C, α1-embryonic globin; D, band3; and E, pax2.1.

investigated. Surprisingly, expression of the early erythroid markers, gata1 (Fig. 6B) and embryonic α-globin (Fig. 6C), was relatively intact at the 16–21-somite stage (17–19 hpf) in scl MO-injected embryos. Embryos injected in the same experiment as those used for in situ hybridizations were observed beyond 48 hpf, and over 80% never developed circulating blood.

Although expression of gata1 and embryonic globin demonstrated intact early erythroid development, the erythroid progenitors did not appear to develop into hemoglobinized erythrocytes, as shown by the lack of circulating blood and the lack of o-dianisidine staining. Expression of band3, a marker of mature erythrocytes, was absent in both the single and the double scl MO-injected embryos, whereas there was strong expression in 100% of the control embryos (Fig. 6D). Therefore, erythroid development was arrested prior to expression of band3.

Injection of MOs into a gata1 promoter DsRed transgenic line, Tg(gata1::DsRed) (26), allowed continued observation of the fluorescently marked erythroid progenitor cells through 3 dpf. Due to the stability of DsRed, DsRed fluorescence was visible beyond 4 dpf. Using the DsRed line, erythroid cells could be distinctly seen in ex 2 or ex 3 MO-injected embryos in the ICM/PLM between 24 hpf (Fig. 7A) and 2 dpf (Fig. 7F), although gata1(+) cells never entered circulation (Fig. 7, E and F). In control embryos, DsRed (+) cells entered circulation by 28 hpf (Fig. 7A), with few cells remaining in the ICM (Fig. 7B), but DsRed (+) cells were clearly seen in circulation as seen both in the tail (Fig. 7B) and over the yolk sac (Fig. 7C). Although control embryos continued to have strong DsRed expression in circulating cells up to 4 dpf, the DsRed (+) cells in the ICM of the scl MO embryos were no longer visible by 3 dpf. Acridine orange staining was done at 3-h intervals from 26 hpf to 3 days, but no excess apoptosis was seen in the single scl MO-injected embryos (data not shown). Since the loss of DsRed expression appeared to occur slowly between 2 and 3 dpf, apoptosis may be occurring asynchronously so that few cells may stain with acridine orange at any given time point.

Primitive Erythroid Maturation Requires scl DNA Binding Activity—To confirm that the hematopoietic defects in scl MO embryos were due to the loss of functional Scl, rescue experiments were performed with scl mRNA. Synthesized scl mRNA (100 pg) was co-injected with scl ex 2, ex 3, or the combined ex 2/ex 3 MOs. Since scl splice MOs target exon-intron boundaries, synthetic mRNA lacking introns will not be affected by either MO. Injection of mRNA with the ex 2/ex 3 splice site MOs resulted in rescue of gata1 (Fig. 8A), globin (Fig. 8B), band3 (Fig. 8C), lps C (Fig. 8D), and l-pl (Fig. 8E). Similar rescue was obtained for each scl MO separately (data not shown), excluding gata1, since its expression was intact in the single MO-injected embryos.

The finding of erythroid specification, but not maturation in single scl MO-injected embryos, suggested that zebrafish Scl is needed at two independent steps in erythroid differentiation, namely for erythroid specification and again for maturation. In mammals, the DNA binding function of Scl has been shown to be required for definitive erythroid maturation but not for erythroid specification by in vitro studies (36, 38). Zebrafish Scl has 100% identity to the human and mouse bHLH domain of SCL. An Sc1 construct encoding the identical changes in the bHLH domain to a previously described mouse mutant ScL was used (36). The mutant ScL was unable to bind DNA with its binding partner.
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E12 (Ref. 36 and data not shown). This mutant mRNA, which encodes AAA residues replacing the basic RER sequence within the bHLH domain (scl-RER mRNA), was able to rescue gata1 (Fig. 8A), globin (Fig. 8B), lys C (Fig. 8D), and l-pl (Fig. 8E) in ex 2/ex 3 MO-injected embryos. However, scl-RER mRNA was unable to rescue band3 expression in ex 2/ex 3 MO-injected embryos (Fig. 8C). band3 expression was examined from 21 somites to 28 hpf and was absent at all time points in RER-scl mRNA-injected embryos (data not shown). These results demonstrated that DNA binding of Scl is needed for erythroid maturation but is not required for its other hematopoietic functions. These data further strengthened the finding that Scl is required at two points in primitive erythroblastic development, both the erythroid specification and the maturation steps of hematopoiesis, since rescue of erythroid specification does not also rescue the subsequent erythroid developmental steps. These results demonstrated conservation between the roles of Scl in primitive and definitive erythroblastic development and were the first in vivo demonstration of both the DNA-dependent and the DNA-independent aspects of Scl function.

**DISCUSSION**

Using the zebrafish model, we showed that varying threshold levels of Scl are required for different functions in primitive and definitive hematopoiesis. Further, we demonstrated that there are distinct requirements for Scl in primitive erythroblastic specification and maturation, with only maturation requiring intact DNA binding function. The zebrafish provides a number of unique capabilities for these studies. First, the presence of both primitive erythroid and myeloid lineages is unique in comparison with mammals, in which primitive hematopoiesis is largely erythropoietic. Further, unlike mouse models, which require creation of transgenic or knock-out lines and multiple generations to create allelic combinations, use of MOs in zebrafish allows rapid in vivo modeling and assessment of phenotypes.

In this report, we demonstrated an essential role for Scl in vivo in the development of primitive and definitive hematopoiesis, consistent with two other recent studies in zebrafish (23, 24). Our work, however, examined not only the Scl-ablated state but also the Scl-reduced condition. A unique phenotype was observed when we reduced Scl rather than ablated Scl using single MOs. We showed isolated development of erythroid progenitors that were unable to mature. In contrast, primitive myelopoiesis and definitive hematopoiesis did not occur in the presence of severely reduced Scl levels. The relatively intact expression of **dra**
followed by $gata1$ and $\alpha$-globin in the PLM/ICM marked erythroid progenitors. In contrast, $dra$ and $pu.1$ were not expressed in the ALM, demonstrating a lack of myeloid progenitors. These results showed that very low levels of functional Scl sustain primitive erythroid specification but not primitive myeloid development. The absence of $c-myb$ expression in the ventral aorta beyond 30 hpf supported the loss of definitive hematopoiesis under these conditions.

The finding of erythroid specification, but not maturation, in Scl-reduced embryos supported a role for Scl at two distinct stages in primitive erythroid development. If Scl was only required during specifica-
tion, then erythroid progenitors would continue to mature once they had been successfully specified from mesoderm. Previous data have supported an independent role for Scl in mammalian definitive erythroid specification and maturation (36, 38, 39). Our studies revealed that Scl also plays independent roles during primitive erythropoiesis.

In vitro cell differentiation studies have demonstrated that the DNA binding function of SCL is required for definitive erythroid maturation but not for initiation of primitive or definitive hematopoiesis (36, 38). To expand upon these findings using our in vivo model, we tested the rescue abilities of a dimerization-competent/DNA binding-defective Scl. Our results demonstrated that a DNA binding-defective Scl could rescue primitive erythroid specification but could not rescue erythroid maturation.

Our work has been the first, to our knowledge, to examine the role of the Scl DNA binding mutants in vivo rather than through in vitro differ-
entiation assays that may not fully recapitulate the in vivo state. Fur-
ther, our data demonstrated a DNA binding requirement for primitive erythroid maturation, whereas other studies found this requirement for definitive erythroid maturation (36, 38).

These findings added another dimension to the hematopoietic func-
tions of Scl. Our observations demonstrated strong functional conser-
vation between the role of Scl in primitive and definitive erythroid mat-
uration. The conservation of function between primitive and definitive hematopoiesis cannot be assumed a priori. For example, some tran-
scription factors function differently in primitive and definitive ha-
ematopoiesis, such as Runx1/AML1 (40) and c-Myb (41), which are required for definitive, but not primitive, hematopoiesis. Thus, it is crit-
ical to closely analyze the roles of hematopoietic factors in both stages of hematopoiesis.

The findings furthermore supported the idea that Scl may function in different multiprotein complexes, some requiring DNA binding from Scl and others achieving DNA binding through different factors in the complex. This principle should guide studies of other transcriptional complexes to dissect the functional roles played by all their component proteins.

To date, a large body of work studying Scl null mice or cells derived from these mice has reflected the complete loss of the Scl gene. In contrast, our study has been the first to examine an Scl hypomorphic phenotype. The different phenotype manifested by an Scl hypomorphic state when compared with a null state reveals differential threshold levels of Scl needed for its hematopoietic functions. This type of modula-
tion has been seen with other hematopoietic (42–46) and non-he-
ematopoietic transcription factors (47–49) and provides a level of com-
plexity beyond a simple on-off state.

Examination of hypomorphic phenotypes, either through haploin-
sufficiency (50% levels) or through allelic mutations that reduce quant-
ty or function of proteins, has demonstrated that dosage effects often result in phenotypes that significantly differ from the null state. Such hypomorphic phenotypes have now been shown to contribute to many congenital developmental defects (50, 51), disease states (47, 48), and predisposition to leukemia (52, 53). A number of transcription factors have been shown to have differing threshold levels for various aspects of development, including the homeodomain transcription factor, Pitx2 (49), and another member of the bHLH family, myogenin (54). Thus, our findings with Scl have important implications both for hematopoiesis and for our understanding of widespread developmental and dis-
ease processes.

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