sion of NKG2D on activated T cells and thymocytes may be relevant to T cell activation during development as well as clonal expansion or effector function. Hence, many of the considerations for NKG2D in NK cell biology may apply much more broadly, just as detailed analysis of NK cell inhibitory receptors helped lead to recognition that inhibitory receptors are widely utilized by other hematopoietic cells.

Finally, it is worth noting that retinoic acid is employed for chemotherapy of acute promyelocytic leukemia (APL)[15]. Conventional wisdom suggests that retinoic acid induces differentiation of APL cells to terminal myeloid cells that have limited proliferation potential, creating a population of cells that then die by apoptosis. The recognition that retinoic acid can induce ligands for NK cells and macrophages which results in killing of target cells provides support for another possibility, involving display of these ligands on APL cells. Therefore, it may be feasible to exploit an innate immunotherapy strategy involving therapeutic administration of agents to induce activation ligands on unwanted cells and harness the innate immune system to eliminate such cells *in vivo*.


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*Howard Hughes Medical Institute, Rheumatology Division, Washington University, St. Louis, MO 63110, USA. (yokoyama@imgate.wustl.edu)*



# Intertwining proteins in thymocyte development and cancer

Perturbation of T cell differentiation by overexpression of SCL and LMO can lead to leukemia. This dysregulation may be initiated by inactivation of E2A.

CORNELIS MURRE

During the past decades cellular and molecular analysis has provided a wealth of information about thymocyte development. On pages 138–144 of this issue[1], Herblot *et al.* reveal the actions of some transcriptional regulators that turn out to be critical for early T cell development.

The development of thymocytes can be organized with respect to T cell receptor (TCR) rearrangement – the assembly of V (variable), D (diversity) and J (joining) DNA segments and the expression of surface coreceptors CD4 and CD8. The most immature thymocytes express little or no CD4 or CD8 and are thus termed double negative (DN) cells; these cells comprise less than 5% of the adult thymus. The DN population can be further subdivided based on the expression of CD44 and CD25 (**Fig. 1**). The earliest thymic progenitors, expressing CD44 but lacking CD25, are not committed to the T cell lineage and have not initiated TCR gene rearrangements. Rearrangements of the gene encoding TCRB (*TCRB*) begin while CD44 is being down-regulated. At this stage the expression of pTα (TCRα chain "substitute" for immature T cells) is initiated as well. Upon in-frame rearrangement of the *TCRB*, a pre-T TCR is formed, which consists of TCRβ, pTα and the CD3 chains. Once the pre-TCR is assembled, thymocytes undergo further developmental progression, characterized by the cessation of TCR recombination and cellular expansion. At this stage, thymocytes also undergo changes in surface marker expression. These include the down-regulation of CD25, followed by the expression of high levels of CD8 that characterize immature single positive (ISP) thymocytes. Ultimately *CD4* gene expression is activated leading to double positive (DP) thymocytes. DP cells, which comprise 75–85% of thymocytes, exit the cell cycle and begin rearrangement of the gene encoding TCRα.

A number of transcriptional regulators have been identified that control key steps in thymocyte development. Among these are the basic helix-loop-helix proteins (bHLH) E12, E47 and HEB. E12 and E47 are encoded by the *E2A* gene and arise through differential splicing of the exon encoding the bHLH domain. Both E2A and HEB proteins are broadly expressed and play key roles in the developmental progression of a wide variety of cell types, including muscle, brain and lymphocytes[2]. E2A-deficient thymocytes display a defective thymic phenotype at the DN $CD44^+$ stage, before the onset of *TCRB* rearrangement (**Fig. 1**)[3]. HEB null mutant thymocytes show distinct developmental blocks at the DN and ISP cell stage (**Fig. 1**)[4]. By expression of the antagonist HLH protein Id3, previous studies have shown that expression of the gene encoding pTα requires the activities of either E2A or HEB[5]. To determine directly whether HEB regulates transcription of the gene encoding pTα, Herblot *et al.* examined HEB-deficient thymocytes for the presence of pTα[1]. Indeed, the investigators unambiguously identify the gene encoding pTα as a critical target for HEB.

There has also been a considerable amount

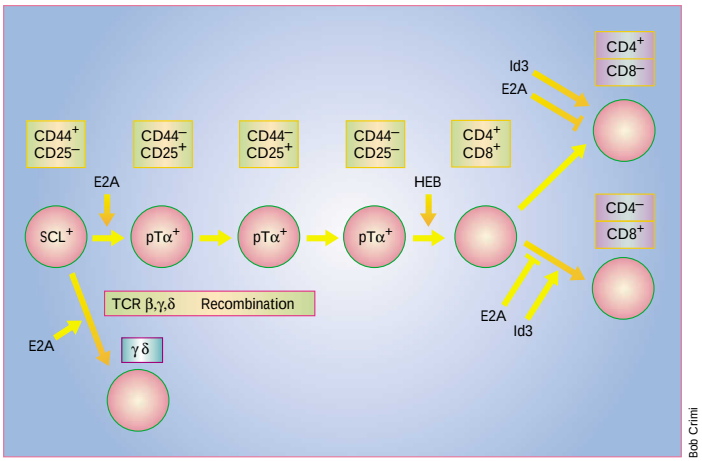


**Figure 1. Thymocyte development regulated by basic helix-loop-helix proteins.** Cell surface expression of CD44 and CD25 and rearrangement of TCR loci are shown. Expression of SCL and pTα is indicated. Roles of E2A, HEB and Id3 at different stages are shown.[3,4,7,11,12]

of effort in examining the roles of HLH proteins in T cell–acute lymphoblastic leukemia (T-ALL)[6]. A large fraction of human T-ALL is characterized by the ectopic expression of the HLH protein, SCL and the LIM domain–containing proteins LMO1 and LMO2. SCL is essential for the development of multipotent hematopoietic progenitor cells. LMO2 has been demonstrated to interact with SCL and LMO2 null mutant embryos show hematopoietic abnormalities that are strikingly similar to that of embryos lacking the *SCL* gene. In erythroid cells, both SCL and LMO2 interact with E2A, GATA-1 and Ldb1, forming a multimeric complex that binds a DNA sequence consisting of E box and GATA sites[7]. These same proteins have also been shown to be present as a higher order complex in T cell lymphomas and strengthen the argument that they act in concert to induce lymphomagenesis by the aberrant activation of critical target genes.

However, there is an alternative way of looking at the contribution of SCL and E47 to the development of lymphoma. SCL has long been known to interfere with the transcriptional activity of E2A. Additionally, mice with targeted mutations in the *E2A* gene locus are also highly susceptible to T cell lymphomas. This has raised the question as to whether inactivation of E2A by ectopic expression of SCL and LMO2 is the key step towards the development of lymphoma.

Herblot *et al*. have now investigated this issue further and created transgenic mice that carry both the *SCL* and *LMO2* genes[1]. Interestingly, thymocyte development in these mice is severely perturbed. Specifically, aberrant expression of SCL blocks T cell development at the same stage as has been observed for HEB null mutant thymocytes. Additionally, Herblot *et al*. show that γδ development is abnormal, as has also been described for E2A-deficient thymocytes[8]. Furthermore, the authors detect large numbers of apoptotic cells in SCL-LMO2 double transgenic mice, a phenotype that is remarkably similar to that of transgenic mice expressing the antagonist HLH protein Id1[9]. Although there are differences in the phenotypes of the pTα null mutant thymocytes and transgenic mice carrying the SCL and LMO1 transgene, there are striking similarities. Herblot *et al*. now demonstrate that indeed overexpression of SCL in the DN thymocyte compartment significantly interferes with expression of the gene encoding pTα. These data suggest that SCL interferes with E2A and HEB function. How, then, does SCL interfere with HEB and E2A function? SCL has the ability to function as a transcriptional repressor and readily forms heterodimers with either E2A or HEB. One possibility is that SCL, upon interacting with either E2A or HEB, blocks the activation of downstream target genes, by converting a transcriptional activator into a repressor.

The observations made by Herblot *et al*. provide further support for the model in which the ectopic expression of SCL and LMO-1 in human T-ALL acts through the inactivation of E2A and HEB. The investigators show that SCL, E2A and HEB are each expressed in the various DN populations. However, their expression patterns differ. SCL is transcribed in the early populations whereas E47 and HEB are activated later, at the onset of expression of the gene encoding pTα. Inappropriate activation of SCL may prevent E2A from properly activating its critical target genes. As E2A has been shown to act as a tumor suppressor, ectopic expression of SCL and/or LMO-1 may lead to the development of lymphoma. The important question now to be addressed is how the E2A deficiency leads to lymphomagenesis.

Finally, it is conceivable that the absence of E2A activity promotes the development of other forms of human T-ALL. Another HLH protein, designated LYL-1 is involved in human T-ALL as well. LYL-1 readily forms heterodimers with E2A to modulate its DNA binding specificity and functionally remove E2A activity. Thus, through the overexpression of LYL-1 in developing thymocytes, E2A may not have the ability to regulate its downstream target genes, ultimately leading to the development of lymphoma. TAN-1, a gene product which belongs to the Notch receptor family, has been shown to be involved in a subset of human T-ALL. A recent study has demonstrated a link between Notch and E47[10]. Activation of the Notch signaling pathway was shown to perturb the transcriptional activity of E47, and it is possible that this subset of lymphomas also relies on the inactivation of E2A as a crucial step towards lymphomagenesis. This line of research will certainly help to clarify the role of bHLH proteins in thymocyte development and perhaps will provide further insight into the development of subsets of human T-ALL.

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*University of California - San Diego, Department of Biology, La Jolla, CA 92093, USA.*
*(murre@biomail.ucsd.edu)*