CHAPTER SIX

# SCL/TAL1 in Hematopoiesis and Cellular Reprogramming

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## Contents



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## Abstract

SCL, a transcription factor of the basic helix–loop–helix family, is a master regulator of hematopoiesis. *Scl* specifies lateral plate mesoderm to a hematopoietic fate and establishes boundaries by inhibiting the cardiac lineage. A combinatorial interaction between *Scl* and *Vegfa/Flk1* sets in motion the first wave of primitive hematopoiesis. Subsequently, definitive hematopoietic stem cells (HSCs) emerge from the embryo proper via an endothelial-to-hematopoietic transition controlled by *Runx1*, acting with *Scl* and *Gata2*. Past this stage, *Scl* in steady state HSCs is redundant with *Lyl1*, a highly homologous factor. However, *Scl* is haploinsufficient in stress response, when a rare subpopulation of HSCs with very long term repopulating capacity is called into action. SCL activates transcription by recruiting a core complex on DNA that necessarily includes E2A/HEB, GATA1–3, LIM-only proteins LMO1/2, LDB1, and an extended complex comprising ETO2, RUNX1, ERG, or FLI1. These interactions confer multifunctionality to a complex that can control cell proliferation in erythroid progenitors or commitment to terminal differentiation through variations in single component. Ectopic *SCL* and *LMO1/2* expression in immature thymocytes activates of a stem cell gene network and reprogram cells with a finite lifespan into self-renewing preleukemic stem cells (pre-LSCs), an initiating event in T-cell acute lymphoblastic leukemias. Interestingly, fate conversion of fibroblasts to hematoendothelial cells requires not only *Scl* and *Lmo2* but also *Gata2*, *Runx1*, and *Erg*, indicating a necessary collaboration between these transcription factors for hematopoietic reprogramming. Nonetheless, full reprogramming into self-renewing multipotent HSCs may require additional factors and most likely, a permissive microenvironment.

## 1. INTRODUCTION

The stem cell leukemia gene (*SCL/TAL1)* is a master regulator of normal and aberrant hematopoiesis (Begley & Green, 1999; Lecuyer & Hoang, 2004). The gene, encoding a transcription factor of the basic helix–loop–helix (bHLH) family (Fig. 1), was independently cloned by several groups from a recurrent chromosomal translocation in T-cell acute lymphoblastic leukemia (T-ALL; Begley, Aplan, Denning, et al., 1989; Bernard et al., 1990; Chen, Cheng, et al., 1990; Finger et al., 1989). In the first case report, however, the disease initiated with an early T-cell phenotype and strikingly underwent phenotype conversion to a myeloid type leukemia following treatment (Begley, Aplan, Denning, et al., 1989). Because of this "stem cell leukemia," the *SCL* name was assigned to the gene. As it turned out, stem cell leukemia was an exception rather than the rule, although the

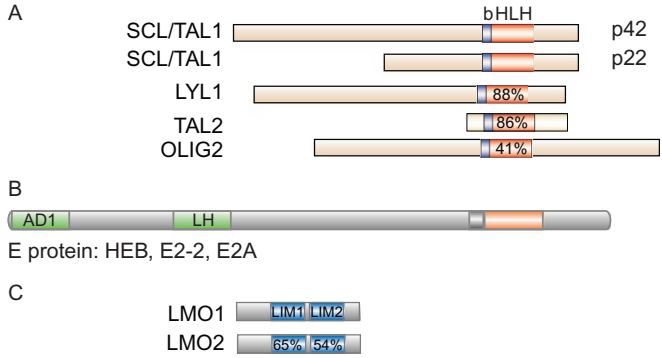


**Fig. 1** *Schematic representation of hematopoietic transcription factors of the basic helix–loop–helix (bHLH) family and LIM-only (LMO) proteins*. (A) bHLH transcription factors encoded by genes that are activated by recurring chromosomal translocations in T-ALL. Two major protein isoforms are produced by translation regulation of *SCL*. Shown are the degrees of homology in the HLH domains of the various factors compared to SCL. (B) E proteins (HEB/TCF12, E2-2/TCF4 and E2A/TCF3) are relatively more ubiquitously expressed than hematopoietic bHLH factors. The *E2A* gene is involved in chromosomal translocations in B-ALL. (C) LMO1 and LMO2 proteins are encoded by genes that are activated in T-ALL, either by recurring chromosomal translocations or by retroviral integration. Shown are the homology within LIM1 or LIM2 between LMO1 and LMO2.

gene name was remarkably premonitory of SCL critical function at the onset of hematopoiesis and in stem cells, as shall be discussed later. The gene was also designated as *TAL1* because of its frequent occurrence in chromosomal translocations in T-ALL (Chen, Cheng, et al., 1990). Additional bHLH oncogenic transcription factors were later cloned from rare chromosomal translocations (Fig. 1).

## 2. SCL FUNCTION DURING DEVELOPMENT

### 2.1 Back to the Origin: Onset of Hematopoiesis

The development of the hematopoietic system occurs in at least three waves: a first wave of primitive erythropoiesis, followed by a second wave of transient multipotent progenitors, and a third wave of definitive or adult hematopoiesis. Exhaustive and critical analyses of the process can be found in recent reviews (Ciau-Uitz, Monteiro, Kirmizitas, & Patient, 2014; Palis, 2014). A brief overview is provided here as a framework for contextualizing SCL function. Primitive erythropoiesis consists in a rapid and transient

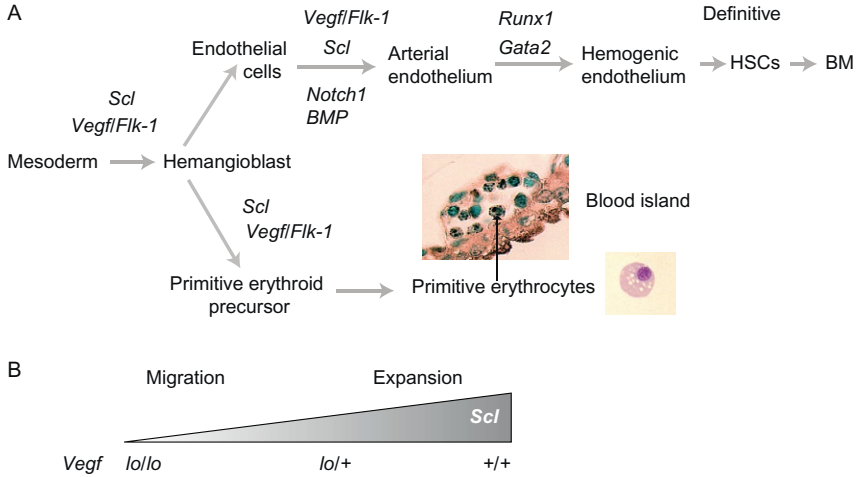


**Fig. 2** *Role of Scl and Vegf during the ontogeny of the hematopoietic system*. (A) Spatial and temporal dynamics during hematopoietic development in the mouse. The lateral plate mesoderm gives rise FLK1$^{+}$ hemangioblasts from which originate both primitive and definitive lineages. The onset of hematopoiesis is characterized by the emergence of primitive erythrocytes within blood islands in the yolk sac (YS; primitive hematopoiesis). Hematopoietic stem cells (HSC) of the definitive myeloid and lymphoid lineages (definitive hematopoiesis) originate from the embryo proper via an endothelial to hematopoietic transition (EHT) to finally seed the bone marrow, the major site of hematopoiesis in the adult. *Vegf*: vascular endothelial growth factor. (B) Different *Vegf* thresholds control the migration of Flk1$^{+}$ hematopoietic and endothelial precursors to reach the yolk sac and their expansion during primitive erythropoiesis. *Scl* is also upregulated by VEGF signaling, in vitro and in vivo. *Vegf* $^{+/+}$: wild-type *Vegf* allele. *Vegf* $^{lo}$: hypomorphic allele.

generation of primitive erythrocytes, which are large, nucleated cells that express embryonic globins (Fig. 2; reviewed in Palis, 2014) and of primitive macrophages (Travnickova et al., 2015). Primitive erythrocytes emerge in the yolk sac after 7.5 days of gestation (E7.5) in the mouse, surrounded by endothelial cells, in structures known as blood islands (Fig. 2; Palis, 2014). At E9.5, erythro-myeloid progenitors (EMP) emerge in the yolk sac and migrate to the fetal liver to produce erythroid and myeloid lineages (McGrath et al., 2015). Definitive hematopoietic stem cells (HSCs) originate in the embryo proper, by budding from the aortic endothelium (Bertrand et al., 2010; Boisset et al., 2010) and begin to colonize the fetal liver at E11.5 (McGrath et al., 2015), which rapidly becomes the main site of hematopoiesis until birth, when self-renewing HSCs finally seed the bone marrow (Fig. 2; reviewed in Ciau-Uitz et al., 2014).

## 2.2 SCL Expression During Development

SCL, a bHLH transcription factor, is first expressed at E7.5, in groups of mesodermal cells of the yolk sac that are fated to generate blood islands (Elefanty, Begley, Hartley, Papaevangeliou, & Robb, 1999; Kallianpur, Jordan, & Brandt, 1994; Silver & Palis, 1997). As blood islands develop, SCL expression is maintained at high levels in primitive erythroid cells and at lower levels in surrounding endothelial cells (Drake & Fleming, 2000). In the developing embryo and in the adult, SCL is detected in all of the major hematopoietic organs, strongly in hematopoietic clusters emerging from the floor of the dorsal aorta (Labastie, Cortes, Romeo, Dulac, & Peault, 1998), fetal liver, spleen and bone marrow, and restricted to a few cells in the thymus (Elefanty et al., 1999; Green, Lints, Visvader, Harvey, & Begley, 1992; Herblot, Steff, Hugo, Aplan, & Hoang, 2000; Kallianpur et al., 1994; Mouthon et al., 1993; Pulford et al., 1995). In the adult, *Scl* levels are highest in HSCs (Lacombe et al., 2010; Moignard et al., 2013), although *Scl* is also present in CFU-S (Elefanty et al., 1998), in hematopoietic precursors of all lineages, including those of the T and B lymphoid pathways (Brady et al., 1995; Herblot, Aplan, & Hoang, 2002; Herblot et al., 2000; Moignard et al., 2013; Zhang, Mortazavi, Williams, Wold, & Rothenberg, 2012), and coexpressed with GATA1 during erythroid, megakaryocytic, and mast cell differentiation (Green et al., 1992; Hoang et al., 1996; Mouthon et al., 1993; Visvader, Begley, & Adams, 1991). Outside of the hematopoietic system, SCL is found within specific regions of the midbrain, hindbrain, and spinal cord (Sinclair et al., 1999; van Eekelen et al., 2003). Thus, the broad, yet specific, expression pattern of SCL suggests that it may be involved at the onset of hematopoiesis, in hematopoietic stem and progenitor cells (HSPCs) and during lineage differentiation.

## 2.3 Soloist of the First Wave: *Scl*

When *Scl* is inactivated, embryos die at E9.5 due to the complete absence of blood cells and to vascular defects (Robb et al., 1995; Shivdasani, Mayer, & Orkin, 1995; Visvader, Fujiwara, & Orkin, 1998). Conversely, *Scl* overexpression expands hematopoietic and endothelial cells at the expense of other mesoderm derived tissues (Ema et al., 2003; Gering, Rodaway, Gottgens, Patient, & Green, 1998; Martin et al., 2004; Mead, Deconinck, Huber, Orkin, & Zon, 2001; Mead, Kelley, Hahn, Piedad, & Zon, 1998). Together, these results clearly establish that *Scl*

determines mesoderm to a hematopoietic fate and acts as master regulator of primitive erythropoiesis. With current understanding of gene ensemble and functional nodes within intricate gene networks, it is highly surprising that invalidation of a single gene should have such a dramatic effect at the onset of primitive hematopoiesis. As it turns out, this narrow window of non-redundancy at the onset of hematopoiesis is not the norm for *Scl* which has multiple functions in hematopoiesis, as shall be discussed later. But what acts upstream of *Scl* to activate the genetic program for hematopoiesis?

## 2.4 Combinatorial Interaction Between Cell Intrinsic and Noncell Intrinsic Processes at the Onset of Primitive Erythropoiesis: Role of *Scl* and VEGF/FLK1 Signaling

Hematopoiesis is activated in extraembryonic mesoderm by diffusable signals emanating from the visceral endoderm (VE) layer of the yolk sac. Ablation of VE through the genetic targeting of *Gata4* abrogates the development of hematopoietic cells during the in vitro differentiation of embryonic stem (ES) cells (Bielinska, Narita, Heikinheimo, Porter, & Wilson, 1996). Indian hedgehog (IHH) has emerged as a candidate VE hematopoietic-inducing signaling molecule, by reprogramming prospective neuroectoderm to hematopoietic and endothelial cell fates to induce blood formation in explant cultures (Dyer, Farrington, Mohn, Munday, & Baron, 2001). Since BMP4 is upregulated in extraembryonic mesoderm following treatment with IHH, it is possible that IHH mediates its effect on hematopoiesis in part through BMP signaling. BMP4 is required for the formation of blood islands (Winnier, Blessing, Labosky, & Hogan, 1995) and BMP signaling is known to enhance hematopoietic development in vitro and in vivo (Chadwick et al., 2003; Crisan et al., 2015; Johansson & Wiles, 1995; Kanatsu & Nishikawa, 1996; Mead et al., 1998; Nakayama, Lee, & Chiu, 2000; Park et al., 2004). Activin A (Kanatsu & Nishikawa, 1996), TGFβ (Dickson et al., 1995; Pardanaud & Dieterlen-Lievre, 1999), and bFGF (Faloon et al., 2000; Pardanaud & Dieterlen-Lievre, 1999) have also been implicated in blood island development, although their relationship to IHH signaling has yet to be defined. Interestingly, the hematopoietic defects caused by defective IHH signaling in culture of differentiating ES cells and in zebrafish can be rescued by Notch signaling or by *Scl* expression (Kim et al., 2013), thereby placing *Scl* downstream of *Hh* during embryonic EHT.

VEGF is also expressed by VE and extraembryonic mesoderm and can respecify somatopleural mesoderm for hematopoietic and endothelial development (Pardanaud & Dieterlen-Lievre, 1999). Through aggregation

studies using tetraploid and wild-type embryos, Damert et al. demonstrated that the expression of VEGF by VE is prerequisite for the formation of blood islands and cannot be compensated for by expressing VEGF in the extra-embryonic mesoderm or in the embryo proper (Damert, Miquerol, Gertsenstein, Risau, & Nagy, 2002). Considering that VEGF and BMP4 synergize during the formation of hematopoietic cells in vitro (Nakayama et al., 2000; Park et al., 2004) and that hedgehog signaling activates the expression of VEGF in a mouse hindlimb ischemia model (Pola et al., 2001), it is conceivable that the effects of IHH at the onset of hematopoiesis are mediated through the combined action of BMP4 and VEGF.

Hematopoietic and endothelial cells develop from a common precursor, the hemangioblast (Keller, Lacaud, & Robertson, 1999; Kinder et al., 1999), expressing the VEGF receptor FLK1/KDR (Eichmann et al., 1997; Kabrun et al., 1997; Nishikawa, Nishikawa, Hirashima, Matsuyoshi, & Kodama, 1998; Ogawa et al., 1999; Pardanaud et al., 1996; Yamashita et al., 2000). At a clonal level, ES cell derived hemangioblasts (BL-CFC) originate exclusively from $FLK1^{+}$ cells and require VEGF for their proliferation (Choi, Kennedy, Kazarov, Papadimitriou, & Keller, 1998; Chung et al., 2002; Kennedy et al., 1997; Martin et al., 2004; Wang et al., 2004). Embryos lacking a single *Vegf* allele (Carmeliet et al., 1996; Ferrara et al., 1996) or *Flk1* (Shalaby et al., 1995) die between E8.5–9.5, due to the absence of blood islands (Fig. 2A). Furthermore, $Flk1^{-/-}$ ES cells fail to reach sites of hematopoiesis in chimeras (Shalaby et al., 1997), indicating a tight dose-dependent regulation of embryonic vessel and hematopoietic development by VEGF. Surprisingly, $Flk1^{-/-}$ ES cells generate BL-CFC colonies and differentiate into hematopoiesis in vitro, although BL-CFC are reduced in number and do not expand as efficiently in response to VEGF (Hidaka, Stanford, & Bernstein, 1999; Schuh, Faloon, Hu, Bhimani, & Choi, 1999). Therefore, VEGF/FLK1 signaling is not essential for hematopoietic and endothelial specification. Rather, VEGF is required for the migration of hemangioblasts from the mesoderm to sites that are permissive to hematopoiesis.

Once $FLK1^{+}$ hematopoietic and endothelial precursors have reached the yolk sac, they must expand and differentiate into blood islands. We showed that different thresholds of VEGF are required for the migration of hematopoietic and endothelial precursors to the yolk sac and for their subsequent expansion into primitive erythrocytes, through a dose-dependent anti-apoptotic effect of VEGF (Martin et al., 2004; Fig. 2B). In vitro, the addition of VEGF to differentiating ES cells expands the number of primitive

erythroid precursors ($Ery^P$) in a graded fashion, whereas adult hematopoietic precursors remain largely unaffected, as reported for human ES cells (Cerdan, Rouleau, & Bhatia, 2004). Conversely, we observed a progressive reduction in the number of $Ery^P$ in the yolk sacs of *Vegf* hypomorph heterozygous ($Vegf^{lo/wt}$) and homozygous ($Vegf^{lo/lo}$) embryos, as compared to wild-type littermates. Since *Flk1* expression is detected in the yolk sacs of homozygous ($Vegf^{lo/lo}$) embryos, these results suggest that low levels of VEGF is sufficient for the migration of $FLK1^+$ hematopoietic precursors to the yolk sac, whereas higher levels of VEGF are required for their expansion. Remarkably, different *Vegfa* isoforms have different functions (Leung et al., 2013). Thus, the medium/long isoform controls *Notch1* expression in somites and specifies HSCs in dorsal aorta whereas the short isoform is sufficient for arteriogenesis. Even though both lineages originate from hemangioblasts via VEGF/FLK1 signaling, these observations uncouple *Vegfa* functions in the two lineages.

Several lines of evidence indicate that SCL functions downstream of VEGF/Flk-1 signaling in putative hemangioblasts. First, *Scl*, *Flk1*, and *Vegf* are required for hematopoietic and endothelial cell development in vivo and for hemangioblast development in vitro (BL-CFC; reviewed in Ciau-Uitz et al., 2014). Second, overexpression of *Scl* expands hematopoietic and endothelial cells at the expense of other mesodermal cell fates (Ema et al., 2003; Gering et al., 1998; Gering, Yamada, Rabbitts, & Patient, 2003; Mead et al., 2001; Mead et al., 1998). Third, during development, *Scl* expression follows that of *Flk1* (Drake & Fleming, 2000; Park et al., 2004; Robertson, Kennedy, Shannon, & Keller, 2000) and is absent in $Flk1^{-/-}$ embryos (Ema et al., 2003). Fourth, *Scl* can rescue the hematopoietic and vascular defects of the Zebrafish mutant cloche (Liao et al., 1998), which acts upstream of *Flk1* (Liao et al., 1997), and allows BL-CFC to grow in the absence of *Flk1* (Ema et al., 2003). Furthermore, *Scl* levels are increased by VEGF during the in vitro differentiation of ES cells (Cerdan et al., 2004; Martin et al., 2004) and severely compromised when VEGF activity is reduced, as seen in *Vegf* hypomorph embryos. Finally, a gain of function of *Scl* partially complements the hematopoietic defect caused by a *Vegf* hypomorph allele, to reestablish the survival of erythroid cells and the expression of primitive erythroid genes (Martin et al., 2004). Since increased levels of SCL do not enhance survival and the expression of hematopoietic genes in a wild-type background, the results therefore suggest that SCL functions downstream of VEGF signaling to ensure an expansion of the primitive hematopoietic compartment by sustaining the survival of

primitive erythrocytes (Martin et al., 2004). Consistent with this hypothesis, *Scl* was subsequently placed downstream of FLK1 signaling and of *Etv2* (Liu et al., 2015), a transcription factor that marks the hemogenic endothelium (Wareing, Eliades, Lacaud, & Kouskoff, 2012). Indeed, deletion of *Etv2* abrogates *Flk1* expression and VEGF response as well as the hematopoietic and endothelial fates (Wareing, Mazan, et al., 2012). While *Etv2* controls the expression of *Scl*, *Gata2*, and *Fli1* (Liu et al., 2015), ectopic *Scl* expression was sufficient to rescue hematopoietic differentiation in *Etv2*$^{-/-}$ ES cells while *Gata2* and *Fli1* expression had a partial effect only (Wareing, Mazan, et al., 2012). Given the importance of VEGF in cell migration, it remains to be seen whether *Scl* rescue would be sufficient to reestablish hematopoiesis in vivo. Nonetheless, these rescue experiments confirm the primary and nonredundant role of *Scl* at the onset of hematopoiesis, as shown by gene invalidation (Porcher et al., 1996; Shivdasani et al., 1995; Visvader et al., 1998).

## 2.5 Controlling Boundaries: *Scl* Drives the Hematopoietic Fate at the Expense of the Cardiac Lineage

Blood cells, the vasculature and the cardiac lineage originate from the lateral plate mesoderm, most likely from a common FLK1$^{+}$ multipotential progenitor (Kattman, Huber, & Keller, 2006). During embryogenesis, *Scl* acts as a master regulator that specifies the hematopoietic fate from mesoderm (Lancrin et al., 2009) and in the absence of *Scl*, mouse embryos die at E9.5 due to the complete absence of primitive erythrocytes and an abnormal vasculature (Porcher et al., 1996; Shivdasani et al., 1995; Visvader et al., 1998). These functional results confirm the common embryonic origin of endothelial cells and hematopoietic cells (Kattman et al., 2006) and a common role for *Scl* in both lineages (Deleuze et al., 2012; Ema et al., 2003; Lazrak et al., 2004; Porcher, Liao, Fujiwara, Zon, & Orkin, 1999). Contrary to the above, the cardiac and hematopoietic lineages proved to be antagonistic (Schoenebeck, Keegan, & Yelon, 2007) despite sharing a common ancestry (Kattman et al., 2006). Unexpectedly, this antagonism is controlled by *Scl* that acts to repress the cardiac fate (Org et al., 2015). Indeed, in the absence of *Scl*, cardiomyocytes develop ectopically in the yolk sac vasculature, normally a specialized hematopoietic site, and in the endocardium (Van Handel et al., 2012). These results are consistent with previous reports indicating that *Scl* has mesodermal patterning activity in murine ES cells in vitro (Ismailoglu, Yeamans, Daley, Perlingeiro, & Kyba, 2008) and that *Scl* and *Etv2/Er71* spatially restrict heart size in zebrafish in vivo (Schoenebeck

et al., 2007). Remarkably, these results point to a recurring theme in lineage restriction in hematopoiesis, revealing an intrinsic plasticity in progenitors even after lineage diversification. Hence, *Scl* as a master regulator has multiple roles: in specifying the hematopoietic and endothelial fates from mesoderm; in lateral inhibition to prevent alternative fate(s); i.e., cardiomyogenesis; and in consolidating of the hematopoietic fate.

## 2.6 First Wave Duet: *Gata1* and *Gata2*

GATA1, a zinc finger transcription factor that drives erythroid gene expression, is essential for erythroid cell maturation (Pevny et al., 1991). Surprisingly, mouse embryos lacking *Gata1* die between E10.5 and E11.5 (Fujiwara, Browne, Cunniff, Goff, & Orkin, 1996), 1–2 days later than $Scl^{-/-}$ embryos (Shivdasani et al., 1995). In $Gata1^{-/-}$ embryos, primitive erythropoiesis was initiated but cells failed to progress beyond the proerythroblast stage (Fujiwara et al., 1996) while erythropoiesis was completely blocked in $Scl^{-/-}$ embryos (Shivdasani et al., 1995). This is because of a functional overlap between *Gata2* and *Gata1* in the yolk sac as the combined absence of both *Gata* genes completely ablated primitive erythroid cells (Fujiwara, Chang, Williams, & Orkin, 2004). This raises the question whether *Scl* is upstream of *Gata1* and *Gata2* at the onset of primitive erythropoiesis. Genome-wide occupancy determined by ChIP-seq analysis later revealed that SCL binds to the promoter region close to the transcription start site (TSS) of *Gata1* in $Lin^-$ fetal liver hematopoietic progenitors (Kassouf et al., 2010) and both *Gata1* and *Gata2* in multipotent hematopoietic progenitor cells (HPC; Wilson et al., 2010). While genome occupancy does not warrant functional consequence, Org et al. showed that *Gata1/2* expression is nearly abolished in $FLK1^+$ cells in the absence of *Scl,* while *Tal1/Scl* expression is unaffected by *Gata1/2* inactivation (Org et al., 2015). These observations suggest that at this time point in development, *Scl* acts upstream of both *Gata1* and *Gata2* to initiate primitive erythropoiesis.

## 2.7 Third Wave Trio: The *Runx1–Gata2–Scl* Connection Unfolded

The evidence that HSCs emerge from the AGM region to seed the fetal liver was paradigm shifting (Bertrand et al., 2010; Medvinsky & Dzierzak, 1996), and this endothelial-to-hematopoietic transition (EHT) was attributed to *Runx1* at the onset of definitive hematopoiesis (Chen,

Yokomizo, Zeigler, Dzierzak, & Speck, 2009). It is now recognized that GATA2 and SCL share this privilege with RUNX1. Indeed, *Runx1* is not required during primitive erythropoiesis while the deletion of *Runx1* in vascular endothelial cells prevented the emergence of HSCs from endothelium. These observations established the functional importance of *Runx1* during EHT (Chen et al., 2009). In contrast, the early lethality of $Scl^{-/-}$ or $Gata2^{-/-}$ embryos hindered a precise assessment of their function at later developmental stages. Interestingly, *Gata2* expression during development was assigned in part to a cis-regulatory enhancer at +9.5, which then controls *Scl* et *Gfi1* expression in the AGM (Gao et al., 2013). Moreover, the generation of HSCs from the AGM depends on the integrity of the +9.5 element in vivo. Concurring with the view that RUNX1, GATA2, and SCL are often found in association in ChIP-seq experiments (Wilson et al., 2010), the above functional studies point to the importance of all three transcription factors for the emergence of definitive HSCs from endothelium (Fig. 2A). Additionally *Scl* expression and the onset of definitive HSCs is also controlled by transcription factor genes of the Ets family, *Etv2* (Liu et al., 2015), *Etv6*, and Fli1 (Ciau-Uitz, Pinheiro, Gupta, Enver, & Patient, 2010; Ciau-Uitz, Pinheiro, Kirmizitas, Zuo, & Patient, 2013).

These results also suggest that *Gata2* is upstream of *Scl* during EHT (Gao et al., 2013). Since master regulators are often autoregulatory, or involved in positive feed-forward loops, it remains to be seen whether *Scl* may in turn upregulate *Gata2* during EHT. Indeed, gene regulatory networks inferred enhancer sequences driving *Scl*, *Gata2*, and *Fli1* in embryonic day-11.5 dorsal aorta in the mouse during EHT that implicate all three genes in a recursive wiring (Pimanda et al., 2007; Sive & Gottgens, 2014). Moreover, the +9.5 sequence controlling *Gata2* expression during EHT was shown to be occupied by SCL in fetal liver hematopoietic progenitors, in a binding site-dependent manner (Sanalkumar et al., 2014). These observations suggest that *Scl* and partners may indeed form a coherent feed-forward loop with *Gata2* during EHT. Gene knockout and conditional invalidation have revealed major insight into gene function. In addition to these gene targeted approaches, the report by Gao et al. suggests that modulation of cis-regulatory sequences based on the specificity of protein DNA interactions may bring more fine-tuned instruments into the study of gene function, by integrating the timing of expression as well as spatial regulation (Gao et al., 2013; Hewitt et al., 2015).

## 3. THE LONG, THE INTERMEDIATE, AND THE SHORT (TERM): HSC FUNCTION AND *SCL*

### 3.1 A Hierarchy Within HSCs

The life-long production of blood cells depends on the dual capacity of HSCs to commit to multilineage differentiation and to self-renewal. Both properties are assessed in transplantation assays which also unraveled intrinsic heterogeneity within HSCs based on their differing capacities to sustain hematopoiesis in host mice (reviewed in Rojas-Sutterlin & Hoang, 2013). Initially, HSCs were classified as short term (ST-HSCs) and long term (LT-HSCs), based on the durability of engraftment (Fig. 3). ST-HSCs

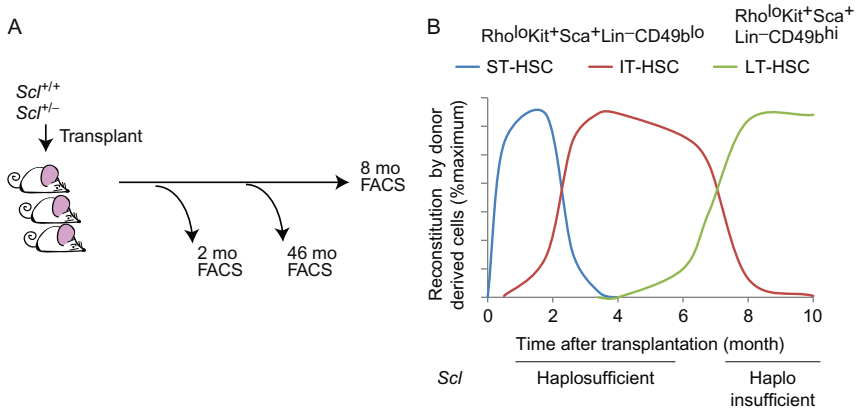


**Fig. 3** *Time-line of hematopoietic reconstitution by transplanted hematopoietic stem cells.* (A) Mice are transplanted with adult bone marrow cells of different genotypes. Illustrated is a competitive assay in which HSCs of two different genotypes are transplanted in competition, typically at 1:1 ratio. Mice are sacrificed at the indicated times and analyzed for reconstitution in all hematopoietic organs, blood, bone marrow, thymus, and spleen. Test cells and competitor cells can be distinguished on the basis of CD45 alleles. (B) HSCs can be resolved into different populations based on the duration of reconstitution posttransplant. Short term HSCs (ST-HSCs) provide a transient wave of hematopoietic reconstitution (1–2 month) while long term HSCs (LT-HSCs, >4 month) can confer more durable reconstitution. These two cell types can be separated based on surface markers. Phenotypically, HSCs are enriched in the $Kit^{+}Sca^{+}Lin^{-}$ (KSL). ST-HSCs and LT-HSCs are found within the $CD48^{+}CD150^{-}$ and $CD48^{-}CD150^{+}$ fractions, respectively (Kiel et al., 2005). More recent evidence indicates that the population of LT-HSCs is heterogenous, containing $CD49b^{lo}$ cells that indeed provide sustained reconstitution (>8 month), but mostly $CD49b^{hi}$ cells providing intermediate reconstitution (IT-HSCs, <6 month) (Benveniste et al., 2010). *Scl* is haploinsufficient in these very long term HSCs (>8 month) while haplosufficient in IT-HSCs (Lacombe et al., 2010).

provided lymphomyeloid reconstitution that peaked at 3–4 weeks while LT-HSCs were defined as stem cells that generated circulating blood cells beyond 16 weeks. These cells are separable based on cell surface markers, in particular SLAM family members (Kiel et al., 2005). More recent evidence indicates that the population previously considered as LT-HSCs indeed contains cells that provide permanent and sustained life-time reconstitution but these rare cells are outnumbered by intermediate clones that self-maintained for 6–8 months and disappeared afterward (Benveniste et al., 2010).

## 3.2 Hierarchy Within a Hierarchy: Very Long Term HSCs Unraveled by *Scl* Haploinsufficiency—Role in Stress Response

The enhanced reconstitution ability of *SCL* overexpressing-human HSCs (Kunisato et al., 2004; Reynaud et al., 2005) suggested a role for *SCL* in HSCs, an observation that was not mirrored by loss of function studies. Although *Scl* is required for the generation of all hematopoietic cells during ontogeny (Porcher et al., 1996; Robb et al., 1996), analysis of conditional knockouts indicated that the loss of *Scl*, specifically within the adult hematopoietic compartment, did not compromise the ability of HSCs to self-renew and to reconstitute lethally irradiated recipients (Curtis et al., 2004; Mikkola et al., 2003). This unexpected observation suggests several possibilities that need not be mutually exclusive. First, the genetic program for the specification and function of a tissue may differ, implying the existence of distinct transcription factors which stabilize the hematopoietic fate, similar to the role of *Pax-5* during B cell commitment (Nutt, Heavey, Rolink, & Busslinger, 1999; Schaniel, Bruno, Melchers, & Rolink, 2002; Schaniel, Gottar, Roosnek, Melchers, & Rolink, 2002), and others that regulate self-renewal, such as *HoxB4* (Sauvageau et al., 1995) and *Bmi-1* (Lessard & Sauvageau, 2003; Park et al., 2003). Along these lines, *Runx1* and *Gata2* are shown to be essential for adult HSCs. Second, *Scl* may be redundant with other factors. Indeed, HSC survival requires both *Scl* and *Lyl1* in a gene dosage-dependent manner (Souroullas, Salmon, Sablitzky, Curtis, & Goodell, 2009). This functional redundancy between two highly homologous proteins is not unprecedented, and was previously reported for *E2a* and *Heb* during thymocyte development (Jones & Zhuang, 2007). Interestingly, the *Scl–Lyl1* redundancy occurs in adult HSCs but not in hemangioblasts in which reexpression of *Lyl1* cannot rescue the lethality of $Scl^{-/-}$ embryos (Chan et al., 2007). Finally, a third alternative is the possibility that different classes of HSCs may have different requirements for *Scl*

gene dosage (Fig. 3). With the recognition of heterogeneity within HSCs and the existence of a highly dormant subclass that may take up to 90 days per cell division (Trumpp, Essers, & Wilson, 2010), we reasoned that the activity of these highly dormant HSCs may be revealed only after very long term reconstitution or after a prolonged proliferative stress.

We compared host reconstitution by donor cells at 4–8 months and found that stem cell functions at 4 months posttransplantation are independent of *Scl* gene dosage. However, *Scl* is haploinsufficient when HSC functions were monitored at 8 months posttransplant (Lacombe et al., 2010). Thus, our observations provide genetic evidence for a difference between intermediate term- and LT-HSCs. Furthermore, the transcription of the *Scl gene itself* was highest in LT-HSCs that are in G0 and decreased in ST-HSCs that are committed into the cell cycle. This conclusion was independently confirmed through the study of gene expression in LTR-HSCs that were purified through another protocol (Benveniste et al., 2010). Interestingly, removing one allele of *Scl* was sufficient to allow dormant LT-HSCs to transit through G0–G1, and the cells undergo exhaustion upon serial transplantation under conditions where wild-type HSCs do not. These observations suggest that quiescence control is an important determinant of the maintenance of the stem cell pool in vivo (reviewed in Nakamura-Ishizu, Takizawa, & Suda, 2014; Rojas-Sutterlin & Hoang, 2013; Sorrentino, 2010).

## 3.3 Role of SCL During Lineage Differentiation

The role of SCL during lineage commitment and differentiation remains unclear, mainly as a consequence of the embryonic lethality of $Scl^{-/-}$ embryos. The generation of conditional knockouts has clearly established an essential role for SCL during erythroid and megakaryocytic differentiation, as the loss of SCL results in a complete lack of differentiated cells in both lineages (Hall et al., 2003; Mikkola et al., 2003). In agreement, overexpression of SCL in immature primary hematopoietic cells enhances the output in erythroid and megakaryocytic cells (Elwood, Zogos, Pereira, Dick, & Begley, 1998; Ravet et al., 2004; Valtieri et al., 1998). These findings also concur with earlier studies describing an upregulation of *Scl* during erythroid differentiation and enhanced spontaneous erythroid differentiation upon overexpression of SCL in cell lines (Aplan, Nakahara, Orkin, & Kirsch, 1992; Green, Salvaris, & Begley, 1991; Hoang et al., 1996). The question arising is whether SCL operates at lineage branchpoints. Initially, overexpression of SCL in cell lines enhances erythroid differentiation, as seen in primary cells, but blocks myeloid differentiation

(Condorelli et al., 1997; Hoang et al., 1996; Tanigawa et al., 1993; Tanigawa, Nicola, McArthur, Strasser, & Begley, 1995). However, observations in cell lines should be interpreted with caution, as cell lines often have an ambiguous lineage status and a poor differentiation response (Socolovsky, Lodish, & Daley, 1998; Wu, Liu, Jaenisch, & Lodish, 1995). Indeed, increasing levels of SCL in primary hematopoietic cells expand the erythroid and megakaryocytic compartments, without affecting myeloid development (Elwood et al., 1998; Hall et al., 2003; Ravet et al., 2004; Valtieri et al., 1998). *Scl* was later shown to control the expansion of primary monocyte progenitors (Dey, Curtis, Jane, & Brandt, 2010). Therefore, unlike the "lateral" inhibition of the cardiac fate during development (Van Handel et al., 2012), *Scl* is permissive for both myeloid and lymphoid lineage commitment.

Within the lymphoid compartment, SCL is expressed in the earliest committed precursors of the B and T cells lineages, but is rapidly downregulated as cells mature (Herblot et al., 2002; Herblot et al., 2000), suggesting that SCL downregulation may be prerequisite to lymphoid differentiation. Indeed, the analysis of *SIL–SCL* transgenic mice ($SCL^{tg}$), which express SCL ubiquitously, or in *Ly6–Scl* transgenic mice, indicated that sustained expression of SCL severely compromises the earliest committed precursor of the B (Herblot et al., 2002), but not the T-cell lineages (Herblot et al., 2000). Since the number of common lymphoid progenitors (CLPs) in $Scl^{tg}$ mice are unaffected (R. Martin & T. Hoang, unpublished data), the results suggest that SCL specifically hinders B cell differentiation after the commitment stage to CLPs.

With regards to the initial decision of HSCs to commit to either myeloid or lymphoid compartments, Kunisato et al. proposed that SCL levels skews differentiation of HSCs toward common myeloid progenitors (CMPs), at the expense of CLPs (Kunisato et al., 2004). Nonetheless, unlike its decisive role in favoring the hematopoietic fate at the expense of the cardiac fate during embryonic development, current evidence does not favor such a decisive role for SCL in establishing lineages in HSCs. Rather, as exemplified by the ability of SCL to specifically block B cell differentiation at the pro-B stage (Herblot et al., 2002), SCL may be acting not at the level of lineage choice in HSCs but later during differentiation.

## 3.4 Erythroid and Megakaryocyte Lineages: A Matter of SCL Isoforms?

The *Scl* gene encodes different protein isoforms (Aplan et al., 1990; Calkhoven et al., 2003). The major product is a 42-kD full length SCL

protein, which contains an N-terminal transactivation domain (Hsu, Wadman, Tsan, & Baer, 1994; Sanchez-Garcia & Rabbitts, 1994; Wadman, Hsu, Cobb, & Baer, 1994), a basic domain which binds to specific DNA sequences known as E-boxes (CANNTG; Hsu, Huang, Tsan, et al., 1994) and an HLH domain (Fig. 1A) required for heterodimerization with widely expressed class-I bHLH proteins, which include E2A, HEB, and E2-2 (Goldfarb, Lewandowska, & Shoham, 1996; Hsu, Cheng, Chen, & Baer, 1991; Hsu, Wadman, & Baer, 1994; Voronova & Lee, 1994; Fig. 1B). Shorter SCL isoforms (40, 34, and 22 kD) retain the bHLH domain, but present N-terminal deletions of the transactivation domain (Aplan et al., 1990; Calkhoven et al., 2003; Cheng, Hsu, Hwang, & Baer, 1993; Pulford et al., 1995). The expression of SCL isoforms are regulated primarily through translational control (Calkhoven et al., 2003).

Structure function studies suggest that different domains of SCL are required at distinct stages of hematopoiesis. While the N-terminal and basic domains of SCL are dispensable for hematopoietic specification (Porcher et al., 1999) and transactivation of the c-kit gene in immature cells (Lecuyer et al., 2002), binding of SCL to DNA is required for the survival of HSPCs (Krosl et al., 1998; Lacombe et al., 2013; Ravet et al., 2004), for the activation of the erythroid gene *glycophorin A* (Lahlil, Lecuyer, Herblot, & Hoang, 2004; Ravet et al., 2004) and for erythroid and megakaryocytic differentiation (Porcher et al., 1999). Our observations suggest that SCL isoforms differentially regulate hematopoiesis. We have found that short isoforms are preferentially upregulated during erythroid differentiation, while remaining at low levels during megakaryocytic differentiation (Calkhoven et al., 2003). When expressed in bone marrow cells, short isoforms favor erythroid differentiation, whereas longer isoforms are favor megakaryocytic differentiation (Calkhoven et al., 2003). It would be interesting to examine the expression pattern of SCL isoforms throughout the hematopoietic hierarchy in order to determine whether specific isoforms may influence lineage output at different branchpoints.

## 4. THE SCL COMPLEX

### 4.1 Networking via LMO2

SCL does not bind DNA on its own but requires heterodimerization with E proteins, preferentially E2A/TCF3 and HEB/TCF12, for DNA binding (Hsu et al., 1991; Lecuyer & Hoang, 2004). Surprisingly, despite binding with high affinity to a consensus E box (Hsu, Huang, Tsan, et al., 1994),

the SCL–E2A heterodimer does not activate transcription but instead, tempers transcription activation by E2A homodimers or E2A–HEB heterodimers (Hsu, Wadman, Tsan, et al., 1994). It was later found that the missing partner in these experiments is LMO2 (Fig. 1C) that directly interacts with SCL, GATA1, and LDB1 (El Omari et al., 2013; Wadman et al., 1997; Xu, Huang, Chang, Agulnick, & Brandt, 2003) to activate transcription. In the erythroid lineage, we showed that transcription activation of erythroid genes occurs if and only if all components of the complex are properly assembled on the promoter (Lahlil et al., 2004), resulting in a tight control of gene expression(Lecuyer & Hoang, 2004). Interestingly, the SCL–LMO2 interaction is essential for nucleation of this pentameric SCL complex (Fig. 4), a specific property that clearly distinguishes SCL from the neuronal homologue NSCL/NHLH1 (Lecuyer et al., 2007; Schlaeger et al., 2004). Furthermore, interaction with SCL protects LMO2 from proteasomal degradation (Lecuyer et al., 2007), with the resulting consequence that LMO2 is always stoichiometric with SCL and involved in complex formation.

Analysis of preferred binding sites of the SCL complex ChIP-seq indicated preferred binding to a composite E box-GATA motif (Kassouf et al., 2010; Wilson et al., 2010), a modest variation from the initial motif identified by in vitro selection (Wadman et al., 1997). The motif was found

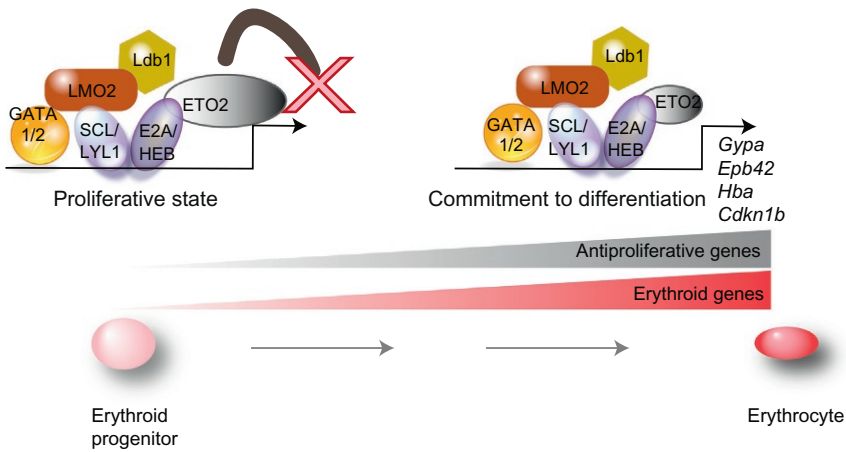


**Fig. 4** *Dynamic variations in the stoichiometry of the ETO2/MTGR1 corepressor and SCL/E2A* within the SCL complex determine whether erythroid progenitors proliferate or commit to terminal differentiation. *Cdkn1b*: p27Kip; *Gypa*: glycophorin A; *Epb42*: p4.2; *Hba*: hemoglobin α (Goardon et al., 2006).

in individual SCL targets (Cohen-Kaminsky et al., 1998; Vyas et al., 1999) or more recently, via genome-wide cistrome analysis (Hewitt et al., 2015). The complex can also assemble on GATA motif in the absence of an Ebox (Kassouf et al., 2010; Lahlil et al., 2004; Wilson et al., 2010) or CACC box (Kassouf et al., 2010). Using a mutant devoid of the basic domain, Kassouf et al. showed that one fifth of SCL targets does not require direct SCL DNA binding (Kassouf et al., 2010), indicating that SCL can be recruited to DNA via protein–protein interactions (Kassouf, Chagraoui, Vyas, & Porcher, 2008; Lecuyer et al., 2002; reviewed by Lecuyer & Hoang, 2004). Resolution of the structure of the SCL complex at the atomic level revealed an unexpected function for LMO2 in strengthening the SCL–E2A heterodimer that reduces DNA binding and favors additional protein–protein interactions (El Omari et al., 2013). Among SCL direct targets, *Kit* (Kassouf et al., 2010; Lecuyer et al., 2002; Wilson et al., 2010) establishes with *Scl* a coherent feed-forward loop in multipotent and megakaryocyte-erythroid progenitors (Krosl et al., 1998; Lacombe et al., 2013; reviewed in Rojas-Sutterlin, Lecuyer, & Hoang, 2014).

## 4.2 Building Multifunctionality

The concept of "building multifunctionality" (Fujiwara, Lee, Sanalkumar, & Bresnick, 2010) is illustrated by the proteomics of the SCL complex that confirmed members of the core complex and revealed the recruitment of novel corepressors, in particular ETO2 (Cai et al., 2009; Goardon et al., 2006; Kolodziej et al., 2014; Schuh et al., 2005). Since SCL can both activate and inhibit gene expression, dynamic change in the stoichiometry of SCL–ETO2 could govern the switch between an activating to a repressive complex in transcription regulation. In the erythroid lineage, this switch was shown to govern the transition between a proliferative state in progenitors and growth cessation required for commitment to terminal differentiation (Goardon et al., 2006). That ETO2 should be a corepressor was not a surprise and *Eto2* knockdown resulted mostly in upregulation of gene expression in G1E erythroid cells (Fujiwara et al., 2010). The repressive function of ETO2 is well documented and occurs via HDAC1 and 2, causing E protein silencing (Zhang, Kalkum, Yamamura, Chait, & Roeder, 2004). The identification of ETO2 within the SCL complex raised much interest, since SCL also inhibits E protein activity (Chervinsky et al., 1999; Goardon et al., 2002; Herblot et al., 2000; O'Neil, Shank, Cusson, Murre, & Kelliher, 2004; Sanda et al., 2012). Contrary to ETO2, LMO2 is expected to convert this inhibitory state into an activator of transcription via its capacity to recruit transcription

factors such as GATA1/2 and coactivators into the SCL pentameric complex. Unexpectedly, knocking down *Lmo2* in the same cells also derepressed gene expression (Fujiwara et al., 2010). It is possible that knocking down *Lmo2* prevents the assembly of the pentameric complex, leaving SCL in heterodimer with E2A and thereby, inhibiting E2A target genes. Nonetheless, the gene ensembles controlled by *Lmo2* and *Eto2* are significantly divergent (Fujiwara et al., 2010). These results bring into light the multifunctionality of protein complexes formed on DNA. It is possible that specific genes may have differing tolerance to a twofold decrease dosage of ETO2 and LMO2, depending on the chromatin context or on the local composition of the complex formed on DNA. Notwithstanding the exact molecular mechanism, these observations call for a reassessment of the activity of the SCL complex that may have more functions beyond the inhibition of E proteins.

SCL can also repress or activate gene expression (Lecuyer & Hoang, 2004; Palomero et al., 2006) depending on interaction with the coactivator proteins CBP/P300, P/CAF (Huang, Qiu, Shi, Xu, & Brandt, 2000; Huang, Qiu, Stein, & Brandt, 1999) or the corepressor LSD1 (Hu et al., 2009). More recent affinity purification of SCL/TAL1 coupled with quantitative mass spectrometry revealed the distinctive association of SCL with the peptidylarginine deiminase IV (PADI4) that counteracts the repressive histone mark induced by PRMT6 to enhance the active H3K4me3 mark (Kolodziej et al., 2014).

## 4.3 A Pentameric Complex Evolves into a Heptad

Novel technological advance brings novel insights into gene regulation. The capacity to systematically map genome-wide binding profiles of 10 hematopoietic transcription regulators via ChIP-sequencing in a multipotent model cell line (HPC7) confirmed the proximity of SCL and LMO2 on DNA (highest Z' score). Interestingly, LMO2 was found to be the major nucleation factor with pair-wise proximity to GATA2 and LYL1 as expected, and additionally, with two Ets transcription factors, ERG and FLI1, as well as RUNX1 (Wilson et al., 2010). SCL also mapped close to the same factors, although Z' scores were slightly lower. These results led to the identification of a "heptad" as illustrated in Fig. 5. Since the genome binding profile of E proteins and of LDB1 remained to be assessed in the same cells, it is predicted that the heptad may turn out to be a Decad, a composition of 10 transcription factors that control hematopoietic cell fate.

Indeed, nine of these ten factors have been shown individually to promote HSC maintenance(Knudsen et al., 2015; Kruse et al., 2009; Lacombe

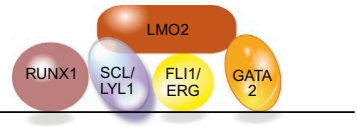


**Fig. 5** *Transcription factor heptad*, as revealed by the proximity of DNA-bound peaks in multipotent HPC7 cells (Wilson et al., 2010).

et al., 2010; Ravet et al., 2004; Rodrigues et al., 2005; Semerad, Mercer, Inlay, Weissman, & Murre, 2009; Souroullas et al., 2009; Taoudi et al., 2011). These observations are consistent with the view that an extensive transcription factor network controls HSC function, thereby explaining HSC robustness and tolerance to fluctuations in *Scl* alleles (Mikkola et al., 2003), except under extensive proliferative stress when *Scl* turned out to be haploinsufficient (Lacombe et al., 2010). Similarly, *Runx1* is not essential in adult HSCs but required in stress response (Cai et al., 2015). Finally, SCL complexes can form higher order structures via the self-association capacity of LDB1, which could lead to DNA looping and thus facilitating communication between enhancers and promoters (Love, Warzecha, & Li, 2014; Yun et al., 2014).

### 4.4 The Importance of Being Noncell Autonomous

In another series of experiments in zebrafish embryos, Gering et al. showed that coinjection of *Scl* and *Lmo2* is sufficient to induce blood and endothelial genes along the anteroposterior axis (Gering et al., 2003). Nonetheless, erythroid cells were not produced in areas such as the head and heart, and this is due to the absence of *Gata1*. Together, these experiments indicate that transcription activation by SCL minimally requires a molecular context whereby LMO2 and GATA factors are present, possibly requiring a permissive microenvironment as well, as discussed later.

## 5. SCL AND CELLULAR REPROGRAMMING

### 5.1 The SCL Complex in Thymocyte Reprogramming

A number of functional studies indicate a role for SCL/TAL1 in suppressing apoptosis in erythroid cells during development (Martin et al., 2004) or in the adult (Palii et al., 2011), in normal HSPC (Krosl et al., 1998; Lacombe et al., 2013) and in leukemic T cells (Leroy-Viard et al., 1995; Sanda et al., 2012). In addition, SCL also exerts cell cycle control (Chagraoui et al., 2011; Lacombe et al., 2010; Palii et al., 2011), indicating a multifunctional control of

hematopoietic cell fate. With the possibility of somatic cell reprogramming and given the importance of SCL in HSCs, the question arises whether SCL or its protein partners can confer de novo stem cell properties to nonstem cells? We will examine this question in the T-lineage in which SCL and LMO1 or LMO2 act as an oncogene, and extend this notion to somatic cell reprogramming. The first area addresses mechanism(s) of cell transformation, the second one would have a major impact on cell-based therapy. Indeed, bone marrow transplantation has emerged as the most promising therapeutic approach in adult acute leukemias. Nonetheless, this is limited by the availability a compatible HLA-matched donor as well as the number of cells that can be obtained for transplantation. The reader is referred to several exhaustive reviews on the molecular basis of T-ALL (Aifantis, Raetz, & Buonamici, 2008; Look, 1997; Sive & Gottgens, 2014; Van Vlierberghe & Ferrando, 2012; Van Vlierberghe, Pieters, Beverloo, & Meijerink, 2008), on preleukemia (Greaves & Maley, 2012; Nowell, 1977; Shlush & Minden, 2015) and hemogenic reprogramming (Ebina & Rossi, 2015; Vo & Daley, 2015). The current section is focused on SCL.

### 5.1.1 Thymocyte Differentiation

Thymocytes lack intrinsic self-renewal capacities (Bhandoola & Sambandam, 2006). Thymic output depends on the continuous influx of multipotent progenitors that are released from the bone marrow and settle in the thymus where they are directed toward the T-lineage by the thymic microenvironment, in particular Notch1 signaling (Allman et al., 2001; Petrie & Zuniga-Pflucker, 2007; Fig. 6). Rearrangement of *Tcr* genes represent the first commitment step in the thymus, which is initiated at the $CD4^{-}CD8^{-}$ double

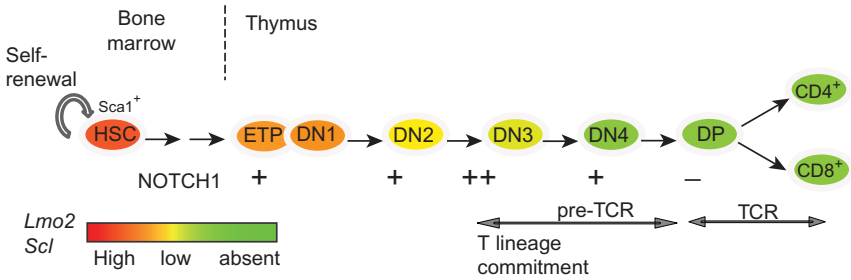


**Fig. 6** *Schematic diagram of thymocyte differentiation.* Upon entering the thymus, multipotent hematopoietic progenitors that are produced in the bone marrow are guided toward a T-lineage commitment point controlled by the pre-TCR in concert with elevated Notch1 signaling at the DN3 stage ($Thy1^{+}CD4^{-}CD8^{-}CD44^{-}CD25^{+}$).

negative 2 (DN2) stage. *Trb/Tcrβ* rearrangement is completed at the DN3 stage. Subsequently, signaling through a functional pre-TCR and Notch1 represents an irreversible T-lineage commitment into the α/β lineage, associated with cell survival and proliferation. Cells having passed this beta selection checkpoint progress through the DN4 stage to the double positive (DP) stage to acquire CD4 and CD8 expression while rearranging their *TRA/TCRA* genes (reviewed in Ciofani & Zuniga-Pflucker, 2010; Rothenberg, 2014; Yang, Jeremiah Bell, & Bhandoola, 2010).

*Scl* and *Lmo2* mRNA levels are highest in DN1 thymocytes and decrease to levels that are below the limit of detection in DN3 thymocytes (Herblot et al., 2000; Zhang et al., 2012; Fig. 6). Chromosomal translocations driven by illegitimate RAG activity associated with t(1;14), t(1;7) juxtapose *TRA/TCRA or TRB/TCRB* regulatory sequences to the *SCL* gene respectively, thereby driving *SCL* transcription in all *TRA or TRB* expressing thymocyte populations (Begley, Aplan, Davey, et al., 1989; Bernard et al., 1990; Chen, Yang, et al., 1990). Another mechanism in t(1;3) translocation (Aplan et al., 1992) possibly involves Z DNA structure associated with alternating purine and pyrimidine residues on chromosome 3 together with illegitimate recombination events. These recurring chromosomal translocations never altered *SCL* coding sequences but caused ectopic *SCL* expression in thymocytes.

### 5.1.2 SCL *Collaborates with* LMO1 *to Reprogram Thymocytes into Preleukemic Stem Cells*

Transgenic mouse model expressing the human *SCL* and *LMO1* or *LMO2* oncogenes in the thymus closely reproduces the natural history of the human disease, with a preleukemic state evolving into aggressive T-ALL that recapitulates the same mutations found in patients' blasts (Lin, Nichols, Letterio, & Aplan, 2006; Tremblay et al., 2010). Using this mouse model, McCormack et al. and our group showed that *LMO2* or *SCL–LMO1* establish a preleukemic state in thymocytes by conferring de novo self-renewal activity to DN3 thymocytes and converting them into preleukemic stem cells (pre-LSCs; Gerby et al., 2014; Hoang, 2010; McCormack et al., 2010; Fig. 7). SCL has a dual activity in transcription regulation, either to inhibit the activity of the HEB–E2A transcription factors (Hsu, Wadman, Tsan, et al., 1994; Murre, 2000) that control normal thymocyte differentiation (Jones & Zhuang, 2007) or to activate transcription via direct association with LMO2 to nucleate the assembly of the SCL core complex (Lecuyer & Hoang, 2004). To distinguish between these two properties

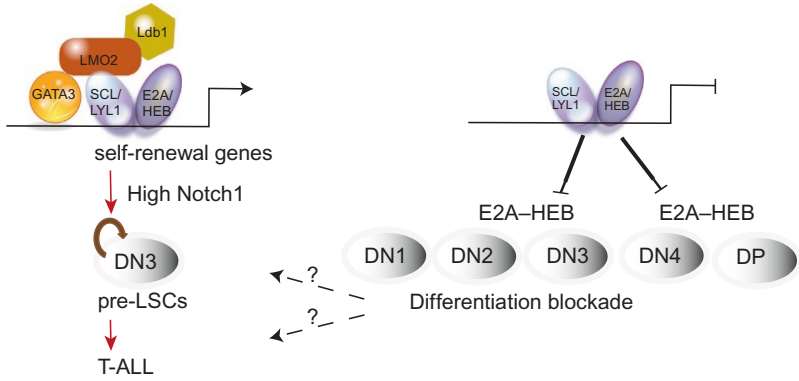


**Fig. 7** *SCL acts in two parallel pathways in DN thymocytes.* SCL activates the transcription of self-renewal genes if and only if all members of the transcription complex are present. The SCL complex collaborates with high levels of Notch1 in DN3 thymocytes to reprogram these cells into self-renewing preleukemic stem cells (pre-LSCs). DN3 cells are the only targets of transformation by the SCL complex because Notch1 levels in other DN subpopulations are insufficient for collaboration. These long lasting cells acquire collaborating mutations leading to T-ALL. When bound to E2A or HEB in the absence of other transcription partners, SCL inhibits the activity of E2A/HEB heterodimers or homodimers, thereby causing differentiation blockade via decreased expression levels of E protein target genes that are required for thymocyte differentiation.

in thymocyte reprogramming, we designed an LMO2-binding defective SCL mutant that no longer activates transcription but retains its capacity to inhibit E2A, based on the interface of the SCL–LMO2 interaction (El Omari et al., 2013; Lecuyer et al., 2007). When expressed in transgenic mice, the SCL mutant fails to reprogram thymocytes and exhibits much impaired capacity to induce T-ALL, as assessed by decreased penetrance and a threefold delay in time of disease onset. Therefore, acquisition of self-renewal activity is a major determinant of T-ALL. Our genetic and genomic approaches indicate that self-renewal activity depends not on inhibition of HEB–E2A but on transcription activation by the SCL complex to drive expression of a self-renewal gene network in thymocytes. We do not rule out the possibility that SCL inhibits HEB–E2A, but this leads to differentiation blockade (Chervinsky et al., 1999; Goardon et al., 2002; Herblot et al., 2000) and not to self-renewal activity (Gerby et al., 2014; Fig. 7), nor the hypothesis that inhibition of E2A could facilitate T-ALL since removal of one *E2a* or one *Heb* allele accelerates *SCL/TAL1*-induced T-ALL (O'Neil et al., 2004). Interestingly, this genetic complementation suggests that both *E2a* and *Heb* function in a parallel pathway to *SCL*, not

downstream as initially proposed by Hsu, Wadman, and Baer (1994). This is further supported by the fact that ectopic E47 expression in four LMO2-induced leukemic T-cell lines caused proliferation arrest in two of lines only, indicating that inhibition of E proteins is not a consistent feature (Goodings et al., 2015), as would be the case if *Lmo2* was to enforce E2A deficiency. Rather, these observations suggest that E2A deficiency is a collaborating event, frequently occurring in *Lmo2*-induced leukemias.

### 5.1.3 A Notch Up from the Microenvironment

The cell of origin of acute leukemia, which is distinct from leukemic stem cells, has been a matter of debate as it could be a self-renewing HSC or a progenitor. Our results show that the target cells of transformation by these two oncogenes are immature DN3 thymocytes, concurring with the stunning report by McCormack et al. for LMO2 (McCormack et al., 2010) which was independently confirmed in another mouse model (Cleveland et al., 2013). Consequently, the question becomes: what determines such specific targeting of DN3 cells, given that both transgenes are well expressed in all thymocyte populations (Tremblay et al., 2010)? By exploiting the ImmGen database (Heng, Painter, & Immunological Genome Project Consortium, 2008) and the Haemcode/Codex ChIP-seq resource (Wilson et al., 2010), we analyzed gene sets that are significantly increased at each transition, e.g., DN1–DN2, DN2a–DN2b, for enrichment in particular transcription factor targets. We identified *Notch1*-regulated genes as the most highly induced at the DN2b–DN3a transition. Accordingly, overexpressing an activated *Notch1* gene in transgenic mice converts all *SCL–LMO1*-expressing DN1–ISP8 thymocyte subsets into self-renewing cells. Therefore, NOTCH1 activation levels determine the susceptibility of thymocytes to reprogramming by SCL–LMO1.

These self-renewing thymocytes are considered preleukemic because they engraft the thymus and give rise to functional T cells. Furthermore, pre-LSCs can be serially transplanted 3× without giving rise to T-ALL in most cases. Finally, pre-LSCs remain T-lineage restricted and exclusively engraft in the thymus of recipient mice. Therefore, SCL–LMO1 reprogram DN3 thymocytes into T-lineage committed pre-LSCs, not into multipotent HSCs (Gerby et al., 2014). These long lasting pre-LSCs subsequently acquire *Notch1* mutations and a competitive advantage to become leukemia propagating cells (Tremblay et al., 2010). Interestingly, in leukemic T cells, SCL forms a regulatory circuit involving HEB, E2A, LMO1/2, and RUNX1, much like the complex found in normal HSPC (Sanda et al.,

2012). In the T-lineage, GATA3 is associated with the core complex instead of GATA1 or GATA2 (Sanda et al., 2012), suggesting that GATA3 can compensate for the lack of GATA1/2 in these cells.

In summary, SCL and LMO1 operate cell autonomously to activate a self-renewal program in differentiating thymocytes, and this reprogramming activity requires high levels of Notch1 activation in DN3 cells via noncell autonomous pathways.

## 5.2 Hemogenic Reprogramming

Can SCL confer de novo hemogenic potential to nonhematopoietic cells? Recent evidence (Doulatov et al., 2013; Riddell et al., 2014) indicates that there are at least "Two routes to make blood" (Singbrant et al., 2015).

*SCL* overexpression in human ES cells accelerates hemangioblast formation while *SCL* knockdown prevented this process, as described in murine ES cells (Real et al., 2012). Furthermore, transduction of human pluripotent stem cells in culture with either *Etv2/Gata2* or *Gata2/Scl* induced robust hematopoiesis which was predominantly myeloid or erythroid/megakaryocytic, respectively (Elcheva et al., 2014; Fig. 8). Nonetheless this was not sufficient for engraftment, which requires a tailored set of transcription factors to produce mature hematopoietic cells from induced pluripotent cells

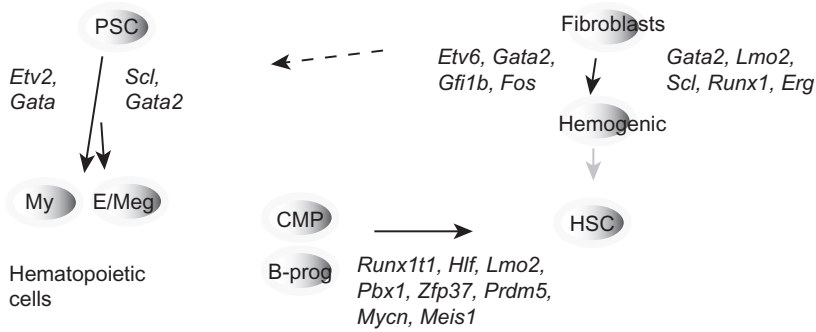


**Fig. 8** *Hemogenic reprogramming*. Different starting cell types can be converted to a hematopoietic fate via the expression of a limited number of transcription factors. Human pluripotent stem cells (embryonic or induced) can be converted to endothelium to produce myeloid cells (My) by ETV2 and GATA2, or erythro-megakaryocytic (E/Meg) cells by GATA2 and SCL (Elcheva et al., 2014). In parallel, fibroblasts are directed toward hematoendothelial cells by *Etv6*, *Gata2*, *Gfi1b*, and *Fos* (Pereira et al., 2013), or by *Gata2*, *Lmo2*, *Scl*, *Runx1*, and *Erg* (Batta, Florkowska, Kouskoff, & Lacaud, 2014). Nonetheless, these cells do not engraft. Committed hematopoietic cells, i.e., common myeloid progenitors (CMPs) or B-progenitor cells (B-prog) can be reprogrammed toward HSCs that engraft in vivo by allowing for HSC selection in their niches (Riddell et al., 2014).

(Doulatov et al., 2013). In parallel, a screen for transcription factor combinations that can induce hemogenic reprogramming in murine fibroblasts revealed a combination of four factors that did not include *Scl* : *Gata2*, *Gfi1b*, *cFos*, and *Etv6* (Pereira et al., 2013; Fig. 8). Given the critical importance of *Scl* at the onset of hematopoiesis, the fact that *Scl* did not score as a hit in the screen was unexpected. It is conceivable that loss of function studies and gain of function studies do not assess the same properties. Hence, *Scl* gene knockout revealed the nonredundant function of *Scl* at a specific time point, whereas gain of function studies indicate that *Scl* might be necessary but not sufficient. In addition, the first wave hematopoiesis is geared mostly toward the production of primitive erythroid cells while the establishment of a complex hemogenic program of the third wave is more complex and requires an intricate gene network. Given that transcription factors and SCL in particular, do not function in solo but within ensembles and that networking (Kerenyi & Orkin, 2010) may be a recurring theme in cell fate decision that extends well beyond erythropoiesis, perhaps the missing factor in experiments relying on ectopic expression is the timing of SCL expression and/or the cellular/molecular context for SCL activity.

Since *Etv6* is upstream of *Scl* during hemangioblast formation (Ciau-Uitz et al., 2013) and that *Gata2* is upstream of *Scl* and *Gfi1b* (Gao et al., 2013) during EHT, these transcription factors may induce *Scl* as part of their hemogenic specification, a possibility that remains to be addressed. However, *Etv6* or *Gata2* have additional targets that may complement *Scl* function. Indeed, the study of thymocyte reprogramming indicates that SCL–LMO activity strictly depends on the microenvironment of target cells, suggesting that hemogenic reprogramming of fibroblasts may also require a permissive microenvironment. Consistent with this hypothesis, *Etv6* activates *Vegfa* expression in lateral plate mesoderm and in somites, which then upregulates *Scl* expression via FLK1 signaling, indicating a noncell autonomous role for *Etv6* in HSC development (Ciau-Uitz et al., 2010). The upregulation of *Scl* by *Vegf* in HSC development is reminiscent of the genetic interaction between *Vegf* and *Scl* at the onset of the primitive erythroid lineage (Ema et al., 2003; Martin et al., 2004). Together, these results suggest a complementation of cell autonomous and noncell autonomous pathways converging on *Scl* induction during hemogenic reprogramming. Furthermore, these results clearly indicate that fate conversion can be dictated by a limited number of transcription factors (Pereira et al., 2013), possibly through modification of the gene expression landscape and the chromatin architecture. Reprogrammed cells nonetheless did not engraft in vivo.

Another approach using a combination of 19 transcription factors delivered into E14.5 murine embryonic fibroblasts identified a minimal combination of five factors, *Erg*, *Gata2*, *Lmo2*, *Runx1c*, and *Scl* to generate multipotent hematopoietic progenitors (Batta et al., 2014; Fig. 8). In this experiment, three hematopoietic-specific components of the SCL complex scored as hits. This approach did not assess more ubiquitous protein partners encoded by *Tcf3* or *Tcf12* and *Ldb1* that are required for the activity of the SCL complex. It may be postulated that these factors are expressed at sufficiently high levels in fibroblasts to be recruited within the SCL complex. In addition, complementation by *Erg* and *Runx1c* suggests that these factors are not redundant with the SCL complex, a possibility that remains to be addressed. Finally, these reprogrammed fibroblasts showed limited self-renewal activity compared to committed common myeloid progenitors (CMP) or B-progenitor that are reprogrammed by a set of six factors (Riddell et al., 2014; Fig. 8). The latter cocktail includes *Lmo2* but not *Scl*. Since these progenitors express *Scl* (Herblot et al., 2002), it is possible that *Lmo2* is the limiting factor that needs to be supplied in trans. Interestingly, Riddell et al. took into account the importance of the microenvironment for the production of HSCs and, therefore, transplanted cells immediately after gene transfer for selection of HSCs in their physiological niches. As a consequence, the authors were able to select for HSCs that are capable of engraftment in vivo, although the efficiency of gene transfer was low (Riddell et al., 2014).

Akin to the inhibition of the cardiac fate at the onset of hematopoiesis, SCL together with LMO2 may direct cell fate conversion by driving a hematopoietic gene expression program and by inhibiting the fate of the starting cell type, be it a fibroblast or a B-progenitor. Furthermore, the combinatorial interaction of *Scl* and *Vegf* or other signaling pathways during embryonic development as well as the reliance of *SCL–LMO1* reprogrammed pre-LSCs on high Notch1 signaling indicate that the microenvironment is an important determinant in the reprogramming equation, as suggested by the successful reprogramming of B-progenitors into self-renewing HSCs via in vivo selection (Riddell et al., 2014). In addition, the requirement for coincidence detection (if and only if) of the necessary factors on DNA and the need for stoichiometry may be met at low frequency and therefore impact on the efficiency of conversion. These factors may also explain the difference in fate conversion between fibroblasts and B-progenitors as starting cell types. Finally, if cellular reprogramming is to be considered as a source of stem cells for transplantation, a strategy for

regulated self-renewal activity may be needed, as the precedence in the T-lineage clearly point to an increased probability of cell transformation when these transcription factors endowed with reprogramming activity are constitutively expressed.

## 6. CONCLUDING REMARKS

Multidisciplinary approaches targeting *Scl* have revealed major molecular and biological insights. Molecular studies revealed that SCL interacts with an extensive network of transcription regulators, coactivators, and corepressors via its association with LMO2. These multiprotein complexes confer surprising flexibility in DNA binding, which is not limited to the canonical E box and may explain the multifaceted properties of SCL at the molecular level and in multiple hematopoietic cell types. One of the distinctive properties of this complex is the requirement for coincidence detection of at least five protein partners for transcription to occur. Since several members of the initial pentameric complex are well-established transcription factors, GATA1/2 and E2A/HEB, the requirement for all five binding to promoters for activation was initially surprising, but later explained by the structure of the complex. These extensive protein–protein interactions could explain the large numbers of SCL- or LMO2-bound sequences in the genome. The significance of these large numbers of peaks remain to be assessed, as only a limited subset of genes that are in the vicinity of these peaks are regulated by SCL levels. With the advent of quantitative proteomics, it will also be interesting to determine dynamic variations in the composition of the complex formed on DNA, not only in terms of which proteins are present or absent, but with regards to the stoichiometry of protein partners during processes such as erythroid differentiation and in T-cell transformation. Another open question is the different protein complexes in which SCL and its protein partner LMO2 are involved. For example, RUNX1, ERG, and FLI1 are often found in the vicinity of LMO2 on DNA. It is not known whether these factors associate with SCL–LMO2 in the same complex or in distinct complexes. Is there a role beyond transcription regulation for those genes that are bound by SCL or LMO2 but are not affected by *Scl* or *Lmo2* knockdown? Another aspect that remains to be documented is the importance of SCL isoforms.

Functional studies with *Scl* revealed the surprising robustness of HSCs during steady state. These genetic studies also unraveled an unexpected hierarchy within LT-HSCs and unearthed a rare subpopulation of very long

term and dormant HSCs that are recruited into action under stress conditions. These cells represent the only subpopulation of LT-HSCs that depends on *Scl* gene dosage. Finally, the study of transgenic mice expressing the human *SCL* and *LMO1* oncogenes provides evidence for a preleukemic state which is initiated by the reprogramming of cells with a finite life span into self-renewing preleukemic stem cells. These cells eventually evolve into leukemia propagating cells. *Scl* and *Lmo2* are identified in strategies aimed at hematopoietic reprogramming of diverse cell types into blood or blood stem cells, consistent with the role of *Scl* as a master regulator that specifies the hematopoietic fate and prevents alternate fates. These successful approaches indicate that fate conversion can be achieved and open highly promising perspectives in cell therapy. The next question is how to achieve regulated expression, in particular for transcription factors that can confer de novo self-renewal properties.

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