SCL/TAL1: a multi-faceted regulator from blood development to disease

Catherine Porcher, Hedia Chagraoui and Maiken S. Kristiansen

Medical Research Council Molecular Haematology Unit, Medical Research Council Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, John Radcliffe Hospital, University of Oxford, Oxford, UK.

Running title: SCL/TAL1 in hematopoiesis and oncogenesis

Corresponding author

Catherine Porcher
Medical Research Council Molecular Haematology Unit
Medical Research Council Weatherall Institute of Molecular Medicine
John Radcliffe Hospital
Oxford OX3 9DS
United Kingdom
catherine.porcher@imm.ox.ac.uk

Abstract

SCL/TAL1 is an essential transcription factor in normal and malignant hematopoiesis. It is required for specification of the blood program during development, adult hematopoietic stem cell (HSC) survival and quiescence, and terminal maturation of select blood lineages. Following ectopic expression, SCL contributes to oncogenesis in T-cell acute lymphoblastic leukemia (T-ALL). Remarkably, SCL's activities are all mediated through nucleation of a core quaternary protein complex (SCL:E-protein:LMO2:LDB1) and dynamic recruitment of conserved combinatorial associations of additional regulators in a lineage- and stage-specific context. The finely tuned control of SCL's regulatory functions (lineage priming, activation and repression of gene expression programs) provides insight into fundamental developmental and transcriptional mechanisms, and highlights mechanistic parallels between normal and oncogenic processes. Importantly, recent discoveries are paving the way to the development of innovative therapeutic opportunities in SCL-positive T-ALL.

Introduction

Lineage specification, commitment and differentiation are essential biological processes underlying all cellular pathways. They involve, among other regulatory mechanisms, progressive acquisition of tissue-specific programs of gene expression. In hematopoiesis, this directs specification of mesoderm into blood-fated cells, production of hematopoietic stem cells (HSCs) and differentiation into highly specialized blood cells. Emphasizing the intricate control of these processes, their deregulation often leads to aberrant cell proliferation/differentiation and cancer.

Transcription factors (TFs) are essential effectors at the end of a cascade of extra- and intracellular regulatory mechanisms that establish gene expression networks conferring and progressively sealing cell fates. The basic Helix-Loop-Helix (bHLH) protein SCL/TAL1 (thereafter referred to as SCL) is a pivotal hematopoietic transcriptional regulator. This protein not only lies at the apex of the hierarchy of TFs involved in hematopoietic specification, but it is also required in adult HSCs and for terminal maturation of select blood lineages. When ectopically expressed, it is involved in the physiopathology of T-cell acute lymphoblastic leukemia (T-ALL). Mechanistically, SCL engages with a large array of protein partners to establish activating and repressive transcriptional activities in a lineage- and stage-specific manner. At the heart of these regulatory complexes are conserved combinatorial protein associations and a subtle interplay of DNA-binding activities. Therefore, studying SCL provides an excellent window on versatility of TFs and their modular responses to distinct cellular environments, both in normal and malignant milieus.

Here, we review the function and mechanisms of action of SCL in mesoderm to HSCs, blood lineages and leukemia, contrasting its activities in specification, maturation and oncogenic processes. We discuss how these findings are shaping the development of targeted drug therapies.

SCL confers endothelial and hematopoietic fate to mesodermal cells

The *SCL* gene was cloned by virtue of its involvement in chromosomal translocation t(1;14)(p33;q11) from a cell line derived from a patient presenting with T-ALL.[1] Because of its capacity to differentiate into lymphoid and myeloid lineages, the leukemia cell line was referred to as a "stem cell" line and the newly cloned gene was termed "**S**tem **C**ell **L**eukemia". Since then, studies have progressively unveiled SCL's critical mechanistic role in normal and malignant hemopoiesis, confirming initial

hypotheses about parallel functions in HSC development and leukemic transformation.

In vivo ablation of SCL activity in murine models provided the first evidence that SCL functions at early stages of blood development. *Scl*$^{-/-}$ embryos died at day E9.5 from absence of yolk sac (YS) primitive erythropoiesis and myelopoiesis (Wave 1, see Box 1).[2,3] Moreover, all adult definitive hematopoietic lineages (Wave 3) were absent in *Scl*$^{-/-}$ mouse chimeras.[4,5] This complete block in hematopoiesis suggested a function in either the first differentiation steps from blood stem/progenitor cells or the specification of mesodermal cells towards a blood fate.

Box 1

Blood development: **the three waves**

During vertebrate embryogenesis, blood development occurs in three successive waves (Figure 1).[6,7] The first two waves (1 and 2) take place in the extra-embryonic yolk sac (YS) and give rise to transient blood populations; the third wave (3) develops in the intra-embryonic dorsal aorta (DA) and gives rise to hematopoietic stem/progenitor cells (HSPCs), providing the organism with lifelong blood production. Whilst Wave 1 produces primitive red blood cells (expressing embryonic globins), megakaryocytes and macrophages, Waves 2 and 3 give rise to definitive multipotent erythro-myeloid progenitors (EMPs) in the YS, and HSPCs in the DA, respectively. Waves 2 and 3 produce a highly specialized endothelium, referred to as hemogenic endothelium (HE), from which hematopoietic progenitors bud in a process known as the endothelial-to-hematopoietic transition (EHT).[8-11] This is consistent with the hypothesis, first framed 100 years ago, of an endothelial origin for blood cells.[12] EMPs and HSPCs subsequently migrate to and differentiate in the fetal liver to produce adult-type blood cells.

Subsequent studies in series of experimental models pointed to a role for SCL in endothelial and blood development rather than from already established blood progenitors. Immunolabeling revealed SCL expression in (i) dispersed mesodermal endothelial cell precursors (angioblasts) expressing FLK1 (receptor for vascular endothelial growth factor A, VEFGA) in days E6.5-E7.5 mouse embryos, (ii) angioblasts in the splanchnic mesoderm of avian embryos and (iii) FLK1^{-} hematopoietic cells in mouse YS blood islands.[13,14] Enforced SCL expression in zebrafish and *Xenopus* embryos was sufficient to induce mesoderm to hematopoietic and endothelial fates.[15,16] Finally, differentiation of *Scl*-null cells in murine embryonic stem (ES) cell models, that recapitulate Waves 1 and 2 of YS hematopoiesis,[7] established that SCL was required for generation of both blood and endothelial components of colonies derived from FLK1^{+} mesodermal cells (also called blast

colony-forming cells, BL-CFC).[17,18] Taken together, this suggested a role for SCL at the onset of blood/endothelial development.

Conditional deletion of *Scl* through Tie2-Cre recombinase-mediated pan-endothelial excision refined these observations.[19] In this model, hematopoietic specification was not affected, but maturation of primitive and definitive erythroid cells as well as megakaryocytes was defective, leading to lethality at days E13.5/14.5. This study informed on three key aspects of SCL's functions: (i) SCL is required before Tie2-Cre becomes functional, (ii) SCL activity in blood fate specification can be uncoupled from a later role in blood cell maturation, (iii) after specification of the blood lineage, SCL is not required for HSPC production (Figure 1).

SCL is also necessary for development of the vascular network after angioblast formation. Ablation of *Scl* expression disrupted extra-embryonic angiogenic remodeling in mouse embryos.[20,21] In zebrafish and *Xenopus*, *Scl* loss led to disorganization of major blood vessels, including the dorsal aorta (DA).[22-24]

In summary, during development, SCL is essential for (i) specification of the three hematopoietic waves; (ii) maturation of select blood lineages; (iii) remodeling of the vascular network.

In which cell types does SCL exert its function? Regarding extra-embryonic hematopoiesis, the prevailing hypothesis is that SCL acts in the precursor of YS blood and endothelial populations giving rise to Waves 1 and 2, the hemangioblast, located in the primitive streak (PS). However, recent lineage tracing studies propose independent origins for blood and endothelial lineages, in the epiblast, before gastrulation and formation of the PS (see Box 2).[10] This supports earlier fate mapping studies describing ingression of erythroid-fated cells prior to endothelial-fated cells into the PS of gastrulating embryos.[25] Of note, lineage restriction of cardiovascular progenitors is also believed to occur before gastrulation.[26] Together, this suggests that mesoderm patterning and lineage specification decisions might take place pre-PS formation. Importantly, this model does not exclude a common origin for progenitors of Waves 1 and 2, but in early epiblast stages, before restriction of potentiality and regional segregation as cells ingress into the PS.[10,25] Further lineage tracing studies are required to identify this cell and refine our understanding of blood fate determination.

Box 2

The hemangioblast: towards a new definition?

The hemangioblast was first defined a century ago as a cell giving rise to both blood and endothelial lineages in the yolk sac (YS) of chick embryos.[27,28] This cell was later identified in the primitive streak (PS) of mouse embryos and described as a bi- (blood/endothelial) or tri- (blood/endothelial/vascular smooth muscle) potential clonal progenitor both *in vitro* and *in vivo*.[29,30] However, a recent retrospective clonal lineage tracing study based on conditional activation of CreERT2 disputes this model and reports independent blood and endothelial progenitors in the epiblast, before formation of the PS and migration of cells to the YS.[10] This finding, whilst not excluding the existence of a common blood and endothelial progenitor before separation of the two lineages in the epiblast, questions the existence of the hemangioblast as originally defined.

Therefore, we propose that SCL acts on the progenitors of the YS blood cells (Wave 1) and blood-fated angioblasts (Wave 2) after their ingression into the PS and before their migration into the YS where they form the blood islands (Figure 1). Similarly, in this model, SCL is required to instruct the angioblasts at the origin of Wave 3 (as demonstrated in *Xenopus* embryos, see below). Full examination of the function and spatio-temporal expression of SCL in early stages of gastrulation is required to confirm this model.

Vascular endothelium, cardiomyocytes and endocardium: an intricate relationship

Manipulation of SCL expression during development has highlighted complex relationships between endothelial cells and the cardiovascular lineage.

1. *Blood-fated vascular endothelium and cardiomyocytes* - In zebrafish embryos, forced expression of *Scl* mRNA expanded blood and endothelial tissues at the expense of somitic and myocardial tissues.[15,23,31] Conversely, down-regulation of *Scl* led to ectopic cardiomyocyte production and unveiled an FGF-mediated repression of a latent cardiac potential in blood and vessel progenitors.[32,33] In agreement with this, absence of SCL revealed cardiac potential in FLK1$^+$ blood-fated endothelial cells of murine YS.[34] Corroborating these phenotypes, SCL not only activates vascular- and blood-specific transcriptional programs in FLK1$^+$ cells, but also represses expression of cardiac-specific regulators and structural proteins to prevent ectopic cardiac activity (Org et al.[35] and HC, MSK and CP, unpublished data).

2. *Endocardium and myocardium*. Whilst endocardial precursors are specified in *Scl* mutant embryos, endocardial cell migration and maturation are impaired, leading to

defective intercellular junctions and heart tube formation.[36,37] Interestingly, absence of SCL in the endocardium also induces ectopic myocardial differentiation.[34,37] Therefore, as for vascular endothelium, SCL is required for the biology and identity of the endocardium.

Altogether, these studies open a window into intricate lineage relationships in early embryogenesis. Expression of SCL in vascular endothelium and endocardium suggests a shared origin from a common endothelial progenitor. Separately, the requirement for SCL to restrict a cardiomyocyte fate in blood-fated vascular endothelial cells and endocardium suggests a developmental relationship between myocardium and endothelial cells. Whether the cardiovascular and blood/endothelial systems share a common origin is, however, still under debate.[26,38-40] Understanding the detailed mechanistic basis to these processes is likely to provide principles to a recurring question in developmental biology: at what stage do lineage-fated cells arise and what is the extent of multi-potentiality of common progenitors?

SCL lies at the top of the hematopoietic transcriptional hierarchy

Studies of other TFs required in early stages of mesoderm/blood development have helped position SCL in the hierarchy of regulatory events leading to endothelial and blood specification. An important regulator of vascular and hematopoietic development is the ETS family TF, ETV2. *Etv2*$^{-/-}$ mouse embryos die at days E9.5/10.5 from complete absence of vasculature and YS hematopoiesis,[41] inferring a role in mesoderm specification towards blood/endothelial lineages. Indeed, in response to VEGFA signaling, ETV2 is transiently required for progression of FLK1^{+}PGFRα^{+} early mesoderm to FLK1^{+}PDGFRα^{-} lateral plate mesoderm, the population containing the angioblasts.[42-45] ETV2 is believed to control segregation of a heterogeneous population of angioblasts into distinct lineages through differential activation of genetic programs: ETV2's target gene *miR-130a* directs angioblasts towards endothelial cells, but not blood-fated endothelial cells,[46] whilst SCL and the transcriptional regulators GATA2 and FLI1 (ETS family), both also involved in endothelium/blood development, are critical for induction of blood-fated cells.[42,44,47,48] As SCL, GATA2 and FLI1 participate in an interconnected regulatory loop,[49] they are able to sustain a genetic program after the initiating events (VEGFA-ETV2) have ceased. This mechanism, known to specify and maintain cell identity,[50] confers hematopoietic fate to the angioblasts at the origin of the blood lineage.

We are now starting to appreciate aspects of SCL function in angioblasts giving rise to the adult HSPC lineage, from studies in *Xenopus* embryos. As in the mouse, ETV2 initiates expression of *Scl* and additional endothelial and blood markers in response to VEGFA.[51] Upon acquisition of an endothelial identity, the angioblasts migrate from the dorsal lateral plate (DLP) mesoderm towards the embryo midline where they form the DA. Knock-down of *Scl* negatively affects endothelial gene expression in the DLP. This does not prevent endothelial cell migration but disrupts DA formation and hematopoietic development.[24,51] As *Scl* mRNA expression is down-regulated in endothelial cells migrating towards the embryo midline,[52] SCL is likely to instruct endothelial cell progenitors in the DLP before their migration. The mechanisms of how SCL directs these processes are not clear but may involve the establishment of appropriate epigenetic/transcriptional landscapes. Indeed, recent genome-wide ChIP analyses of ES cell-derived cellular intermediates have shown SCL binding to genomic targets in GFP-Brachyury^{+}/FLK1^{+} cells, prior to endothelial development and appearance of DNase I hypersensitive sites.[53] This suggests that SCL may prime key target loci in DLP cells; these become expressed at high levels at the time of DA formation and HE production, possibly through remodeling of TF binding patterns by RUNX1, the key regulator of EHT processes.[54]

Altogether, SCL is one of the early-acting hematopoietic TFs likely to prime *cis*-acting elements in gene loci involved in hemopoietic and endothelial development.

SCL in adult hematopoiesis

Although *Scl* is broadly expressed in adult blood cells, its mRNA levels mark distinct branches of the hematopoietic tree. *Scl* is expressed in the HSC compartment, myeloid progenitors and mature myeloid cells (red cells, megakaryocytes, mast cells and basophils; at lower levels in eosinophils, macrophages and neutrophils) and at low levels in lymphoid progenitors. *Scl* is absent in differentiating T and B lymphocytes (Figure 2).[55-57]

SCL and HSCs: survival and quiescence

SCL's functions in adult long-term repopulating HSCs have been complex to decipher,[58-60] as these roles are shared with another bHLH protein, LYL1. Whilst LYL1 does not compensate for SCL in specification processes,[61,62] *Scl/Lyl1* conditional double KO mouse models revealed redundant, anti-apoptotic and dose-dependent functions in HSC survival.[63] Using transplantation assays in proliferative

stress conditions, Lacombe et al. were then able to demonstrate a gene dosage effect in *Scl*$^{+/-}$ mouse bone marrow HSCs. SCL negatively regulates the G_0-G_1 transition in adult KLS/CD150$^+$/CD48$^+$ HSCs, in part by controlling expression of the genes encoding TF ID1 and cyclin-dependent kinase (CDK) inhibitor P21/CDKN1A.[64] HSC quiescence and long-term activity are further maintained through a positive feedback loop involving the cytokine receptor c-KIT.[65] Interestingly, SCL exhibits opposite functions in human cord blood HSCs where it is reported to positively control proliferation through the mTOR pathway.[66] Whether this difference is species- or developmental stage-related is not known. Altogether, these studies have highlighted an important facet of SCL in survival and self-renewal mechanisms to preserve adult HSC integrity.

SCL and adult endothelium: a role in vascular repair

Reminiscent of its functions in vascular remodeling during development, SCL controls post-natal angiogenesis. While low in quiescent endothelial cells, its expression is up-regulated during morphogenesis events.[67] Mechanistically, SCL regulates the development of human adult endothelial progenitors through activation of genes involved in cell adhesion and migration, conferring vascular repair functions.[68]

SCL and lineage maturation: balance between proliferation and differentiation

Gain- and loss-of-function studies have highlighted SCL's roles in proliferation and differentiation of erythroid cells, megakaryocytes, mast cells and monocytes/macrophages.[19,69-72] In contrast to adult HSCs, SCL activates cell cycle progression in lineage progenitors. Through control of *p21* and *p16/Ink4a* expression, SCL regulates the onset of red cell differentiation, polyploidization and terminal maturation of megakaryocytes, as well as proliferation of monocyte progenitors.[71-73] SCL also promotes cell survival through anti-apoptotic activities. In physiological conditions requiring control of red cell production, caspase-mediated cleavage of SCL leads to reduced expression of TF GATA1 and survival factor BCL-X_L, thereby triggering apoptosis.[74]

Genome-wide interrogation of SCL's direct target genes provided further insight into its activities in lineage differentiation. ChIP-sequencing combined with gene expression analyses from primary erythroblasts revealed that SCL controls both general processes (transcription, cell cycle, proliferation) and erythroid-specific pathways (redox processes, heme biosynthesis, cytoskeleton organization).[75]

Comparative studies of SCL ChIP-seq data from multipotent blood progenitors, proliferative lineage-specific precursors and terminally differentiating erythroid and megakaryocytic cells reported dramatic changes in SCL occupancy through the progressive stages of commitment and differentiation.[76] In progenitor cells, SCL target genes were enriched in processes associated with proliferation and apoptosis. Megakaryocytic genes were bound by SCL in progenitor cells, suggesting priming of genetic loci associated to low-level expression, as in mesodermal precursors. In contrast, erythroid-specific genes were typically bound in the erythroid lineage only, but the binding pattern of SCL varied as the cells matured, often reflecting changes in levels of target gene expression.[76] These dynamic changes are central to lineage commitment and terminal maturation. What drives these processes is unclear but likely to stem from the adaptable nature of SCL-containing transcriptional complexes.

SCL and multi-protein regulatory complexes: an elaborate chemistry

To fully appreciate SCL's mechanisms of action, it is essential to consider SCL-containing combinatorial multi-protein complexes and their recruitment to genomic targets.

SCL's DNA-binding activity is not the primary determinant of genome occupancy

SCL binds the consensus Ebox DNA motif, CANNTG, as a heterodimer with ubiquitously expressed bHLH E-proteins (E12/E47, HEB, E2-2).[77] Non DNA-binding proteins (bridging LIM-only (LMO) and LIM domain-binding (LDB) proteins) are then recruited to form the SCL core complex: SCL:E-protein:LMO2:LDB1. This quaternary module, described in all SCL-expressing cells, recruits additional DNA-bound TFs and co-factors. Amongst the best-described partners are the GATA proteins: GATA2, involved in HSPC specification/survival and the early stages of lineage differentiation, and GATA1, required in lineage maturation.[47,78,79] *In vitro* enrichment of DNA-binding sites initially demonstrated the assembly of the pentameric complex (SCL:E47:LMO2:LDB1:GATA1) on a bipartite Ebox-GATA DNA motif.[80] Interestingly, analyses of the sequences underlying SCL ChIP-seq peaks invariably describe GATA motifs as the most frequent SCL-bound sequences.[35,75,76,81] Therefore, throughout development, GATA proteins appear as a major determinant of SCL genomic occupancy. Together with the identification of ½ Ebox-GATA motifs in sequences underlying SCL ChIP peaks (CTG(N_{9-10})A/WGATAA, the ½ Ebox CTG being bound by E47),[75,82,83] this put into perspective the importance of Ebox motifs in recruiting SCL-containing complexes.

SCL bridges transcriptional activities to other DNA-bound regulators

The X-ray structure of SCL quaternary complex (SCL:E47:LMO2:LDB1) bound to an Ebox revealed how binding of LMO2 strengthens the interactions between SCL and E47 through creation of new hydrogen bonds.[82,84] This, in turn, induces a rotation of E47 that weakens the affinity of the heterodimer for DNA. Through its adaptor function, LMO2 links the heterodimer to other DNA-bound proteins, such as the GATA proteins,[82] leading to recruitment of SCL:E-protein:LMO2 complexes to GATA1/2-bound genomic loci. Interaction with additional DNA-bound proteins therefore provides DNA-binding specificity, more so than Ebox or ½ Ebox motifs, the latter merely acting as an anchoring point for the heterodimer when associated to GATA sites. Altogether, this explains, in part, why some of SCL's functions, especially in blood specification, are independent of SCL's own ability to bind DNA.[62,75,85] As co-factors such as p300/CBP and ETO2 (possibly mSIN3A and pCAF) interact directly with E-proteins,[82,86] SCL's main role may be to act as a linker connecting ubiquitously-expressed E-proteins and associated transcriptional activities to tissue-specific DNA-binding proteins through interaction with LMO2 (Figure 3). Finally, the SCL complex is involved in chromatin architecture through DNA looping. In erythropoiesis, looping occurs upon dimerization of LDB1 to juxtapose enhancers and promoters. This triggers recruitment of cofactors and RNA polymerase II at promoters, leading to gene activation.[87,88]

bHLH, GATA, ETS and RUNX proteins: the winning combination

Following the observation that SCL/GATA2/FLI1 coordinately control hematopoietic development, parallel analyses of TF ChIP-seq assays identified a similar combination of regulators in adult hematopoiesis. A "heptad" of TFs, namely SCL, LYL1, GATA2, FLI1 and ERG (ETS proteins), RUNX1 and LMO2, function cooperatively in HSPCs and cell intermediates as they differentiate into erythroblasts and megakaryocytes.[89,90] Whilst composition of the protein complexes binding to regulatory elements at any one time still remains to be identified, pairwise combinations and motif search analyses demonstrated the combinatorial nature of TF interactions. This has unveiled previously uncharacterized combinations, such as SCL/RUNX1, and a strong GATA/ETS correlation in most genomic regions bound by the heptad.[90,91] Comparative analyses of the megakaryocytic and erythroid lineages suggested overlapping and divergent roles for these seven TFs. They highlighted distinct binding of SCL, distinct combinations of TF-binding motifs and distinct patterns of GATA1 and GATA2 binding in the two lineage branches. The switch in

GATA1/GATA2 binding is likely to play an important role in the shifts in SCL genomic occupancy.[76,89] Altogether, these data reinforce the critical requirement for the bHLH/GATA/ETS protein triad throughout hematopoietic development, that also involves RUNX1 in HSPCs and megakaryocytes.

The pivotal role of SCL-containing complexes in specification and differentiation processes was highlighted in recent reprogramming experiments. Forced expression of SCL with various combinations of GATA1/2, ERG, RUNX1 and LMO2 induces differentiation of fibroblasts and pluripotent stem cells into multipotent,[53,92] erythroid (with cMYC)[93] or erythro-megakaryocytic[94] progenitors through a HE intermediate. Direct fate conversion reflects the capacity of these regulators to overcome epigenetic barriers and robustly establish lineage-specific transcriptional programs, suggesting that some of them may act as pioneering factors or interact with such factors.[95]

Repressive versus activating functions

In addition to activation of gene expression, repression mechanisms play an increasingly recognized role in lineage determination. This reflects the fact that (i) uni-lineage–fated cells derive from multi-lineage–primed progenitors and, therefore, need to establish repressive mechanisms to adopt a unique cell fate, and (ii) genes required for terminal maturation are often primed in early stages of lineage commitment but their expression needs to be restrained to prevent premature high-level expression and precocious differentiation. During development, SCL prevents expression of cardiac-affiliated genes through active repressive mechanisms (HC, MSK, CP, unpublished data) and by occupation of cardiac enhancers hampering activation by cardiac-specific TFs.[35] In red cells, SCL interacts with an extended network of co-repressors, comprising ETO2/GFI1B/NCOR1/mSIN3A, to repress primed genes.[73,96,97] Subsequent gene activation necessitates interaction with co-activators, such as p300/CBP and pCAF. The shift from repressive to activating complexes has been documented for ETO2 and P300 where competitive binding to the AD1 domain of E-proteins regulates complex formation.[86] Moreover, the nature of other protein partners, such as GATA proteins, influences multiprotein complex transcriptional activity. In erythropoiesis, SCL complexes exhibit repressive properties in progenitors through association with GATA2, whilst interaction with GATA1 activates genes required for terminal maturation.[79,89,98]

Altogether, these studies invite further identification of the mechanisms orchestrating dynamic recruitment of regulatory complexes to their genomic targets as

hematopoietic differentiation proceeds. In particular, the relationship between SCL and chromatin structure[76] merits investigation at a mechanistic level. Changes in the epigenetic landscape, upon expression/repression of pivotal regulators, could generate an environment propitious to *de novo* enhancer formation or eradication of existing enhancers, leading to TF-binding pattern remodeling and alteration of gene expression programs.

Specification versus maturation

A parallel can be drawn between SCL's functions and (i) its DNA-binding activities, (ii) expression levels and (iii) protein isoforms. Studies employing *Scl* hypomorphic zebrafish embryos and mouse embryos expressing a DNA-binding mutant form of SCL have shown that low levels of SCL and DNA-binding independent activities were sufficient for lineage specification. Conversely, higher levels and direct DNA-binding activities were required for lineage maturation.[85,99] In zebrafish, the SCL isoform required in HSPC specification is subject to rapid degradation resulting in low protein levels.[100] Altogether, this suggests that low levels and DNA-binding independent activities of SCL are sufficient for lineage priming and gene repression in specification processes, and may be a pre-requisite to prevent precocious hematopoietic differentiation in blood-fated angioblasts. In contrast, higher levels of SCL protein together with direct DNA-binding activities are required for robust gene expression of primed genes in terminal differentiation processes.

SCL in leukemogenesis: parallels with normal hematopoietic processes

Aberrant expression of transcriptional regulators often triggers oncogenic processes. In normal conditions, SCL expression is down-regulated during T-cell differentiation (Figure 2).[101] However, SCL expression can be activated in T-cells through chromosomal translocations (interstitial deletions in the *SIL-SCL* locus) and mono/bi-allelic transcriptional mechanisms.[102] This ectopic thymic expression is seen in 60% of childhood and adult T-ALL cases, often associated with poor prognosis.[103,104] Expression of SCL is however insufficient to induce overt leukemia. Collaboration with additional oncogenic events is required for full leukemic transformation with short latency periods. In ~45% of SCL-positive T-ALL cases, the cooperating genetic event is ectopic expression of LMO1 or LMO2 through chromosomal rearrangement.[103] In murine transgenic models,[101,105,106] SCL and LMO1/2 co-expression confers aberrant self-renewal to $CD4^{-}CD8^{-}$ double negative (DN3) pre-

leukemic thymocytes (Figure 2).[107] This self-renewal capacity is enhanced by activation of a major contributor to T-ALL, the NOTCH pathway.[108,109] This leads to acquisition of additional mutations, differentiation arrest and full-blown leukemia.[107]

Parallels with normal hematopoiesis

Remarkably, the oncogenic SCL protein complexes are replicas of those observed in normal hematopoiesis. This occurs as genetic mutations activating *Scl* result in wild-type SCL protein expression. Moreover, members of the protein families normally interacting with SCL are co-expressed in T-cells, either endogenously (RUNX1/GATA3) or ectopically (LMO1/2). Recent genome-wide and structural approaches have refined our understanding of SCL's mechanisms of action in T-ALL and provided further compelling evidence for parallel functions in hematopoiesis and leukemogenesis.

- Direct DNA-binding independent mechanisms, sequestration and relocation

In normal thymocytes, E-protein homodimers direct progression of T-cell differentiation through activation of tissue-specific genes.[110] When ectopically expressed, SCL sequesters E-proteins in heterodimers.[101,111] Recruitment of LMO1/2 stabilises heterodimerization, reinforcing a shift in equilibrium from E-protein homodimers to more stable heterodimers.[82] Because of weaker interactions at the heterodimer:DNA interface,[82] SCL:E-protein:LMO1/2 complexes are directed to new sets of genomic targets through interaction with additional DNA-bound regulators, such as GATA3, ETS proteins or RUNX1. This results in repression of pro-apoptotic and T-cell differentiation transcriptional programs and activation of self-renewal and anti-apoptotic genes.[81,112-115] This sequestration/relocation model is particularly relevant as, as in hematopoietic specification,[85] SCL's mechanisms of action in T-ALL do not require direct DNA-binding activities.[115] By analogy, in specification processes, SCL may sequester E-proteins in blood-fated cells away from cardiac or paraxial bHLH proteins to favor a hematopoietic gene expression program and prevent promiscuous development of alternative lineages (Figure 3).

- Auto-regulatory interconnected loops: SCL, GATA3, RUNX1, ETS1 and MYB

As in normal hematopoiesis, recursive circuits establish and maintain the oncogenic program controlled by SCL. In T-ALL, SCL, GATA3 and RUNX1 auto-regulate each other and positively control expression of key target genes, such as *MYB* which, in turn, contributes to maintaining the oncogenic transcriptional program.[114] The discovery of mutations creating a *de novo* MYB binding site in the *SCL* locus and

triggering formation of a broad enhancer (termed super-enhancer) not only provided a genetic mechanism for *SCL* mono-allelic expression, but also helped refine the composition of SCL regulatory complexes.[116] Mechanistically, MYB binding drives *SCL* auto-regulation through recruitment of CBP, broad H3K27 acetylation, chromatin opening and nucleation of SCL-containing multiprotein complexes involving RUNX1, GATA3 and ETS1. MYB/CBP association, together with SCL, GATA3 and RUNX1, positively regulates transcription of each component of the complex, thus placing MYB in the regulatory kernel. It would be interesting to determine whether MYB is also part of the recursive loops documented in normal hematopoietic development and functionally contributes to some of SCL protein complexes. Finally, not only does SCL positively regulate expression of MYB, but it also prevents its degradation through *miR-223*-mediated repression of the tumour suppressor gene *FBXW7*.[117]

Towards new treatments of T-ALL?

ALL is a heterogeneous group of malignancies, covering a broad range of subtypes of B- and T-lymphocyte origin. The main therapeutic treatment for ALL relies on repeated cycles of chemotherapy, irrespective of the chromosomal abnormalities. This leads to ~90% remission in children, but to only 10-40% survival in adults due to toxicity and relapse.[118] Dissecting the molecular mechanisms underlying the physiopathology of SCL-positive T-ALL provides a basis for the development of novel therapies focusing on distinct aspects of SCL's activities.

As oncogenic transformation relies on gene expression, pharmacological inhibition of components of the general transcriptional machinery has been explored. Low-dose inhibition of CDK7 kinase activity, necessary for phosphorylation of the C-terminal domain of RNA polymerase II, successfully reduced proliferation of an SCL-positive T-ALL cell line.[119] Importantly, the inhibitor predominantly affected expression of genes involved in the core regulatory circuitry (*SCL, GATA3, RUNX1*), possibly due to high sensitivity of the super-enhancers regulating their expression.[120] Epigenetic modifications have been the focus of a recent investigation. UTX, a histone H3K27 demethylase, is a member of the SCL complex in T-ALL that confers oncogenic properties.[121] Remarkably, exposure of patient-derived xeno-transplanted SCL-positive T-ALL to H3K27 demethylase inhibitors selectively suppresses leukemic blast growth. Finally, the recent discovery that SCL regulates miRNA expression[117,122] could pave the way to developing new therapeutic opportunities targeting miRNAs or their targets.

Protein/protein interactions (PPIs) are currently a major therapeutic focus. Although PPI interfaces are often large and featureless, making them difficult to target with small molecules, integration of structural, biochemical and computational methods has opened the way to developing PPI inhibitors, providing a greater level of specificity.[123] The X-ray structure of SCL quaternary complex allows the design of such inhibitory molecules.[82] In particular, the small size of SCL:LMO2 interface (620 Å^2), together with the presence of defined secondary structures and identification of residues directly involved in SCL:LMO2 interaction, makes it an attractive target.[82] As a prelude to these developments, both anti-LMO2 peptide aptamers and single domain intra-cellular anti-LMO2 antibodies successfully inhibit LMO2-dependent T-cell tumor growth.[124,125] Going forward, it will be essential to disrupt SCL-containing oncogenic complexes in a way that does not functionally affect the complexes required in normal hematopoiesis. Differences in affinities between partners in distinct cellular contexts or tissue-specific PPIs would allow oncogenic-specific design or dosage of small inhibitory molecules.

Conclusion

Recent years have seen significant advances in our understanding of SCL's transcriptional mechanisms. The regulatory processes controlled by SCL are complex and many aspects remain to be investigated. In particular, the signaling pathways initiating cellular transitions during lineage development are not clear; the mechanistic relationships between SCL multi-protein complexes and chromatin remodelers need to be defined; the role of LYL1 should be further analyzed: redundancy with SCL in the HE could explain the apparent lack of SCL function in HSC specification; finally, functional correlation between SCL and miRNAs warrants further exploration. Ultimately, unraveling SCL-dependent normal and oncogenic processes may expose unsuspected lineage-specific pathways that will contribute to developing high efficacy targeted therapies in SCL-positive T-ALL.

Acknowledgements

We apologize to our colleagues whose work could not be cited due to space constraints. We thank Paresh Vyas, Claus Nerlov and Aldo Ciau-Uitz for critical review of the manuscript. The work performed in C.P.'s laboratory is funded by the Medical Research Council. The authors declare no conflict of interest.

Authorship

C.P., H.C. and M.S.K. wrote the manuscript.

References

1. Begley CG, Aplan PD, Davey MP, et al. Chromosomal translocation in a human leukemic stem-cell line disrupts the T-cell antigen receptor delta-chain diversity region and results in a previously unreported fusion transcript. *Proc Natl Acad Sci U S A*. 1989;86(6):2031-2035.
2. Shivdasani RA, Mayer EL, Orkin SH. Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL. *Nature*. 1995;373(6513):432-434.
3. Robb L, Lyons I, Li R, et al. Absence of yolk sac hematopoiesis from mice with a targeted disruption of the scl gene. *Proc Natl Acad Sci (USA)*. 1995;92(15):7075-7079.
4. Porcher C, Swat W, Rockwell K, Fujiwara Y, Alt FW, Orkin SH. The T cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. *Cell*. 1996;86(1):47-57.
5. Robb L, Elwood NJ, Elefanty AG, et al. The scl gene product is required for the generation of all hematopoietic lineages in the adult mouse. *EMBO J*. 1996;15(16):4123-4129.
6. Yoder MC. Inducing definitive hematopoiesis in a dish. *Nat Biotechnol*. 2014;32(6):539-541.
7. McGrath KE, Frame JM, Fegan KH, et al. Distinct Sources of Hematopoietic Progenitors Emerge before HSCs and Provide Functional Blood Cells in the Mammalian Embryo. *Cell Rep*. 2015;11(12):1892-1904.
8. Kissa K, Herbomel P. Blood stem cells emerge from aortic endothelium by a novel type of cell transition. *Nature*. 2010;464(7285):112-115.
9. Boisset JC, van Cappellen W, Andrieu-Soler C, Galjart N, Dzierzak E, Robin C. In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. *Nature*. 2010;464(7285):116-120.
10. Padron-Barthe L, Temino S, Villa Del Campo C, Carramolino L, Isern J, Torres M. Clonal analysis identifies hemogenic endothelium as the source of the blood-endothelial common lineage in the mouse embryo. *Blood*. 2014;124(16):2523-2532.
11. Swiers G, Rode C, Azzoni E, de Bruijn MF. A short history of hemogenic endothelium. *Blood Cells Mol Dis*. 2013;51(4):206-212.
12. Jordan HE. Evidence of hemogenic capacity of endothelium. *Anat Rec*. 1916;10:417-420.
13. Drake CJ, Brandt SJ, Trusk TC, Little CD. TAL1/SCL is expressed in endothelial progenitor cells/angioblasts and defines a dorsal-to-ventral gradient of vasculogenesis. *Dev Biol*. 1997;192:17-30.
14. Drake CJ, Fleming PA. Vasculogenesis in the day 6.5 to 9.5 mouse embryo. *Blood*. 2000;95(5):1671-1679.
15. Gering M, Rodaway AR, Gottgens B, Patient RK, Green AR. The SCL gene specifies haemangioblast development from early mesoderm. *Embo J*. 1998;17(14):4029-4045.

16. Mead PE, Kelley CM, Hahn PS, Piedad O, Zon LI. SCL specifies hematopoietic mesoderm in xenopus embryos. *Development*. 1998;125(14):2611-2620.

17. D'Souza SL, Elefanty AG, Keller G. SCL/Tal-1 is essential for hematopoietic commitment of the hemangioblast but not for its development. *Blood*. 2005;105(10):3862-3870.

18. Lancrin C, Sroczynska P, Stephenson C, Allen T, Kouskoff V, Lacaud G. The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage. *Nature*. 2009;457(7231):892-895.

19. Schlaeger TM, Mikkola HK, Gekas C, Helgadottir HB, Orkin SH. Tie2Cre-mediated gene ablation defines the stem-cell leukemia gene (SCL/tal1)-dependent window during hematopoietic stem-cell development. *Blood*. 2005;105(10):3871-3874.

20. Visvader JE, Fujiwara Y, Orkin SH. Unsuspected role for the T-cell leukemia protein SCL/tal-1 in vascular development. *Genes Dev*. 1998;12:473-479.

21. Elefanty AG, Begley CG, Hartley L, Papaevangeliou B, Robb L. SCL expression in the mouse embryo detected with a targeted lacZ reporter gene demonstrates its localization to hematopoietic, vascular, and neural tissues. *Blood*. 1999;94(11):3754-3763.

22. Patterson LJ, Gering M, Patient R. Scl is required for dorsal aorta as well as blood formation in zebrafish embryos. *Blood*. 2005;105(9):3502-3511.

23. Dooley KA, Davidson AJ, Zon LI. Zebrafish scl functions independently in hematopoietic and endothelial development. *Dev Biol*. 2005;277:522-536.

24. Ciau-Uitz A, Monteiro R, Kirmizitas A, Patient R. Developmental hematopoiesis: ontogeny, genetic programming and conservation. *Exp Hematol*. 2014;42(8):669-683.

25. Kinder SJ, Tsang TE, Quinlan GA, Hadjantonakis AK, Nagy A, Tam PP. The orderly allocation of mesodermal cells to the extraembryonic structures and the anteroposterior axis during gastrulation of the mouse embryo. *Development*. 1999;126(21):4691-4701.

26. Lescroart F, Chabab S, Lin X, et al. Early lineage restriction in temporally distinct populations of Mesp1 progenitors during mammalian heart development. *Nat Cell Biol*. 2014;16(9):829-840.

27. Sabin FR. Preliminary note on the differentiation of angioblasts and the method by which they produce blood-vessels, blood-plasma and red blood-cells as seen in the living chick. 1917. *J Hematother Stem Cell Res*. 2002;11(1):5-7.

28. Murray PDF. The development in vitro of the blood of the early chick embryo. *Proc R Soc Lond B*. 1932;111:497-520.

29. Choi K, Kennedy M, Kazarov A, Papadimitriou JC, Keller G. A common precursor for hematopoietic and endothelial cells. *Development*. 1998;125(4):725-732.

30. Huber TL, Kouskoff V, Fehling HJ, Palis J, Keller G. Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. *Nature*. 2004;432(7017):625-630.

31. Gering M, Yamada Y, Rabbitts TH, Patient RK. Lmo2 and Scl/Tal1 convert non-axial mesoderm into haemangioblasts which differentiate into endothelial cells in the absence of Gata1. *Development*. 2003;130(25):6187-6199.

32. Schoenebeck JJ, Keegan BR, Yelon D. Vessel and blood specification override cardiac potential in anterior mesoderm. *Dev Cell*. 2007;13(2):254-267.

33. Simoes FC, Peterkin T, Patient R. Fgf differentially controls cross-antagonism between cardiac and haemangioblast regulators. *Development*. 2011;138(15):3235-3245.

34. Van Handel B, Montel-Hagen A, Sasidharan R, et al. Scl represses cardiomyogenesis in prospective hemogenic endothelium and endocardium. *Cell*. 2012;150(3):590-605.

35. Org T, Duan D, Ferrari R, et al. Scl binds to primed enhancers in mesoderm to regulate hematopoietic and cardiac fate divergence. *EMBO J*. 2015;34(6):759-777.

36. Bussmann J, Bakkers J, Schulte-Merker S. Early endocardial morphogenesis requires Scl/Tal1. *PLoS Genet*. 2007;3(8):e140.

37. Schumacher JA, Bloomekatz J, Garavito-Aguilar ZV, Yelon D. tal1 Regulates the formation of intercellular junctions and the maintenance of identity in the endocardium. *Dev Biol*. 2013;383(2):214-226.

38. Milgrom-Hoffman M, Harrelson Z, Ferrara N, Zelzer E, Evans SM, Tzahor E. The heart endocardium is derived from vascular endothelial progenitors. *Development*. 2011;138(21):4777-4787.

39. Misfeldt AM, Boyle SC, Tompkins KL, Bautch VL, Labosky PA, Baldwin HS. Endocardial cells are a distinct endothelial lineage derived from Flk1+ multipotent cardiovascular progenitors. *Dev Biol*. 2009;333(1):78-89.

40. Scialdone A, Tanaka Y, Jawaid W, et al. Resolving early mesoderm diversification through single-cell expression profiling. *Nature*. 2016;535(7611):289-293.

41. Lee D, Park C, Lee H, et al. ER71 acts downstream of BMP, Notch, and Wnt signaling in blood and vessel progenitor specification. *Cell Stem Cell*. 2008;2(5):497-507.

42. Kataoka H, Hayashi M, Nakagawa R, et al. Etv2/ER71 induces vascular mesoderm from Flk1+PDGFRalpha+ primitive mesoderm. *Blood*. 2011;118(26):6975-6986.

43. Rasmussen TL, Kweon J, Diekmann MA, et al. ER71 directs mesodermal fate decisions during embryogenesis. *Development*. 2011;138(21):4801-4812.

44. Wareing S, Mazan A, Pearson S, Gottgens B, Lacaud G, Kouskoff V. The Flk1-Cre-mediated deletion of ETV2 defines its narrow temporal requirement during embryonic hematopoietic development. *Stem Cells*. 2012;30(7):1521-1531.

45. Liu F, Kang I, Park C, et al. ER71 specifies Flk-1+ hemangiogenic mesoderm by inhibiting cardiac mesoderm and Wnt signaling. *Blood*. 2012;119(14):3295-3305.

46. Singh BN, Kawakami Y, Akiyama R, et al. The Etv2-miR-130a Network Regulates Mesodermal Specification. *Cell Rep*. 2015;13(5):915-923.

47. Fujiwara Y, Chang AN, Williams AM, Orkin SH. Functional overlap of GATA-1 and GATA-2 in primitive hematopoietic development. *Blood*. 2004;103(2):583-585.

48. Hart A, Melet F, Grossfeld P, et al. Fli-1 is required for murine vascular and megakaryocytic development and is hemizygously deleted in patients with thrombocytopenia. *Immunity*. 2000;13(2):167-177.

49. Pimanda JE, Ottersbach K, Knezevic K, et al. Gata2, Fli1, and Scl form a recursively wired gene-regulatory circuit during early hematopoietic development. *Proc Natl Acad Sci U S A*. 2007;104(45):17692-17697.

50. Faucon PC, Pardee K, Kumar RM, Li H, Loh YH, Wang X. Gene networks of fully connected triads with complete auto-activation enable multistability and stepwise stochastic transitions. *PLoS One*. 2014;9(7):e102873.

51. Ciau-Uitz A, Pinheiro P, Kirmizitas A, Zuo J, Patient R. VEGFA-dependent and -independent pathways synergise to drive Scl expression and initiate programming of the blood stem cell lineage in Xenopus. *Development*. 2013;140(12):2632-2642.

52. Ciau-Uitz A, Walmsley M, Patient R. Distinct origins of adult and embryonic blood in Xenopus. *Cell*. 2000;102(6):787-796.

53. Goode DK, Obier N, Vijayabaskar MS, et al. Dynamic Gene Regulatory Networks Drive Hematopoietic Specification and Differentiation. *Dev Cell*. 2016;36(5):572-587.

54. Lichtinger M, Ingram R, Hannah R, et al. RUNX1 reshapes the epigenetic landscape at the onset of haematopoiesis. *EMBO J*. 2012;31(22):4318-4333.

55. Green AR, Lints T, Visvader J, Harvey R, Begley CG. SCL is coexpressed with GATA-1 in hemopoietic cells but is also expressed in developing brain. *Oncogene*. 1992;6:475-479.

56. Mouthon MA, Bernard O, Mitjavila MT, Romeo PH, Vainchenker W, Mathieu-Mahul D. Expression of tal-1 and GATA-binding proteins during human hematopoiesis. *Blood*. 1993;81(3):647-655.

57. de Graaf CA, Choi J, Baldwin TM, et al. Haemopedia: An Expression Atlas of Murine Hematopoietic Cells. *Stem Cell Reports*. 2016;7(3):571-582.

58. Mikkola HK, Klintman J, Yang H, et al. Haematopoietic stem cells retain long-term repopulating activity and multipotency in the absence of stem-cell leukaemia SCL/tal-1 gene. *Nature*. 2003;421(6922):547-551.

59. Curtis DJ, Hall MA, Van Stekelenburg LJ, Robb L, Jane SM, Begley CG. SCL is required for normal function of short-term repopulating hematopoietic stem cells. *Blood*. 2004;103(9):3342-3348.

60. Brunet de la Grange P, Armstrong F, Duval V, et al. Low SCL/TAL1 expression reveals its major role in adult hematopoietic myeloid progenitors and stem cells. *Blood*. 2006;108:2998-3004.

61. Giroux S, Kaushik AL, Capron C, et al. lyl-1 and tal-1/scl, two genes encoding closely related bHLH transcription factors, display highly overlapping expression patterns during cardiovascular and hematopoietic ontogeny. *Gene Expr Patterns*. 2007;7(3):215-226.

62. Porcher C, Liao EC, Fujiwara Y, Zon LI, Orkin SH. Specification of hematopoietic and vascular development by the bHLH transcription factor SCL without direct DNA binding. *Development*. 1999;126(20):4603-4615.

63. Souroullas GP, Salmon JM, Sablitzky F, Curtis DJ, Goodell MA. Adult hematopoietic stem and progenitor cells require either Lyl1 or Scl for survival. *Cell Stem Cell*. 2009;4(2):180-186.

64. Lacombe J, Herblot S, Rojas-Sutterlin S, et al. Scl regulates the quiescence and the long-term competence of hematopoietic stem cells. *Blood*. 2010;115(4):792-803.

65. Lacombe J, Krosl G, Tremblay M, et al. Genetic interaction between Kit and Scl. *Blood*. 2013;122(7):1150-1161.

66. Benyoucef A, Calvo J, Renou L, et al. The SCL/TAL1 Transcription Factor Represses the Stress Protein DDiT4/REDD1 in Human Hematopoietic Stem/Progenitor Cells. *Stem Cells*. 2015;33(7):2268-2279.

67. Lazrak M, Deleuze V, Noel D, et al. The bHLH TAL-1/SCL regulates endothelial cell migration and morphogenesis. *J Cell Sci*. 2004;117(Pt 7):1161-1171.

68. Palii CG, Vulesevic B, Fraineau S, et al. Trichostatin A enhances vascular repair by injected human endothelial progenitors through increasing the expression of TAL1-dependent genes. *Cell Stem Cell*. 2014;14(5):644-657.

69. Hall MA, Curtis DJ, Metcalf D, et al. The critical regulator of embryonic hematopoiesis, SCL, is vital in the adult for megakaryopoiesis, erythropoiesis, and lineage choice in CFU-S12. *Proc Natl Acad Sci U S A*. 2003;100(3):992-997.

70. Salmon JM, Slater NJ, Hall MA, et al. Aberrant mast-cell differentiation in mice lacking the stem-cell leukemia gene. *Blood*. 2007;110:3573-3581.

71. Chagraoui H, Kassouf M, Banerjee S, et al. SCL-mediated regulation of the cell-cycle regulator p21 is critical for murine megakaryopoiesis. *Blood*. 2011;118(3):723-735.

72. Dey S, Curtis DJ, Jane SM, Brandt SJ. The TAL1/SCL transcription factor regulates cell cycle progression and proliferation in differentiating murine bone marrow monocyte precursors. *Mol Cell Biol*. 2010;30(9):2181-2192.

73. Goardon N, Lambert JA, Rodriguez P, et al. ETO2 coordinates cellular proliferation and differentiation during erythropoiesis. *Embo J*. 2006;25(2):357-366.

74. Zeuner A, Eramo A, Testa U, et al. Control of erythroid cell production via caspase-mediated cleavage of transcription factor SCL/Tal-1. *Cell Death Differ*. 2003;10(8):905-913.

75. Kassouf MT, Hughes JR, Taylor S, et al. Genome-wide identification of TAL1's functional targets: insights into its mechanisms of action in primary erythroid cells. *Genome Res*. 2010;20(8):1064-1083.

76. Wu W, Morrissey CS, Keller CA, et al. Dynamic shifts in occupancy by TAL1 are guided by GATA factors and drive large-scale reprogramming of gene expression during hematopoiesis. *Genome Res*. 2014;24(12):1945-1962.

77. Massari ME, Murre C. Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. *Mol Cell Biol*. 2000;20(2):429-440.

78. Tipping AJ, Pina C, Castor A, et al. High GATA-2 expression inhibits human hematopoietic stem and progenitor cell function by effects on cell cycle. *Blood*. 2009;113(12):2661-2672.

79. Fujiwara T, O'Geen H, Keles S, et al. Discovering hematopoietic mechanisms through genome-wide analysis of GATA factor chromatin occupancy. *Mol Cell*. 2009;36(4):667-681.

80. Wadman IA, Osada H, Grutz GG, et al. The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins. *EMBO J*. 1997;16(11):3145-3157.

81. Palii CG, Perez-Iratxeta C, Yao Z, et al. Differential genomic targeting of the transcription factor TAL1 in alternate haematopoietic lineages. *EMBO J*. 2011;30(3):494-509.

82. El Omari K, Hoosdally SJ, Tuladhar K, et al. Structural basis for LMO2-driven recruitment of the SCL:E47bHLH heterodimer to hematopoietic-specific transcriptional targets. *Cell Rep*. 2013;4(1):135-147.

83. Soler E, Andrieu-Soler C, de Boer E, et al. The genome-wide dynamics of the binding of Ldb1 complexes during erythroid differentiation. *Genes Dev*. 2010;24(3):277-289.

84. El Omari K, Hoosdally SJ, Tuladhar K, et al. Structure of the leukemia oncogene LMO2: implications for the assembly of a hematopoietic transcription factor complex. *Blood*. 2011;117(7):2146-2156.

85. Kassouf MT, Chagraoui H, Vyas P, Porcher C. Differential use of SCL/TAL-1 DNA-binding domain in developmental hematopoiesis. *Blood*. 2008;112(4):1056-1067.

86. Zhang J, Kalkum M, Yamamura S, Chait BT, Roeder RG. E protein silencing by the leukemogenic AML1-ETO fusion protein. *Science*. 2004;305(5688):1286-1289.

87. Krivega I, Dale RK, Dean A. Role of LDB1 in the transition from chromatin looping to transcription activation. *Genes Dev*. 2014;28(12):1278-1290.

88. Deng W, Lee J, Wang H, et al. Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. *Cell*. 2012;149(6):1233-1244.

89. Pimkin M, Kossenkov AV, Mishra T, et al. Divergent functions of hematopoietic transcription factors in lineage priming and differentiation during erythro-megakaryopoiesis. *Genome Res*. 2014;24(12):1932-1944.

90. Wilson NK, Foster SD, Wang X, et al. Combinatorial transcriptional control in blood stem/progenitor cells: genome-wide analysis of ten major transcriptional regulators. *Cell Stem Cell*. 2010;7(4):532-544.

91. Wilson NK, Schoenfelder S, Hannah R, et al. Integrated genome-scale analysis of the transcriptional regulatory landscape in a blood stem/progenitor cell model. *Blood*. 2016;127(13):e12-23.

92. Batta K, Florkowska M, Kouskoff V, Lacaud G. Direct reprogramming of murine fibroblasts to hematopoietic progenitor cells. *Cell Rep*. 2014;9(5):1871-1884.

93. Capellera-Garcia S, Pulecio J, Dhulipala K, et al. Defining the Minimal Factors Required for Erythropoiesis through Direct Lineage Conversion. *Cell Rep*. 2016;15(11):2550-2562.

94. Elcheva I, Brok-Volchanskaya V, Kumar A, et al. Direct induction of haematoendothelial programs in human pluripotent stem cells by transcriptional regulators. *Nat Commun*. 2014;5:4372.

95. Iwafuchi-Doi M, Zaret KS. Pioneer transcription factors in cell reprogramming. *Genes Dev*. 2014;28(24):2679-2692.

96. Schuh AH, Tipping AJ, Clark AJ, et al. ETO-2 associates with SCL in erythroid cells and megakaryocytes and provides repressor functions in erythropoiesis. *Mol Cell Biol*. 2005;25(23):10235-10250.

97. Stadhouders R, Cico A, Stephen T, et al. Control of developmentally primed erythroid genes by combinatorial co-repressor actions. *Nat Commun*. 2015;6:8893.

98. Tripic T, Deng W, Cheng Y, et al. SCL and associated proteins distinguish active from repressive GATA transcription factor complexes. *Blood*. 2009;113(10):2191-2201.

99. Juarez MA, Su F, Chun S, Kiel MJ, Lyons SE. Distinct roles for SCL in erythroid specification and maturation in zebrafish. *J Biol Chem*. 2005;280:41636-41644.

100. Qian F, Zhen F, Xu J, Huang M, Li W, Wen Z. Distinct functions for different scl isoforms in zebrafish primitive and definitive hematopoiesis. *PLoS Biol*. 2007;5(5):e132.

101. Herblot S, Steff AM, Hugo P, Aplan PD, Hoang T. SCL and LMO1 alter thymocyte differentiation: inhibition of E2A-HEB function and pre-T alpha chain expression. *Nat Immunol*. 2000;1(2):138-144.

102. Correia NC, Arcangeli ML, Pflumio F, Barata JT. Stem Cell Leukemia: how a TALented actor can go awry on the hematopoietic stage. *Leukemia*. 2016;30(10):1968-1978.

103. Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. *Cancer Cell*. 2002;1:75-87.

104. Ferrando AA, Herblot S, Palomero T, et al. Biallelic transcriptional activation of oncogenic transcription factors in T-cell acute lymphoblastic leukemia. *Blood*. 2004;103(5):1909-1911.

105. Aplan PD, Jones CA, Chervinsky DS, et al. An *scl* gene product lacking the transactivation domain induces bony abnormalities and cooperates with LMO1 to generate T-cell malignancies in transgenic mice. *EMBO J*. 1997;16:2408-2419.

106. Larson RC, Lavenir I, Larson TA, et al. Protein dimerization between Lmo2 (Rbtn2) and Tal1 alters thymocyte development and potentiates T cell tumorigenesis in transgenic mice. *EMBO J*. 1996;15:1021-1027.

107. Gerby B, Tremblay CS, Tremblay M, et al. SCL, LMO1 and Notch1 reprogram thymocytes into self-renewing cells. *PLoS Genet*. 2014;10(12):e1004768.

108. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science*. 2004;306(5694):269-271.

109. Tremblay M, Tremblay CS, Herblot S, et al. Modeling T-cell acute lymphoblastic leukemia induced by the SCL and LMO1 oncogenes. *Genes Dev*. 2010;24(11):1093-1105.

110. Murre C. Helix-loop-helix proteins and lymphocyte development. *Nat Immunol*. 2005;6(11):1079-1086.

111. O'Neil J, Shank J, Cusson N, Murre C, Kelliher M. TAL1/SCL induces leukemia by inhibiting the transcriptional activity of E47/HEB. *Cancer Cell*. 2004;5(6):587-596.

112. Kusy S, Gerby B, Goardon N, et al. NKX3.1 is a direct TAL1 target gene that mediates proliferation of TAL1-expressing human T cell acute lymphoblastic leukemia. *J Exp Med*. 2010;207(10):2141-2156.

113. Palomero T, Odom DT, O'Neil J, et al. Transcriptional regulatory networks downstream of TAL1/SCL in T-cell acute lymphoblastic leukemia. *Blood*. 2006;108(3):986-992.

114. Sanda T, Lawton LN, Barrasa MI, et al. Core Transcriptional Regulatory Circuit Controlled by the TAL1 Complex in Human T Cell Acute Lymphoblastic Leukemia. *Cancer Cell*. 2012;22(2):209-221.

115. O'Neil J, Billa M, Oikemus S, Kelliher M. The DNA binding activity of TAL-1 is not required to induce leukemia/lymphoma in mice. *Oncogene*. 2001;20(29):3897-3905.

116. Mansour MR, Abraham BJ, Anders L, et al. Oncogene regulation. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. *Science*. 2014;346(6215):1373-1377.

117. Mansour MR, Sanda T, Lawton LN, et al. The TAL1 complex targets the FBXW7 tumor suppressor by activating miR-223 in human T cell acute lymphoblastic leukemia. *J Exp Med*. 2013;210(8):1545-1557.

118. Rowe JM. Optimal management of adults with ALL. *Br J Haematol*. 2009;144(4):468-483.

119. Kwiatkowski N, Zhang T, Rahl PB, et al. Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. *Nature*. 2014;511(7511):616-620.

120. Loven J, Hoke HA, Lin CY, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell*. 2013;153(2):320-334.

121. Benyoucef A, Palii CG, Wang C, et al. UTX inhibition as selective epigenetic therapy against TAL1-driven T-cell acute lymphoblastic leukemia. *Genes Dev*. 2016;30(5):508-521.

122. Correia NC, Durinck K, Leite AP, et al. Novel TAL1 targets beyond protein-coding genes: identification of TAL1-regulated microRNAs in T-cell acute lymphoblastic leukemia. *Leukemia*. 2013;27(7):1603-1606.

123. Ferreira LG, Oliva G, Andricopulo AD. Protein-protein interaction inhibitors: advances in anticancer drug design. *Expert Opin Drug Discov*. 2016;11(10):957-968.

124. Appert A, Nam CH, Lobato N, et al. Targeting LMO2 with a peptide aptamer establishes a necessary function in overt T-cell neoplasia. *Cancer Res*. 2009;69(11):4784-4790.

125. Tanaka T, Sewell H, Waters S, Phillips SE, Rabbitts TH. Single domain intracellular antibodies from diverse libraries: emphasizing dual functions of LMO2 protein interactions using a single VH domain. *J Biol Chem*. 2011;286(5):3707-3716.

126. Adolfsson J, Mansson R, Buza-Vidas N, et al. Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell*. 2005;121(2):295-306.

Figure legends

Figure 1. SCL is required for development of the three hematopoietic waves at specification and maturation stages

Specification, development and maturation stages of hematopoietic Waves 1, 2 and 3 during mouse development are depicted.[7] A common origin for Waves 1 and 2 in the early epiblast is shown, but this is still the subject of debate (see Text and Box 2). The yolk sac (YS) and aorta-gonad-mesonephros (AGM) waves are shown as independently specified, as established in *Xenopus* embryos through elegant lineage tracing studies.[52] However, the origin of the angioblasts giving rise to Wave 3 is not yet established in higher vertebrates. The SCL-dependent cellular transitions are represented by bold arrows. The hematopoietic lineages depending on SCL activity for terminal maturation in the fetal liver are in bold font. The main features of SCL's activities in specification and maturation stages are summarized below the diagram.

PS: primitive streak; AGM: aorta-gonad-mesonephros region; EryP: primitive erythroid cells; EryD: definitive erythroid cells. Mk: megakaryocyte; EHT: endothelial-to-hematopoietic transition. E5.5-E12.5: embryonic days E5.5-E12.5.

Figure 2. *Scl* mRNA domains of expression mark distinct branches of the hematopoietic tree

Schematic representation of the successive stages leading to hematopoietic differentiation from LT-HSCs to mature blood lineages.[126] The branches exhibiting expression of *Scl* mRNA are shaded in grey, darker grey representing higher expression levels. During T-cell differentiation, SCL is down-regulated at the DN3 stage. The stage of T-cell development first sensitive to SCL:LMO1/2 ectopic expression (red zigzag) and the subsequent block in differentiation (red bar) in T-ALL mouse models are shown. In human patients, maturation arrest occurs at the late cortical DP CD3^{high} stage (not shown).[103]

LT-HSC: long-term hematopoietic stem cell; ST-HSC, short-term HSC; MPP: multipotent progenitor; CMP: common myeloid progenitor; MEP: megakaryocytic/erythroid progenitor; GMP: granulocyte/macrophage progenitor; LMPP: lymphoid/myeloid-primed progenitor; CLP: common lymphoid progenitor; ETP: early thymic progenitor; DN1-4: CD4$^-$CD8$^-$ double negative populations, stages 1 to 4; DP: CD4$^+$CD8$^+$ double positive population.

Figure 3. SCL-containing multiprotein complexes in hematopoiesis and leukemogenesis

The quaternary complex (SCL:E-protein:LMO2:LDB1) interacts with members of the GATA, ETS and RUNX families throughout hematopoietic development and in leukemogenesis. The ”kinked” SCL molecule represents a DNA-binding independent form of SCL. The main functions of SCL in each compartment are summarized under each diagram, as detailed throughout the Text.

Figure 1

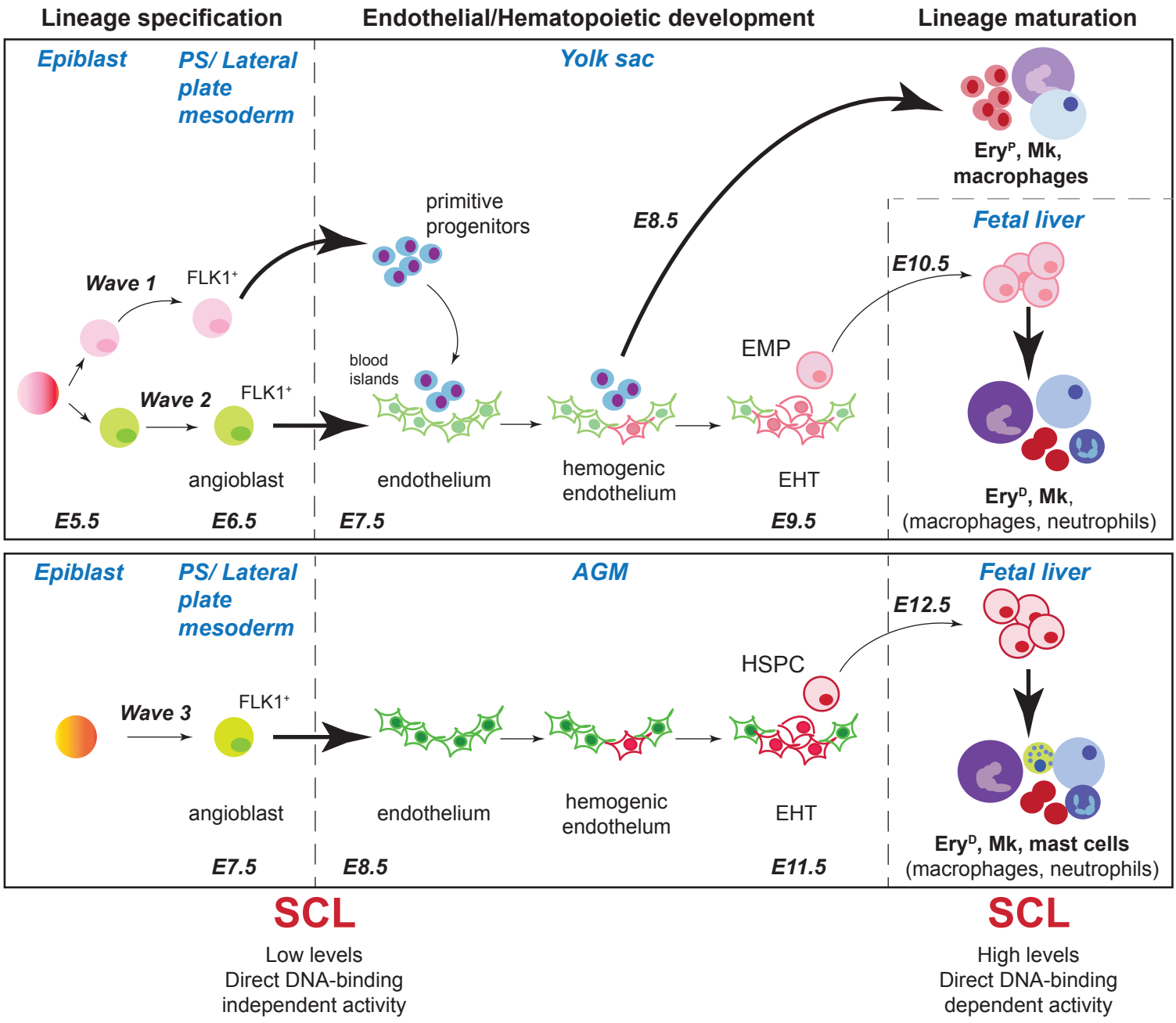

Lineage specification
Endothelial/Hematopoietic development
Lineage maturation
Epiblast
PS/ Lateral plate mesoderm
Yolk sac
Fetal liver
Wave 1
FLK1+
Wave 2
FLK1+
primitive progenitors
blood islands
E8.5
EMP
E10.5
Ery^P, Mk, macrophages
angioblast
endothelium
hemogenic endothelium
EHT
Ery^D, Mk, (macrophages, neutrophils)
E5.5
E6.5
E7.5
E9.5
Epiblast
PS/ Lateral plate mesoderm
AGM
Fetal liver
Wave 3
FLK1+
HSPC
E12.5
angioblast
endothelium
hemogenic endothelium
EHT
Ery^D, Mk, mast cells (macrophages, neutrophils)
E7.5
E8.5
E11.5
SCL
Low levels
Direct DNA-binding
independent activity
SCL
High levels
Direct DNA-binding
dependent activity

Figure 2

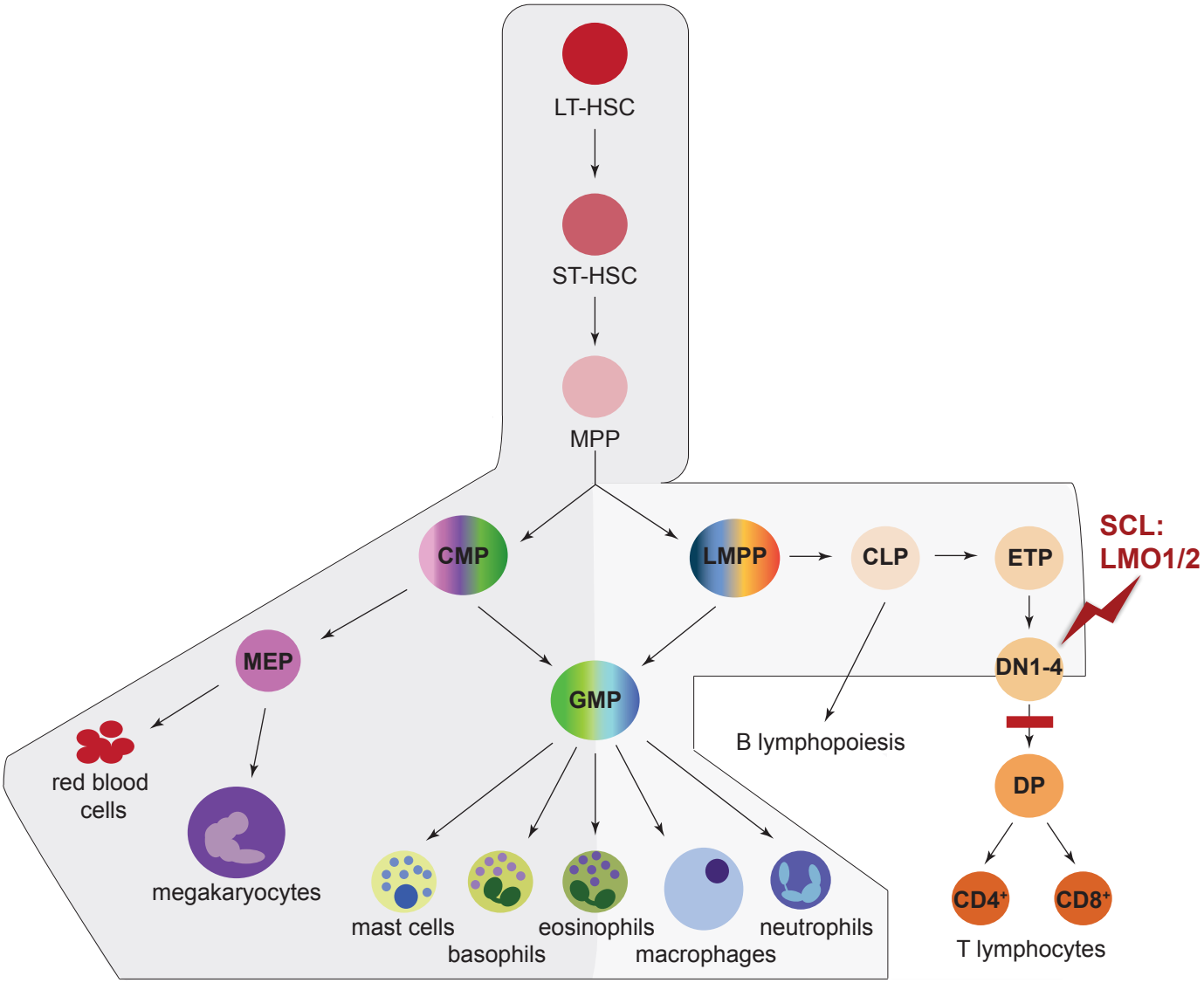
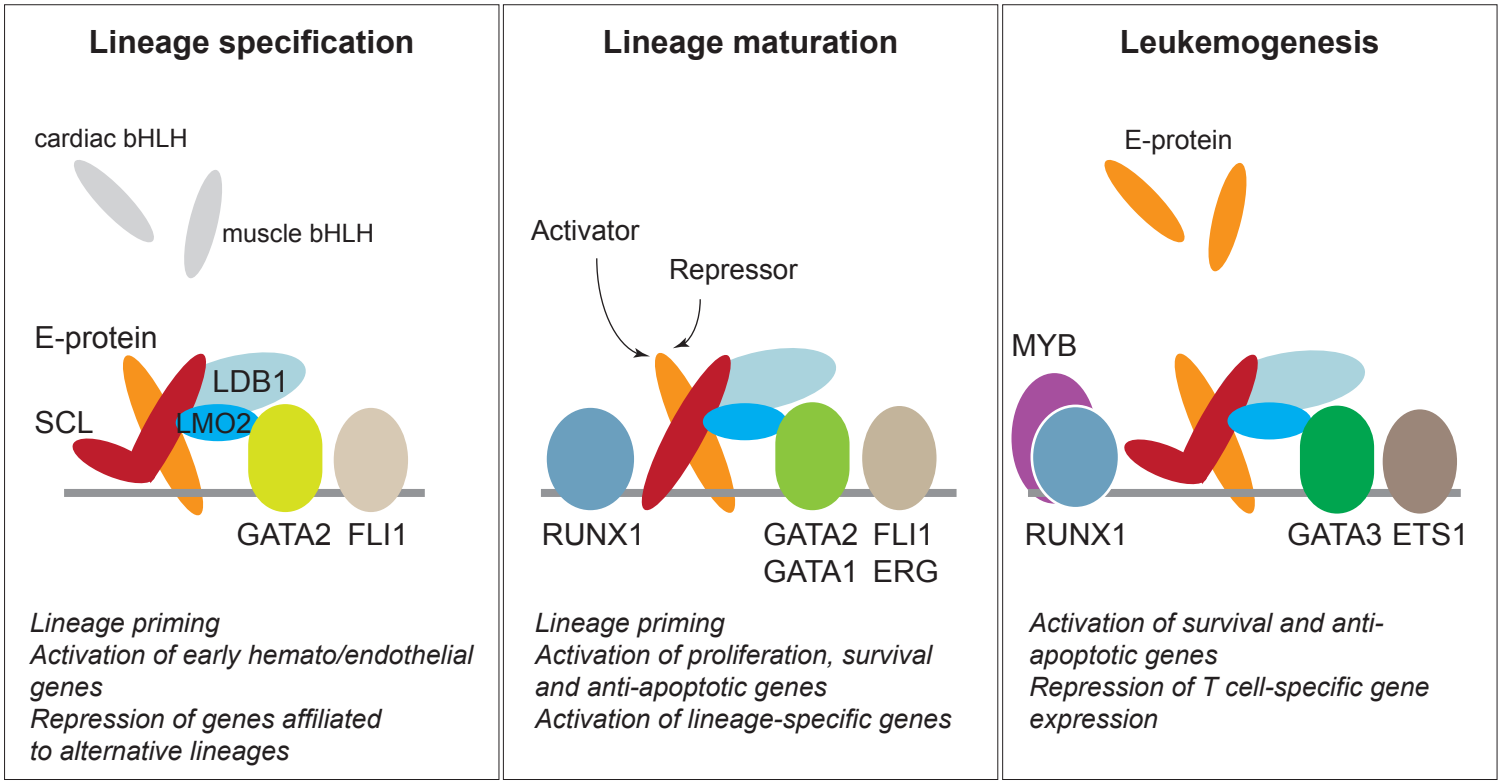

LT-HSC
ST-HSC
MPP
CMP
LMPP
CLP
ETP
SCL:
LMO1/2
MEP
GMP
DN1-4
B lymphopoiesis
DP
red blood
cells
megakaryocytes
mast cells
basophils
eosinophils
macrophages
neutrophils
CD4+
CD8+
T lymphocytes

Figure 3


Lineage specification
cardiac bHLH
muscle bHLH
E-protein
LDB1
SCL
LMO2
GATA2 FLI1
Lineage priming
Activation of early hemato/endothelial genes
Repression of genes affiliated to alternative lineages
Lineage maturation
Activator
Repressor
RUNX1
GATA2 FLI1
GATA1 ERG
Lineage priming
Activation of proliferation, survival and anti-apoptotic genes
Activation of lineage-specific genes
Leukemogenesis
E-protein
MYB
RUNX1
GATA3 ETS1
Activation of survival and anti-apoptotic genes
Repression of T cell-specific gene expression