



The role of E-proteins in B- and T-lymphocyte development

Gretchen Bain* and Cornelis Murre

Development of lymphocytes from hematopoietic stem cells is controlled, in part, by the activity of transcriptional regulatory proteins. In particular, one class of helix-loop-helix proteins, termed E-proteins, have been implicated in the regulation of gene expression during B-cell development. Recent analysis of gene-targeted mice has allowed a direct assessment of the functional roles of several E-protein family members in hematopoiesis. In this review we describe the defects in B- and T-lymphocyte development in mice carrying targeted mutations in the E-protein genes and discuss our current understanding of the role of these proteins in lymphoid development.

Key words: E proteins / lymphoid development / transcription factors

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CELLS OF THE HEMATOPOIETIC system derive from multipotent stem cells through a process that involves progressive restriction of lineage potential. The developmental processes involved in the generation of the hematopoietic system require the ordered activation of a number of transcriptional regulatory proteins. Specific transcription factors may specify individually different lineages or, alternatively, may be required in distinct lineages at later stages of development. For several years, the E-proteins, members of the basic helix-loop-helix family of transcriptional regulators, have been implicated in the regulation of gene expression during lymphopoiesis. In this review we will discuss the role of E-proteins in both B- and T-lineage development (Figure 1) and speculate on the molecular mechanism of E-protein function.

* From the Department of Biology, 0366, University of California, San Diego, La Jolla, CA 92093, USA

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The E-proteins

The E-proteins are members of the highly conserved basic helix-loop-helix (bHLH) family of transcriptional regulatory proteins and have been implicated in the regulation of gene expression during a number of developmental processes. These proteins are characterized by distinct structural features: a protein dimerization domain consisting of two amphipathic α -helices separated by a loop and an adjacent basic region that contacts DNA, both of which are required for DNA binding.¹ The E-proteins were identified by their ability to bind canonical E-box motifs (CANNTG) originally identified in the immunoglobulin κ and μ intronic enhancers.^{2–6} Full activity of the enhancers was shown to be dependent on these E-box elements, suggesting that enhancer activity would also be dependent on the E-protein family.^{7,8} E-box elements have since been identified in all immunoglobulin enhancers, as well as in a number of regulatory elements identified in T-lineage specific genes, including the T-cell receptor α and β enhancers and the CD4 silencer and enhancer elements.^{9–14}

To date, four mammalian E-proteins have been identified (E12, E47, E2-2 and HEB), all with the potential to bind to E-box elements either as homodimers or as heterodimers with tissue-specific bHLH proteins.¹ Two of these proteins, E12 and E47, are encoded by the E2A gene and arise through alternative splicing of the RNA transcript. E12 and E47 possess identical amino-terminal transactivation domains and differ only in the exon that encodes the bHLH domain.² The HEB and E2-2 proteins, although highly homologous to the E2A gene products, are encoded by distinct genes.^{3,4,15}

The isolation of genes encoding *Drosophila* HLH proteins and the genetic analysis of these genes has provided insight into the function of the mammalian homologues. One of the genes identified, *daughterless*

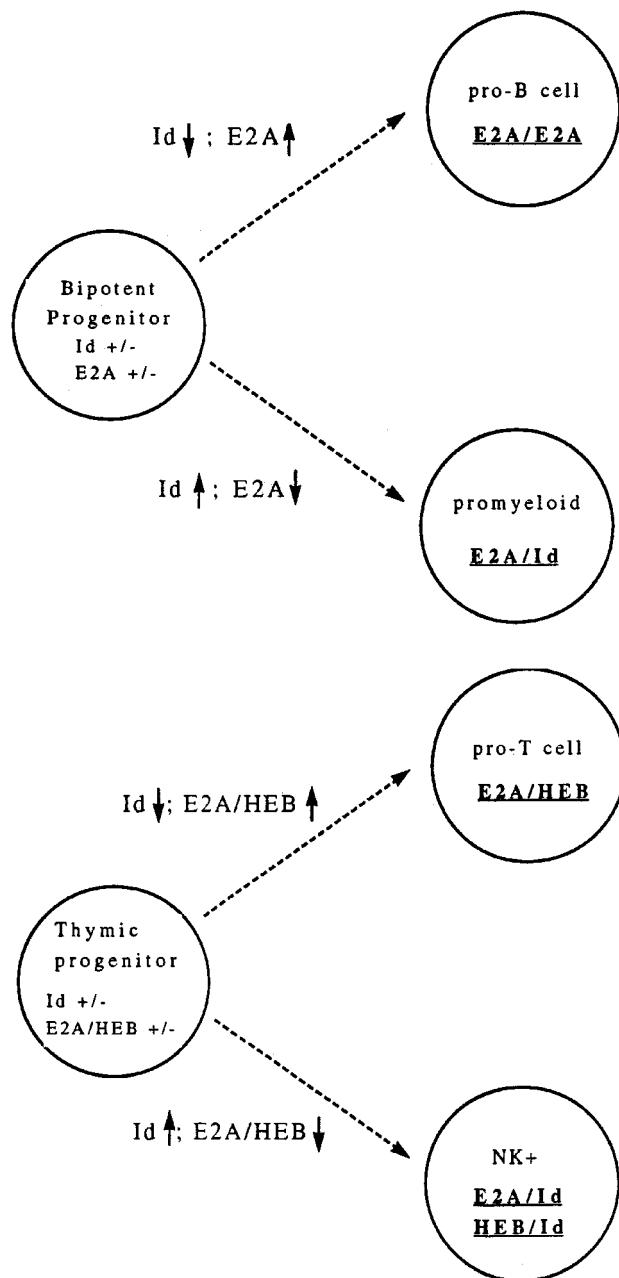


Figure 1. Schematic diagram indicating potential developmental relationships between B-cell/myeloid lineages and T-cell/NK lineages. Indicated are the changes in HLH protein levels that are proposed to influence these lineage decisions. Increasing the functional activity of E2A and/or HEB favors the development of B- or T-lineage cells, whereas decreases in the activity of these E-proteins favors the development of myeloid and NK cells.

(*da*), encodes a bHLH protein homologous to the mammalian E-proteins.¹⁶ *da* and the mammalian E-144

proteins share two highly conserved amino-terminal transactivation domains (AD1 and AD2), in addition to the conserved bHLH domain.¹⁷⁻¹⁹ In *Drosophila*, *da* and bHLH proteins encoded by the *Achaete-Scute Complex* (*AS-C*), control sensory organ precursor formation.²⁰ *da/AS-C* heterodimers act to establish sensory organ precursor cell fate by the formation of a transcriptionally active complex. Loss of function mutations in either gene results in the reduced formation of sensory neuronal precursors.²⁰ In contrast, loss of function mutations in another gene, *emc*, induce ectopic expression of sensory structures.^{21,22} Experiments demonstrating that the *da/AS-C* and *emc* gene products interact in a dosage-dependent manner indicated a genetic interaction between these genes.^{21,22} Subsequent cloning of the *emc* gene revealed that its product encodes an HLH protein lacking a basic region and more recently, *emc* has been shown to interact biochemically with *da/AS-C* to inhibit the DNA-binding activity of *da/AS-C* heterodimers.²³⁻²⁵ Thus, *emc* acts as a negative regulator of *da/AS-C* transcriptional activity through the formation of non-DNA binding heterodimers.

In mice, four gene products have been identified (Id1-4) which show significant homology to *emc* and are members of the Id family of HLH proteins.²⁶⁻²⁹ Importantly, these proteins lack a basic region and so have been proposed to function in a manner analogous to their *Drosophila* homologues. The Id genes have distinct, but overlapping, patterns of expression in both the mouse embryo and in adult organs. All four Id genes are expressed in fetal liver, however, Id2 appears somewhat later in gestation and Id4 is only expressed early in gestation.²⁸ All four genes are expressed in bone marrow, but only Id2, Id3 and Id4 are expressed to a significant degree in the adult spleen.²⁸ Id2 and Id3 show additional expression in the thymus (R. Rivera and C. Murre, unpublished).

Like the Id genes, the E-proteins genes are also ubiquitously expressed. As determined by *in situ* hybridization analysis, E2A transcripts are present in most embryonic and adult rat tissues. Interestingly, the highest levels of E2A expression detectable during embryonic development were evident in areas of rapid cell division.³⁰ Additionally, high levels of E2A transcripts were found in the thymus and bone marrow of day 18 p.c. embryos and in the germinal centers of the adult spleen.³⁰ The expression patterns of the E2A gene products and the presence of E-protein binding sites in the regulatory elements of a

number of B- and T-lineage specific genes suggests that the E-proteins function during lymphoid development. However, in tissues where the Id and E-proteins are co-expressed, the balance between the transcriptional activators and negative regulators will likely determine the activity of the E-box containing elements.

Functional roles for E-proteins in B-cell development

E2A functions during early B-cell development

A further involvement of the E-proteins in B-lymphoid specific gene expression is suggested based on a number of *in vitro* experiments. E47 has been shown to bind as a homodimer in B-cell nuclear extract and binding by E47 preceded the expression of immunoglobulin heavy chain protein in long term bone marrow cultures.³¹⁻³³ Additionally, overexpression of E47 in a pre-T-cell line induced RAG expression, germ-line heavy chain gene transcription and heavy chain D-J rearrangement.³⁴ More recently, overexpression of E47 and to a lesser extent E12, in transfected NIH3T3 fibroblasts resulted in an enormous stimulation of endogenous $I\mu$ transcription and TdT expression.³⁵ These data suggest a role for the E-proteins during the pro-B-cell stages of development.

B-cell development in mouse bone marrow can be divided into discrete stages based, in part, on the differential expression of cell surface molecules and the rearrangement status of the Ig loci. In mouse bone marrow, the earliest cells committed to the B-lineage contain the Ig genes in the germline configuration and express B29, the B-cell specific gene that encodes $Ig\beta$ and μ^0 , an IgH germline transcript initiating upstream of J_H1 .³⁶ Inactivation of the E2A gene products, through gene targeting or overexpression of the negative regulator Id1, results in a complete block in B-cell differentiation.³⁷⁻³⁹ E2A-deficient mice lack pre- and mature-B-cells and have significantly reduced numbers of pro-B-cells which lack D-J and V-DJ rearrangements. Bone marrow and fetal liver from E2A-deficient mice lack expression of mb-1, $\lambda 5$, CD19 and RAG-1, but transcripts for B29, TdT and μ^0 are present.³⁷⁻³⁹ This phenotype is consistent with a block in B-cell development prior to the stage in which Ig gene rearrangement and B-cell specific gene expression is initiated. Furthermore, these data demonstrate a requirement for

the E2A gene products at the approximate time of commitment to the B-cell lineage.

Differential roles for E12 and E47 at the pro-B to pre-B transition

The two proteins encoded by the E2A gene, E12 and E47, are identical, except for the exon that encodes for the bHLH domain.² Mutational analysis of E12 identified an inhibitory domain adjacent to the basic region that prevents efficient homodimerization, while not affecting the potential to heterodimerize.⁴⁰ In addition, while the B-cell specific E-box complexes have been shown to contain E47 homodimers, E12 binding in B-cell nuclear extracts is undetectable, despite the fact that the two proteins are coexpressed.^{32,41,42} The sequence similarity between these two spliced products and the seemingly identical expression patterns raises the question of whether they perform distinct functions during B-lymphopoiesis. Indeed, analysis of E2A-deficient mice expressing transgenes for E12 and E47 reveal distinct roles for the two proteins at the pro-B to pre-B-cell transition.

Expression of the E12 transgene in the E2A-deficient background promotes the development of cells with characteristics of the pro-B-cell population. Development of $B220^+CD43^-$ lymphocytes is blocked in E2A-deficient mice expressing the E12 transgene, however, the population of $B220^+CD43^+$ cells in the bone marrow is increased compared to E2A-deficient non-transgenic mice.⁴³ Transcripts for a number of B-lineage associated genes, including $\lambda 5$, mb-1, VpreB and Rag-1 are detectable in the bone marrow of these mice and both D-J and V-DJ rearrangements are present. However, in the absence of E47, E12 is unable to support the development of mature-B lymphocytes.⁴³

Expression of the E47 transgene in the E2A-deficient background is also able to promote the development of B-lineage committed cells. Surprisingly however, E47 alone is sufficient for the generation of mature surface IgM^+ B-cells, although at significantly reduced numbers compared to wild-type controls.⁴³ The population of mature-B-cells increases dramatically when both the E12 and E47 transgenes are coexpressed in the E2A-deficient background, suggesting that E12 synergizes with E47 in controlling the development of B-lineage cells.⁴³ Taken together, these data demonstrate that either E12 or E47 is sufficient to promote the development of early B-lineage committed cells, but only E47 can support the

transition from the pro-B cell to the pre-B-cell stage. In addition, optimal development of mature B-lymphocytes requires the expression of both E12 and E47, indicating that each performs a unique function during B-lymphopoiesis.

E-proteins and isotype switching

The ability of the E-proteins to bind to and activate transcription from reporter constructs containing the heavy or light chain gene enhancers suggests an important role for these proteins in enhancer function. Activity of the immunoglobulin κ intronic and 3' μ enhancers has been shown to be important at later stages of B-cell development for Ig gene somatic hypermutation and heavy chain isotype switching.^{44,45} The question raised is whether the E-proteins function during these later stages of B-cell maturation. The pattern of E2A expression in the secondary lymphoid tissues supports a role for E2A in the antigen-dependent stages of B-cell differentiation. E2A transcripts are present in the germinal centers of the spleen and E2A protein levels are upregulated in the germinal center dark zones.^{30,46} A role for E-proteins in class switching has recently been demonstrated using a B-cell line which undergoes spontaneous and inducible isotype switching. Forced expression of Id1 in this line blocked spontaneous and to a lesser extent, cytokine inducible class switching.⁴⁶ Taken together, these data indicate a role for the E-proteins in the class switching process.

HEB and E2-2 in B-lymphopoiesis

The HEB and E2-2 proteins are structurally and functionally similar to the E2A gene products. In particular, E2-2, which is expressed predominately in pre-B-cells, is detectable in B-cell specific Ig enhancer binding complexes from pre-B-cells.⁴² However, neither HEB nor E2-2 is required for B-cell development.⁴⁷ Interestingly, fetal livers from mice lacking either HEB or E2-2 have approximately 50% of the wild-type numbers of progenitor B-cells.⁴⁷ A similar decrease in progenitor-B-cell numbers has been observed in the fetal livers of E2A heterozygous mice.³⁷ Mice trans-heterozygous for any two E-protein mutations generated fewer progenitor B-cells than each single heterozygous mutation.⁴⁷ Thus, although neither HEB nor E2-2 is essential for the establishment of the B-lineage, there exists a dosage dependent affect of the E-proteins on differentiation through the B-lineage.

Relationship of E2A to other B-lineage transcription factors

Use of gene targeted mice has led to the identification of a number of other transcription factors that function in regulating the early stages of B-lymphopoiesis. Analysis of their mutant phenotypes has allowed the construction of a hierarchy of transcription factors controlling the development of pro-B-cells. Mice deficient for Pax-5, a member of the paired-box gene family, exhibit an early block that is characterized by the expression of $\lambda 5$, VpreB and HSA and the presence of D_H-J_H rearrangements.⁴⁸ Sox-4 mutants display a similar block in development.⁴⁹ Absence of the Early B-Cell Factor (EBF) results in a block that apparently precedes that of the Pax-5 and Sox-4 mutations, as no D_H-J_H rearrangements can be detected.⁵⁰ Although the more mature $B220^+CD43^-$ populations are lacking in EBF-deficient mice, the $B220^+CD43^+HSA^-BP-1^-$ cells are present in normal numbers.⁵⁰ The pro-B-cells in the E2A-deficient mice display a phenotype almost identical to that of the EBF-deficient pro-B-cells.³⁷⁻³⁹ Significantly however, the population of $B220^+CD43^+$ cells is dramatically reduced in the E2A-targeted mice, which suggests a developmental block at a stage prior to that of the EBF mutation. The fact that E2A-deficient mice lack EBF and Pax-5 transcripts further supports the idea that E2A functions upstream of either of these proteins and identifies the E2A gene products as among the earliest determinants of B-cell development.³⁹

Functional roles for E-proteins in T-cell development

Functionally important E-box elements have been identified in the TCR α and β enhancers and the regulatory elements of the CD4 gene.^{11,12,14,51} In addition, E47 and HEB E-box binding complexes are detectable in T-cell and thymocyte nuclear extracts.^{52,53} These data suggest potential roles for at least two members of the E-protein family in the regulation of gene expression during T-cell development.

E2A functions during early T-cell development

$\alpha\beta$ T-lymphocyte differentiation begins in the fetal thymus and proceeds in an ordered fashion through

a number of well-characterized developmental stages. These stages are defined by the expression of the CD4 and CD8 cell surface molecules and the status of the TCR gene rearrangements.⁵⁴ Analysis of E2A-deficient mice has confirmed a role for the E2A gene products during $\alpha\beta$ T-cell development. Mice lacking E2A display a five to ten fold reduction in total thymocyte numbers, with similar decreases in splenic T-cell numbers.⁵² E2A-deficient thymii are characterized by significantly reduced percentages of double-positive (DP) cells and increased percentages of single-positive (SP) cells. Interestingly, E2A-deficient

mice almost completely lack CD25⁺ double-negative (DN) thymocytes and display an increase in the relative percentage of the CD44⁺CD25⁻ DN thymocytes, a population which contains cells not yet committed to the T-lineage.⁵² Thus, the E2A-deficiency leads to a partial block in T-cell differentiation at a stage that is developmentally similar to the stage in which B-cell differentiation is blocked (Figure 2).

HEB in T-lymphopoiesis

HEB, like E47, is detectable in E-box binding com-

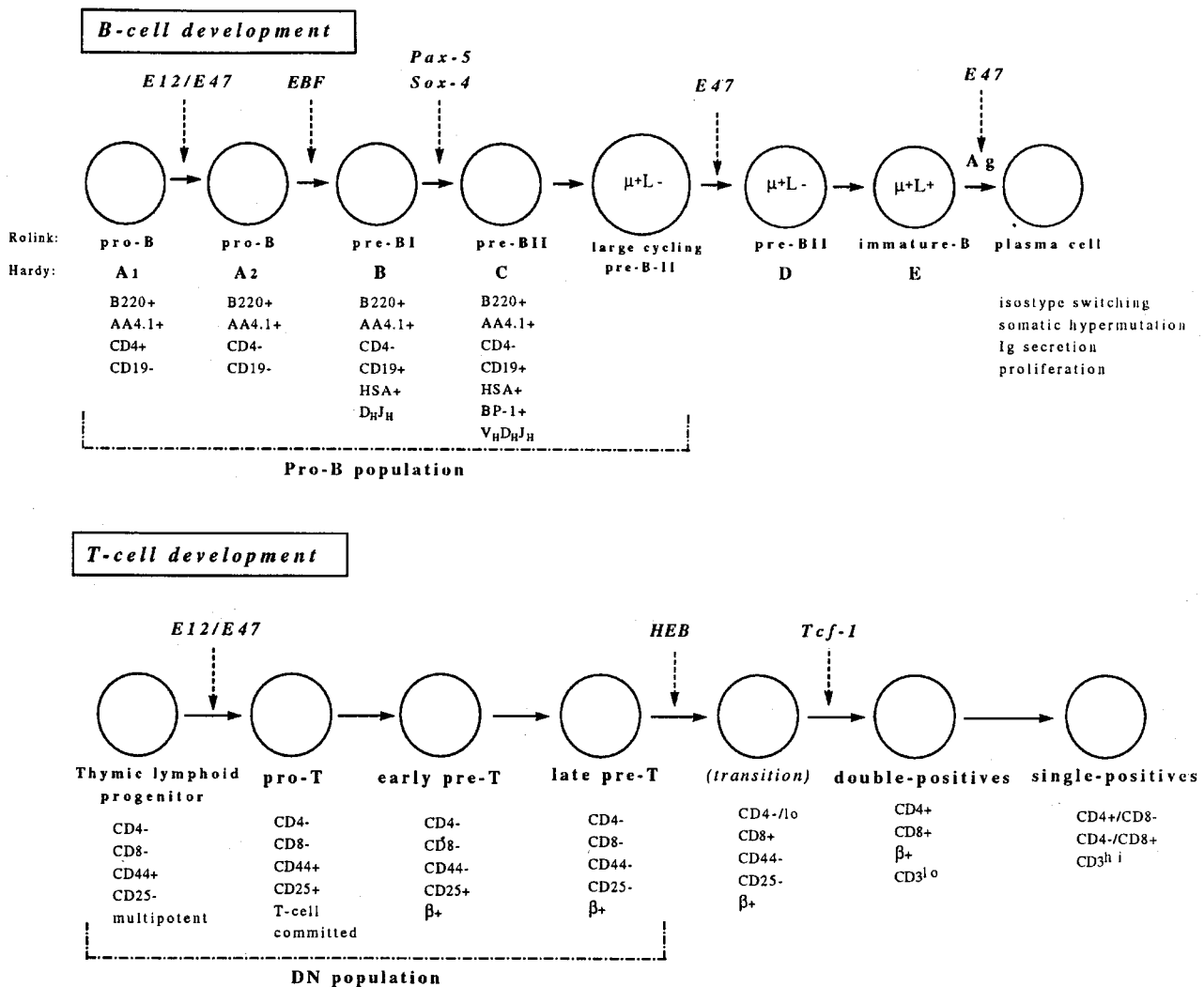


Figure 2. Schematic diagram of murine B- and T-cell development. The different developmental stages are shown together with their characteristic cell surface markers and with the configuration of the Ig and TCR genes. Indicated by the vertical arrows are the approximate developmental stages at which a number of transcriptional regulatory proteins have been shown to function. Some proteins are proposed to function at multiple transcriptional control points.

plexes from thymocyte and T-cell nuclear extracts and in fact the predominate T-cell specific complex is composed of an E47/HEB heterodimer.^{52,53} There are, however, detectable levels of both E47 and HEB homodimers within the T-cell specific complexes (G. Bain and C. Murre, unpublished). Since the majority of the T-cell specific E-box complex consists of E47/HEB heterodimers, it is not surprising that HEB-deficient mice display defects in $\alpha\beta$ T-cell development that are similar to the defects observed in E2A-deficient mice. Like thymii from E2A-deficient, HEB-deficient thymii contain significantly higher percentages of CD8⁺ thymocytes, but show dramatic decreases in the percentages of DP cells.⁴⁷ On the other hand, the HEB-deficient thymus phenotype is distinct from that of the E2A-deficient phenotype in several respects. First, HEB-deficient mice contain apparently normal numbers of thymocytes and do not display increases in the CD4⁺ population.⁴⁷ Additionally, thymii from HEB-deficient mice exhibit enormous increases in the DN population.⁴⁷ Such phenotypes suggest unique functions for the E2A and HEB gene products during T-cell differentiation.

Relationship of E2A to other T-lineage transcription factors

T-cell development is dependent on the expression of a number of other key transcriptional regulatory proteins. Thymocytes from mice deficient for Tcf-1 display a partial block at the transition from the CD4⁻CD8⁺ immature stage to the CD4⁺CD8⁺ DP stage of development.⁵⁵ This defect in maturation results in a marked decrease in the peripheral T-cell population. Intestinal $\gamma\delta$ T-cells are decreased seven to eight fold in Tcf-1-deficient mice, whereas thymic and splenic $\gamma\delta$ cells appear to develop normally.⁵⁵ Ets-1-deficient mice also display marked decreases in the number of mature thymocytes and peripheral T-cells.^{56,57} Interestingly, the thymic phenotype of the Ets-1 mutant mice shows a likeness to the E2A-deficient phenotype. Both deficiencies result in dramatic decreases in the DP percentages and concomitant increases in the SP percentages.^{56,57} It will be interesting to determine whether E2A acts in parallel with the Ets-1 or Tcf-1 transcription factors or functions in similar pathways.

Potential functions for E-proteins

The phenotypes of the gene-targeted mice and the results of previous *in vitro* experiments confirmed a functional role for the E-proteins during both B- and T-lymphocyte development. In this section we would like to speculate on the molecular mechanism of E-protein function during these two developmental processes.

E-proteins as regulators of gene transcription and rearrangement

The E-proteins were originally identified in an expression screen for proteins that could bind to the E2-sites in the κ and μ chain gene enhancers.²⁻⁴ E-box elements have since been identified in all the immunoglobulin enhancers and mutations in these sequences cause significant reductions in enhancer activity.^{7-10,13} The IgH intronic enhancer (E μ) is required for the activation of both transcription and rearrangement of the Ig genes in B-lineage cells, suggesting that the E-proteins might be required for efficient Ig gene transcription and rearrangement.⁵⁸ In support of this idea is the fact that E2A-deficient mice contain significantly reduced levels of I μ germline transcripts and completely lack Ig gene rearrangements.³⁷⁻³⁹ Further evidence that E2A is involved in initiating Ig gene transcription and rearrangement comes from the fact that overexpression of E47 in a pre-T-cell line induced I μ germline transcription and heavy chain D-J rearrangement.³⁴ Additionally, overexpression of E47 and to a lesser extent E12, in NIH3T3 fibroblasts results in an enormous stimulation of endogenous I μ germline transcription, a process preceding rearrangement of the heavy chain locus.³⁵ More recently, activity of the E2A activation domains in yeast has been shown to be dependent on the expression of the Ada proteins, which can function in histone acetylation (M.E. Masari and C. Murre, unpublished). Others have demonstrated a functional collaboration between E47 and p300/CBP, a mammalian transcriptional adaptor protein that also contains histone acetyltransferase activity.^{59,60} We would like to speculate that histone acetylation in the Ig loci may alter the chromatin structure of the Ig genes, thus allowing accessibility of the locus to rearrangement.

While a role for E2A in Ig gene rearrangement

seems likely, it is clear that the E2A-deficient mice are phenotypically different from the RAG-deficient mice.^{61,62} Significantly lower percentages of B220⁺CD43⁺ pro-B-lymphocytes are present in E2A-deficient mice and a number of B-lineage associated transcripts that are present in RAG-deficient mice are undetectable in mice lacking E2A. One possibility is that E2A performs an additional function prior to or at the time of initiation of Ig transcription and rearrangement. That E2A has a distinct and essential function before the initiation of D–J rearrangement has been clearly demonstrated using Ig-transgenic mice. B-cell development is rescued upon expression of an Ig transgene in the RAG-deficient background, but not in the E2A-deficient background (Ref 63 and G. Bain and C. Murre, unpublished).

T- and B-lymphocyte development is similar with respect to the control of rearrangement. The TCR α and β enhancers, like the Ig enhancers, have the ability to promote lineage-specific activation of rearrangement.^{64–67} The presence of E-box sites in the TCR α and β enhancers suggests that the E-proteins may be involved in the regulation of TCR gene rearrangements, although direct evidence for this role is lacking.^{12,14} Of interest, however, is the fact that thymic Rag-1 and Rag-2 expression is decreased by three to six fold in E2A-deficient mice (G. Bain and C. Murre, unpublished). While it is formally possible that the decrease in RAG transcripts is a result of a decreased percentage of DP thymocytes, there is *in vitro* evidence to suggest that the E-proteins may regulate, either directly or indirectly, expression of the RAG genes.³⁴

The strongest evidence that the E-proteins are involved in the regulation of rearrangement comes from the analysis of $\gamma\delta$ TCR gene rearrangements. E2A-deficient mice lack distinct subpopulations of $\gamma\delta$ T-cells (G. Bain and C. Murre, unpublished). Preliminary evidence suggests that the absence of specific $\gamma\delta$ T-cell populations results from an inability to rearrange certain V regions efficiently, suggesting a functional role for the E2A gene products in regulating the accessibility of the DNA to the recombination machinery (G. Bain and C. Murre, unpublished).

While a definitive role for the E-proteins in the rearrangement process remains to be determined, the existing data suggest that the E2A gene products may be involved in several aspects of the rearrangement process, including initiation of RAG expression and germline transcription and regulation of gene region accessibility.

E-proteins as components of lymphocyte signaling pathways

E2A is required close to the time of commitment to the B-cell lineage, however, the molecular events regulating these earliest stages of lymphoid development are predominately unknown. IL-7, a stromal-cell derived cytokine, is important for the survival, proliferation and differentiation of B-cell progenitors.^{68–70} B-cell development in IL-7R α -deficient mice is severely impaired, as characterized by an almost complete block at the pro-B-cell stage of development.⁷¹ The B220⁺CD43⁺ pro-B-cell population in IL-7R-deficient mice displays a phenotype similar to that of the E2A-deficient pro-B cell population. In addition, IL-7 signaling is an important component of the T-cell developmental pathway. Mice lacking IL-7 or IL-7R α or treated with a neutralizing antibody to IL-7 display decreases in thymic cellularity that are consistent with its role in stimulating proliferation of DN thymocytes.^{72,73} Treatment with an anti-IL-7 antibody causes a reduction in the percentage of DP thymocytes and an increase in the CD8⁺ population.⁷³ Both the decreased cellularity and the skewing of the subpopulations are reminiscent of the defects observed in the E2A-deficient mice. The B- and T-cell defects observed in mice lacking E2A expression are unlikely to be at the level of IL-7R receptor expression, since IL-7R α transcripts are present in E2A-deficient mice.⁴³ However, it is possible that E2A functions downstream of the IL-7 signaling pathway to promote survival, proliferation and differentiation of progenitor cells. Signaling through the IL-7 pathway might also result in the down-regulation of Id, a process shown to be necessary for the further development of the progenitor B cells. Alternatively, IL-7 signaling and E47 activity may act in parallel pathways at similar stages in development.

The virtual absence of the IL-2R α ⁺ DN population is strikingly similar to the phenotype of thymocytes that develop in an *in vitro* culture system in the presence of neutralizing antibodies to TNF- α and IL-1 α .⁷⁴ TNF- α and IL-1 α are stromal factors that are required for the progression of CD44⁺CD25⁻ uncommitted thymic precursors to committed CD25⁺ DN thymocytes.⁷⁴ Addition of neutralizing antibodies to both molecules in an *in vitro* culture system completely blocks the development of CD25⁺ cells and results in the generation of fewer numbers of DP and SP thymocytes.⁷⁴ It is conceivable that E2A functions as an effector molecule downstream of the TNF- α

and IL-1 α signaling pathways. One of the next challenges will be to determine whether E2A or Id gene expression is regulated by these signaling pathways during the early stages of T-cell development.

E-proteins and the commitment process

The phenotypes of the E2A-deficient mice raise the question of whether E2A activity induces commitment to the T-cell and B-cell-lineages. *in vivo*, there exists a close developmental relationship between B-cells and macrophages.⁷⁵⁻⁷⁷ *In vitro*, the 70Z/3 pre-B-cell line is able to differentiate into cells expressing a macrophage phenotype, but no culture conditions have yet been identified which allow the pre-B-cell derived macrophage line to revert back to the B-lineage.^{78,79} However, overexpression of E12 in the 70Z/3 macrophage line induces a program of B-cell differentiation, as evidenced by an increased expression of mRNA encoding for IL-7R α , $\lambda 5$, μ and Rag-1 and an ability to induce κ expression in response to mitogenic stimulation (B.L. Kee and C. Murre, unpublished). These data suggest that E12 alone is sufficient to initiate a B-cell program. Myeloid cells express low levels of E2A and reportedly express relatively high levels of Id, whereas B-cells display the reciprocal pattern of HLH gene expression.^{31,33,80} It is conceivable that E2A activity is an important determinant in the decision between the lymphoid and myeloid cell fates (Figure 1). T-cells and NK cells also share a close developmental relationship and given the partial block in T-cell development at a stage prior to T-lineage commitment, one might speculate that the decision between the NK and T-cell lineages is governed by a similar balance between the HLH positive and negative regulatory proteins (Figure 1).^{81,82} In fact, whereas $\alpha\beta$ T-cell numbers are severely decreased in E2A-deficient mice, NK cell numbers are relatively unaffected (G. Bain and C. Murre, unpublished). Clearly, however, the thymic phenotype is 'leaky'. Whereas B-cell development is completely blocked in E2A-deficient mice, mature-T-cells do develop. This difference may be due, in part, to a redundancy in E-protein function. E-box binding complexes from B-cell nuclear extract consist of E47 homodimers, whereas the predominate binding species in thymocyte nuclear extract consists of E47/HEB heterodimers.^{32,52} In addition, E-box binding complexes consisting of HEB homodimers are present in thymocyte nuclear extract from E2A-deficient mice.⁵² Thus, it is conceivable that HEB

activity can partially compensate for the loss of E2A during the development of the T-lineage.

Conclusions and future directions

Numerous studies have identified key transcriptional regulators controlling B- and T-lymphoid development (Figure 2). Members of the HLH family, including the E-proteins and the Id proteins, contribute to the establishment of the B-lineage and to the proper development of the T-cell lineages. Based on a number of observations, it is likely that the E-proteins function at a number of different transcriptional control points along the lymphocyte developmental cascades (Figure 2). However, since knockout phenotypes often show only the earliest block in a pathway, it will be interesting to determine potential E-protein function at subsequent transcriptional control points through the use of targeted mutations that are manifested at later stages of development.

The molecular mechanism by which the E-proteins induce differentiation and the relationship of the E-proteins to other lymphoid regulatory proteins is still not clear. Identification of other relevant E-protein target genes may suggest additional mechanisms by which the loss of E2A interferes with lymphopoiesis. Not discussed in this review is the potential role of the HLH proteins in the regulation of growth control. A number of *in vitro* experiments have suggested that expression of the Id proteins in fibroblasts induces cell cycle entry through the inhibition of E-box DNA-binding activity.^{83,84} In support of this, overexpression of E47 in fibroblasts results in a withdrawal from the cell cycle.⁸⁴ However, there is no evidence that lymphocytes from E2A-deficient mice display increased proliferation. In contrast, thymii of mice lacking E2A actually have a decreased number of cycling cells (G. Bain and C. Murre, unpublished). It is possible that Id and E-proteins function differently in lymphoid cells as compared to fibroblastic cells, or alternatively, that an inhibitory role in the cell cycle is not manifested in the presence of the other phenotypes.

Of further interest is the relationship of the HLH proteins to extrinsic factors shown to be required for the proper development of lymphocytes. For example, are the HLH proteins regulated by signals received from bone marrow or thymic stromal cells which are required for the growth and differentia-

tion of lymphoid progenitors? Connecting the activity of the HLH proteins to extrinsic signals may shed light on the functions of the HLH protein members during early lymphopoiesis.

Whatever the molecular mechanism of E-protein function, it is likely that the developmental processes regulated by the HLH proteins are dependent on the balance between the positive and negative regulators. Indeed, the ratio between the Id and E-proteins may be a critical factor in determining lineage decisions. In addition, if the E-proteins function in activating gene rearrangements, their activity would need to be down regulated after the production of a functional chain in order to prevent further rearrangements. An inhibition of E-protein activity could result from the down-regulation of E-protein expression or the up-regulation of the inhibitor, Id. An important goal for the future is to determine how the expression of these key factors is regulated.

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Note added in proof: A recent paper published by Heemskerk *et al* (1997, *J Exp Med* 186:1597–1602) demonstrated that overexpression of the dominant negative HLH protein Id3 in CD34⁺ progenitor cells blocked development of T cells in fetal thymic organ cultures, but enhanced development of NK cells. These data support the hypothesis that activity of bHLH transcription factors may be involved in lineage decisions.

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