

# Transcription factors in hematopoiesis

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The advent of gene targeting in the mouse has led to rapid advances in the identification of factors controlling gene expression that are essential for normal hematopoietic development. Recent work has also uncovered roles for some of these factors in leukemogenesis and in the global regulation of chromatin structure.

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## Abbreviations

<b>bHLH</b>	basic helix-loop-helix
<b>C/EBP</b>	CCAAT/enhancer binding protein
<b>DN</b>	double negative
<b>EBF</b>	early B-cell factor
<b>ES</b>	embryonic stem
<b>FTOC</b>	fetal thymic organ culture
<b>Hes1</b>	<i>Hairy/Enhancer of split 1</i>
<b>HSC</b>	hematopoietic stem cell
<b>IL</b>	interleukin
<b>NK</b>	natural killer
<b>T-ALL</b>	T-cell acute cell lymphoblastic leukemia
<b>TCR</b>	T-cell receptor

## Introduction

The nineties have been a very good decade for research in hematopoietic development — particularly with respect to the identification of transcription factors that play critical roles in the development of the various lineages that descend from the hematopoietic stem cell (HSC). Clearly, the critical element that has accelerated progress in these areas has been the ability to manipulate the mouse genome via homologous recombination into embryonic stem (ES) cells. The success of this approach in identifying transcription factors with essential roles in hematopoiesis has expanded our knowledge to the extent that a comprehensive review is beyond the scope of this article. Instead, we discuss a few of the areas in which recent progress has been made in further defining the roles of several transcription factors in hematopoiesis.

## Studies in lineage commitment

The ability of gene targeting to reveal requirements for a protein in lineage commitment is often limited by a lack of phenotypic markers for the earliest committed progenitor cells. One approach that has been used to address the role of specific transcription factors in lineage commitment is through the enforced expression of these factors in cells that have at least some capability to differentiate. One such system that has been described employs of primary

transformed multipotent progenitors generated by transducing chicken blastoderm cells with the E26 leukemia virus [1]. Recently, the effects of transduction of an inducible form of the Ets family transcription factor PU.1 into these cultures was examined [2•]. Whereas long-term expression of PU.1 resulted in myeloid commitment, short-term induction of PU.1 activity led to the formation of immature eosinophils. These data were consistent with gene-targeting experiments showing PU.1 to be required for myeloid development and also suggested the existence of a bilineage intermediate capable of progressing down either myeloid or eosinophil developmental pathways [3–5]. In parallel studies involving the transduction of  $\alpha$  and  $\beta$  isoforms of CCAAT/enhancer binding protein (C/EBP), it was demonstrated that these transcription factors could induce the commitment and maturation of eosinophils from multi-potential progenitors, although the  $\beta$  isoform could also induce myeloid differentiation [6•].

Commitment to T- and NK-cell lineages has been examined by retroviral transduction into various purified human multi-potential populations followed by fetal thymic organ culture (FTOC). It was demonstrated that forced expression of Id3 — a dominant negative inhibitor of bHLH transcription factors such as those encoded by E2A and HEB — in multipotential progenitors blocks T-cell development and results in the increased production of NK cells [7]. Subsequently, analogous experiments were reported showing that Id3 also inhibits committed T-cell precursors from developing into TCR $\alpha\beta$  T-cells, but does not affect TCR $\gamma\delta$  T-cell development [8•]. These data are essentially consistent with the phenotypes observed in mice deficient for E2A and HEB, and suggest that bHLH activity may determine thymocyte developmental fates at multiple stages of differentiation. It should be noted, however, that E2A-deficient mice have been reported to have reduced numbers of adult, though not fetal,  $\gamma\delta$  T-cells [9•]. An additional study employing transduced human thymocytes in FTOC demonstrated that blocking of the IL-7 receptor/STAT 5 signaling pathway in pre-T cells also inhibited differentiation [10•].

Another enforced expression approach has been used to distinguish between the roles of E2A and early B-cell factor (EBF) in the differentiation of B-cells. Targeted mutations of the genes encoding these transcription factors have demonstrated that they are both required for progression past a very early stage in B-cell development [11–13] but the reported phenotypes of the knockout mice for these genes failed to indicate clearly whether or not one of these factors acts upstream of the other. This issue was addressed by examining the effects of enforced expression of E12 — one of the two major products of the *E2A* gene — and EBF on the phenotype of a 70Z/3 macrophage

line. It was found that E12 acted largely to convert this line to a pre-B phenotype, as determined by the expression of a number of markers, including EBF [14\*\*]. Enforced EBF expression, however, activated only a subset of the pre-B specific genes induced by E12, and did not result in the induction of E2A. These data suggest that E2A functions upstream of EBF in B-cell development.

### Additional transcription factors controlling T- and NK-cell development

Embryonic lethality is a frequent consequence of the inactivation of transcription factors that are important in hematopoiesis, and often precludes a direct examination of their effect on relatively late developmental stages. Such studies, however, can often be performed by generating chimeric mice whereby ES cells with lethal mutations are implanted into wild-type or Rag1- or 2-deficient blastocysts. The contribution of the ES cells to various lineages can be determined by using allotypic differences to distinguish mutant from wild-type-derived tissue. Rag-deficient blastocysts are frequently used in these studies to block antigen receptor rearrangement, and hence lymphocyte development, in the host tissue. Two reports employing such an approach have recently been published that revealed roles for the bHLH gene *Hes1* (*Hairy/Enhancer of split 1*) and the *c-myb* proto-oncogene in T-lymphocyte development. *Hes1* is a target of the Notch pathway that can function as a transcriptional repressor, and is known to be required for proper nervous system development [15–17]. *Hes1*-deficient thymocytes were unable to progress through the CD4/CD8 double negative (DN) stage of development, during which TCR rearrangements are initiated [18\*\*]. Another recent report demonstrated that *c-myb*, in addition to being essential for fetal erythropoiesis, was also needed for T-cell development [19,20\*\*]. *c-Myb*-deficient thymocytes were arrested at a very early developmental stage, prior to the expression of either the CD44 or CD25 markers that are frequently used to characterize DN thymocytes. Furthermore, it was determined that *c-myb*<sup>-/-</sup> ES cells were also unable to contribute to either the B-cell or macrophage compartments.

Two new mutations affecting NK-cell development have also been reported recently. *Ets-1*-deficient mice were found to have greatly reduced numbers of NK-cells and no detectable NK response either *in vitro* or *in vivo* [21\*\*]. Mice lacking the bHLH-inhibitor protein *Id2* were also found to have reductions in NK-cell number and *in vitro* activity, which is consistent with the findings described above that bHLH activity controls the T/NK lineage decision [7,22\*].

### SCL and LMO proteins in normal and aberrant development

The *SCL/Tal1* gene (*SCL*) encodes a type II bHLH protein that can bind E box DNA sequences by forming heterodimers with type I bHLH proteins encoded by the *E2A*, *HEB* or *E2-2* genes [23,24]. Targeted mutations in *SCL*

revealed that this gene is essential for yolk-sac erythropoiesis, and *SCL* null ES cells fail to contribute to any hematopoietic lineages in chimeric mice [25]. Furthermore, studies with erythroleukemia lines support a role for *SCL* in erythroid development [26]. Recent data suggest that *SCL* is required for vascular differentiation as well. One such line of evidence comes from an attempt to correct the *SCL* null phenotype with a transgenic construct consisting of an *SCL* cDNA under control of a promoter that allows for expression in HSCs. Although the transgene was found to rescue yolk-sac hematopoiesis, the resulting embryos were not viable due to defects in blood vessel formation [27\*\*]. Thus *SCL* has dual roles in hematopoiesis and angiogenesis. In addition, studies in zebrafish have provided evidence for a role for *SCL* in the development of a presumptive hemangioblast. One study demonstrated that overexpression of *SCL* in zebrafish embryos resulted in overproduction of both hematopoietic and endothelial precursors, whereas another group found that *SCL* acts downstream of the zebrafish gene *cloche* to specify both hematopoietic and vascular differentiation [28\*\*,29\*\*].

LMO2/RBTN-2 (*LMO2*), a member of the LIM-only domain family, has been shown to interact with *SCL*, and *LMO2*<sup>-/-</sup> embryos display hematopoietic defects similar to that of embryos with null mutations in *SCL* [30,31\*]. Recently, the complexes containing *SCL* and *LMO2* have been characterized in greater detail. *SCL* and *LMO2* have been shown in erythroid cells to associate with *E2A*, *GATA-1* and the LIM-binding protein *Ldb1/NL1* in a complex that binds a DNA sequence consisting of E box and *GATA* sites separated by ~9 bp [32]. Recently, similar binding sites were immunoprecipitated from fragmented murine erythroleukemia chromatin using anti-*SCL* antibodies [33\*]. One such site was found to be located within a gene whose expression correlated with that of *SCL* and *GATA-1* in both transformed cell lines and an *in vitro* model system of erythroid differentiation.

There have been a number of studies exploring the possibility of a relationship between the roles of *SCL* and *LMO* proteins in normal hematopoiesis and in T-cell acute lymphoblastic leukemia (T-ALL). A large fraction of T-ALL isolates are characterized by aberrantly high expression of *SCL* and either *LMO1* or 2 [34,35]. *SCL* and *LMO* proteins have also been shown to collaborate to induce thymic lymphomas in mouse transgenic models [36]. A binding-site selection protocol was used to demonstrate that *SCL* and *LMO2* present in extracts from *LMO2* transgenic T-lymphomas could interact to form DNA-binding complexes [37\*]. Like the erythroid-specific complex described above, the complex found in T-lymphomas contained the *E2A*-encoded *E47* protein along with *Ldb1/NL1*. It differed from the previously described complex, however, in that it did not include a *GATA* family member and assembled on a DNA binding site that consisted of two E boxes. Interestingly, this site was similar to one in the promoter of the *c-kit* gene, which was found by

Krosil *et al.* [38\*\*] to be positively regulated by SCL in two hematopoietic cell lines. On the other hand, a group that identified retinaldehyde dehydrogenase 2 (RALDH2) as a target of SCL and LMO in human T-ALLs characterized a promoter in the RALDH2 gene that contained a GATA-3 binding site critical for SCL and LMO-dependent expression in T-ALLs [39\*]. Although an E box was found adjacent to this GATA site, SCL binding to DNA was found to be dispensable for promoter activity. Taken together, it appears that there is considerable heterogeneity in the DNA-binding complexes that are formed by SCL and LMO proteins. Nevertheless, the reports of SCL/LMO complexes in T-lymphomas strengthen the contention that these proteins collaborate to induce oncogenic transformation, presumably by the inappropriate activation of critical target genes.

There is an alternative way at looking at the contribution of SCL and LMO proteins to T-ALL, however. SCL has long been known to inhibit E2A activity, as SCL/E2A heterodimers have been shown to have greatly reduced transcriptional activity relative to that of E2A homodimers [40,41]. Mice with targeted mutations in the *E2A* locus are also highly susceptible to T lymphoma [42,43]. Furthermore, both E2A-deficient and SCL/LMO double-transgenic thymocytes exhibit partial blocks during the DN stage of development prior to the onset of lymphoma, although the defects in these two genetic backgrounds differ in several respects [36]. Recently it has been shown that E2A activity can act to kill either the Jurkat T-ALL line — which expresses high levels of SCL and LMO1 — or murine T-lymphoma lines derived from E2A-deficient mice [44\*,45\*]. In addition, E2A activity was also found to inhibit cell-cycle progression in Jurkat cells [44\*]. These data strongly imply that E2A acts a tumor suppressor in thymocytes, and further suggest a relationship between lymphomas induced by either ectopic expression of SCL and LMO or a deficiency in E2A activity. However, the question as to whether SCL and LMO proteins act to induce lymphoma through an inhibition of E2A activity has yet to be answered definitively.

### The Ikaros family in chromatin modulation

*Ikaros* was originally identified as a gene encoding multiple forms of a zinc finger domain protein with potential for both transcriptional activation and repression [46]. Mice deficient in Ikaros activity exhibited a number of severe defects in the development of lymphoid lineage cells [46]. Another member of the *Ikaros* family, *Aiolos*, was recently shown to be required for regulating B-cell activation [47\*\*]. It now appears, however, that the *Ikaros* family of factors may not function as 'conventional' transcription factors — that is, by activating or repressing the expression of a select group of target genes. This was first implied by the finding that Ikaros complexes were found to be associated with centromeric heterochromatin [48]. Recently it was demonstrated that Ikaros formed toroidal structures that colocalized with DNA replication centers in activated

T-cells, and that this property may be important for proper chromosome propagation in these cells as well as for setting general thresholds of activation [49\*\*]. Another report showed that Ikaros and Aiolos were associated with large nuclear complexes that had histone deacetylase and chromatin remodeling activity [50\*]. It will be interesting to see if analogous functions will eventually be ascribed to other hematopoietic transcription factors.

### Conclusion and future directions

The quantum leap in the power of the mouse as a genetic tool through the advent of gene-targeting technology has allowed the identification of a number of transcription factors with critical roles in hematopoietic development. Certainly other similar factors remain to be discovered. In this regard, the development of the zebrafish as a research tool will facilitate the discovery of more hematopoietic regulators through forward genetic approaches. It could be argued, however, that at this point the largest gaps in our understanding of hematopoiesis lie in our knowledge of the critical genes that are regulated by these essential transcription factors. It is likely that the differential screening of arrayed cDNA libraries will be used increasingly to find these target genes. We also anticipate an increased use of retroviral transduction into primary cells as a technique to either ectopically express or repress particular transcription factors, both for the generation of sources of RNA for differential cloning and for lineage commitment studies.

Two other areas of future research deserve mention. One concerns the effect that hematopoietic microenvironments have on gene expression. The importance of this question was demonstrated recently by the finding that globin expression patterns in fetal and adult erythrocytes could be reprogrammed by transfer into the reciprocal microenvironment [51\*\*]. Finally, the regulation of chromatin structure during hematopoiesis is just beginning to be explored. Although questions relating to chromatin modulation have not typically been the main focus of those interested in hematopoietic development, they are likely to become increasingly important. This is suggested by the recent data concerning Ikaros, as well as by recent reports of chromatin modulation upon the activation and differentiation of mature T-cells [52\*\*,53\*].

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These papers [44\*,45\*] suggest that E2A functions a tumor suppressor in the T-cell lineage. Park *et al.* [44\*] enforce in the Jurkat T-ALL line the expression of a chimeric protein consisting of the bHLH domain of SCL and amino-terminal domains of E2A. This protein thus binds DNA only as a heterodimer with endogenous type I bHLH proteins such as those encoded by E2A or HEB (similar to wild-type SCL), but contained the transactivation potential of wild-type E2A. Park *et al.* find that the E2A-SCL construct induces both apoptosis and inhibits DNA synthesis when retrovirally transduced into Jurkat cells. We have transduced both the E47 and E12 products of the E2A gene into cell lines derived from lymphomas that arose in E2A-deficient mice [45\*]. We observe that either E2A-encoded protein act to kill these lines, but unlike Park *et al.*, find no effect on cell-cycle progression.
46. Nichogiannopoulou A, Trevisan M, Friedrich C, Georgopoulos K: **Ikaros in hemopoietic lineage determination and homeostasis.** *Sem Immunol* 1998, **10**:119-125.
47. Wang J-H, Avitahl N, Cariappa A, Friedrich C, Ikeda T, Renold A, Andrikopoulos K, Liang L, Pillai S, Morgan BA, Georgopoulos K: **Aiolos regulates B cell activation and maturation to effector state.** *Immunity* 1998, **9**:543-553.  
See annotation [50\*].
48. Brown KE, Guest SS, Smale ST, Hahn K, Merkschlagler M, Fisher AG: **Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin.** *Cell* 1997, **91**:845-854.
49. Avitahl N, Winandy S, Friedrich C, Jones B, Ge Y, Georgopoulos K: **Ikaros sets thresholds for T cell activation and regulates chromosome propagation.** *Immunity* 1999, **10**:333-343.  
See annotation [50\*].
50. Kim J, Sif S, Jones B, Jackson A, Koipally J, Heller E, Winandy S, Viel A, Sawyer A, Ikeda T *et al.*: **Ikaros DNA-binding proteins direct formation of chromatin remodeling complexes in lymphocytes.** *Immunity* 1999, **10**:345-355.  
Reports from the Georgopoulos group [47\*\*,49\*\*,50\*] suggest new models for how the *Ikaros* family acts to influence hematopoietic development. Avitahl *et al.* [49\*\*] find that *Ikaros* functions to repress T-cell activation induced by a variety of stimuli. As the stimulatory pathways tested are unlikely to converge on the same target genes, Avitahl *et al.* reason that *Ikaros* is probably acting as a general repressor of activation, raising the strength of the stimuli needed to generate a response. Avitahl *et al.* also report that *Ikaros* proteins are found in toroidal chromatin structures that co-localize with DNA replication complexes, and that cycling T-cells with reduced *Ikaros* levels rapidly accumulate chromosomal abnormalities. On the basis of the phenotypes of T-cells with normal and reduced *Ikaros* levels, Avitahl *et al.* propose [49\*\*] that the functions of *Ikaros* to set activation thresholds and maintain chromosomal integrity were likely related to its assembly into higher-order chromatin structures. Wang *et al.* [47\*\*] report that *Aiolos*-deficient mice exhibit hyperactive B-cell responses, suggesting that the role of *Aiolos* in B-cells is likely to have many similarities to that of *Ikaros* in T-cells. Kim *et al.* [50\*] find that both *Ikaros* and *Aiolos* are associated with chromatin-remodeling and histone deacetylation complexes in T-cells.
51. Geiger H, Sick S, Bonifer C, Muller AM: **Globin gene expression is reprogrammed in chimeras generated by injecting adult hematopoietic stem cells into mouse blastocysts.** *Cell* 1998, **93**:1055-1065.  
Geiger *et al.* use transplantation of embryonic/fetal and adult HSCs into adult and blastocyst host environments, respectively, to examine the influence of microenvironment on the expression of a transgenic human globin locus. They find that both adult and embryonic hosts function in a dominant fashion to reprogram gene expression of the transplanted HSCs.
52. Zhao K, Wang W, Rando OJ, Xue Y, Swiderek K, Kuo A, Crabtree GR: **Rapid and phosphoinositide-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling.** *Cell* 1998, **95**:625-636.  
See annotation [53\*].
53. Agarwal S, Rao A: **Modulation of chromatin structure regulates cytokine gene expression during T cell differentiation.** *Immunity* 1998, **9**:765-775.  
These reports provide evidence for chromatin remodeling upon T-cell activation and differentiation. Zhao *et al.* [52\*\*] report that phosphatidylinositol 4,5-bisphosphate (PIP2) generated by treatment of T-cells with anti-TCR antibodies or pharmacological mimics induced the association to chromatin of the BAF complex, which contains subunits related to the yeast SWI/SNF and *Drosophila* Brahma proteins [52\*\*]. Agarwal and Rao [53\*] find that differentiation of naive T-helper populations into different polarized T-helper subsets (i.e. Th1 and Th2) is associated with changes in chromatin structure at the appropriate gene loci, as determined by Dnase hypersensitivity.