

Transcription factor regulation of B lineage commitment

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The past year has provided insight into the mechanisms by which multipotent progenitors commit to differentiation through the B lymphocyte lineage. Mice lacking the Pax5 gene develop pro-B lymphocytes but these cells are not uniquely committed to the B lineage as they can give rise to all hematopoietic cell types if cultured under appropriate conditions. Regulators of lymphocyte proliferation and survival have also been identified that may allow lymphocytes to respond to information provided by the external environment.

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Abbreviations

bHLH	basic helix–loop–helix
BSAP	B-cell-specific activator protein
EBF	early B cell factor
FL	fetal liver
IgH	immunoglobulin heavy chain
Igκ	κ light chain
IL-7R	IL-7-receptor
LEF	lymphoid enhancer-binding factor
M-CSF	macrophage-colony-stimulating factor
Rags	recombinase activating genes
TCF	T-cell-specific transcription factor

Introduction

The development of committed B lineage cells from multipotent progenitors requires the coordinated activity of many transcription factors [1]. Analysis of the regulatory regions of B-lineage-associated genes, such as the

immunoglobulin heavy chain (IgH), the κ light chain (Igκ) and the mb-1/Igα gene, and analysis of differential gene expression have led to the identification of transcription factors that function in early B lineage cells. More recently, the creation of mice that lack expression of these transcription factors has provided a wealth of information regarding the requirements for each of these particular proteins in the regulation of B lymphocyte development. Interestingly, some of these factors are required before the activity of any of the B-lineage-associated genes that they regulate, indicating that novel targets remain to be identified. Elucidation of the processes in which these factors are involved and their essential target genes will provide insight into the mechanisms of lineage determination and early B lineage differentiation. These factors have been shown to function in a transcriptional hierarchy, and also in a combinatorial manner, to establish the expression of the genes that comprise the B lineage differentiation program.

In this review we will briefly discuss recent findings regarding the function and target genes of five transcription factors that are required for the proper development of early B lineage cells: PU.1; E2A; early B cell factor (EBF); B-cell-specific activator protein (BSAP)/Pax5; and lymphoid enhancer-binding factor (LEF)-1. Figure 1 depicts the approximate developmental stage at which each of these factors is known to function during B lineage commitment and differentiation, as elucidated from studies of gene-ablated mice.

PU.1

PU.1 is a member of the *ets* family of transcription factors that is expressed exclusively in hematopoietic cells [2]. PU.1 expression is highest in macrophages but can also be

Figure 1

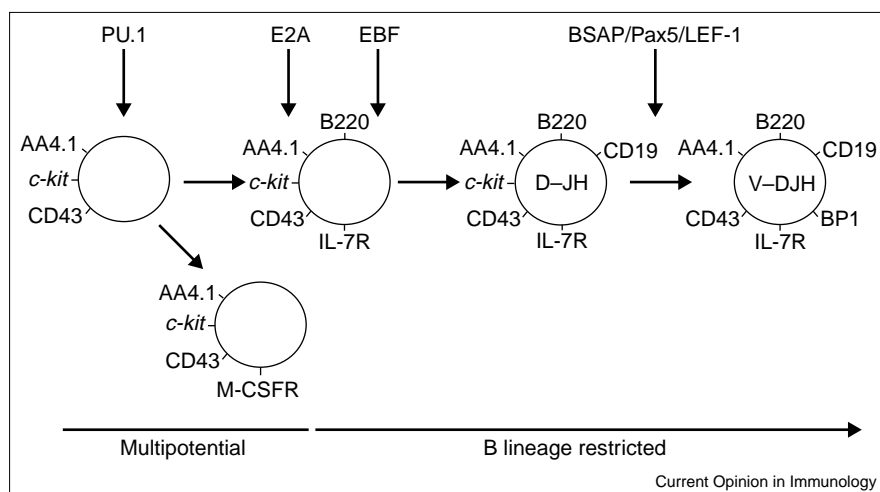


Diagram of the transcription factors required during commitment to the B lineage. Progression from multipotent progenitors to committed B lineage progenitors is depicted. The approximate expression stage of a number of cell surface molecules and rearrangements of the *IgH* loci, which are used to define the stage of differentiation of B lineage progenitors are indicated. Multipotent progenitors are shown giving rise to B lymphocytes (top row) and macrophages (below). The vertical arrows denote the stage of development at which each of the indicated transcription factors is required, as elucidated from studies of gene-ablated mice.

detected in neutrophils, B and early T lymphocytes [2,3]. Two strains of PU.1-deficient mice have been created, both of which show a similar hematopoietic phenotype. The mutation produced by Scott *et al.* [4] results in mice that die at embryonic day 18 and lack macrophages, neutrophils and lymphocytes but contain normal erythrocytes and megakaryocytes. This phenotype is due to defects in the hematopoietic cells themselves and not in the ability of the microenvironment to support hematopoiesis [5]. The PU.1 mutation produced by McKercher *et al.* [6] results in mice that survive for 48 hours after birth but that subsequently die from bacterial infection although treatment with antibiotics can extend their lifespan. These PU.1-deficient mice also lack macrophages, neutrophils and lymphoid cells at birth but T lymphopoiesis and a few neutrophils can be detected in the postnatal period [6]. The absence of lymphoid and myeloid cells in PU.1-deficient mice suggests that PU.1 is required for the development of a progenitor that is common to both the lymphoid and myeloid lineages. Nonetheless, PU.1 may also play a role in the commitment of such progenitors to differentiation down particular hematopoietic lineages, because PU.1 is sufficient to induce myeloid development in chicken multipotent progenitor cell lines [7].

One mechanism by which PU.1 may regulate lymphoid and myeloid development is through transactivation of the receptors encoding lineage-associated growth and survival factors. PU.1 has been implicated in the regulation of expression of the IL-7-receptor (IL-7R), which is required for proper lymphoid development, and the macrophage-colony-stimulating factor (M-CSF), which is required for proper macrophage development [8,9^{••}]. DeKoter and Singh [9^{••}] showed recently that the level of PU.1 expression may determine whether a multipotent progenitor commits to differentiation down the B lymphocyte or macrophage lineage. These authors showed that retroviral infection of PU.1-deficient fetal liver progenitors with a PU.1-expressing retrovirus is sufficient to rescue the development of B lymphocytes and macrophages. The level of PU.1 expression is significantly lower (about five-fold), however, in the resulting B lymphocytes than in the macrophages, even though PU.1 expression is driven by the viral promoter in both cell types [9^{••}].

Expression of PU.1 in PU.1^{+/-} fetal liver (FL) progenitors inhibits the development of B lymphocytes, leading to an increase in macrophages over B cells in the culture, suggesting that B lineage cells do not tolerate high levels of PU.1. Interestingly, a PU.1 transactivation mutant that has reduced activity is highly expressed in B lineage cells and promotes B lymphocyte over macrophage differentiation [9^{••}]. This finding is consistent with the hypothesis that the lower levels of wild-type PU.1 protein in B lineage cells reflects selection against high levels of PU.1 activity. Therefore, one central determinant in B lymphocyte differentiation, as compared with macrophage differentiation, may be related to the expression level of PU.1.

E2A

The E2A gene codes for two proteins, E12 and E47, that are members of the basic helix–loop–helix (bHLH) family of transcription factors [10]. The E2A proteins belong to a subgroup of bHLH proteins, referred to as E-proteins, that includes HEB and E2-2 [11]. These E-proteins bind to a common ‘E-box’ motif, 5'-CANNTG-3', and form homo- and hetero-dimers with other bHLH proteins. E12 and E47 are expressed in many cell types but form a unique homodimeric complex only in B lineage cells [12,13]. Most E2A-deficient mice die before or shortly after birth although some of these animals do survive to adulthood [14,15]. In the absence of E2A, B lymphocytes fail to develop and T cell development is significantly perturbed ([14–16]; for reviews, see [17,18]). The bone marrow and FL of E2A-deficient mice lack CD19⁺ cells, and rearrangement of the *IgH* loci can not be detected. In addition, few B-lineage-associated genes are expressed, with the exception of germline *IgH* transcripts and the signal transducing molecule, B29/Ig β [14,19]. Interestingly, transgenic expression of either E12 or E47 is sufficient to promote the development of B lineage cells, which indicates that these proteins are functionally redundant with respect to their roles at this stage of development [19]. In fact, it appears that any E-protein can partially replace the function of E2A at this stage, as a targeted replacement of the E2A gene with an HEB cDNA is sufficient to rescue the phenotype of E2A-deficient mice [20].

The E2A proteins are involved in the expression and rearrangement of the *IgH* and *Ig κ* gene loci, and are probably required for the proper expression of the *recombinase activating genes (Rags)* [16,21[•],22–24]. Interestingly, the transcription factor EBF, which is required at a similar developmental stage as E2A, and a number of other B-lineage-associated proteins are induced in a macrophage cell line after transfection with E12 [24]. E2A and early B cell factor (EBF) are required, and function cooperatively, to promote the development of B lineage cells at subsequent stages ([25[•]]; see below). Expression of the gene for the surrogate light chain protein, $\lambda 5$, and rearrangement of the *Ig* loci, are probable targets of the coordinated activity of E2A and EBF [21[•],26].

Early B cell factor

EBF is a member of a unique family of transcriptional regulatory proteins, the O/E proteins, that are expressed in olfactory neurons, adipocytes and B lymphocytes [27,28]. To date, three O/E proteins have been cloned but EBF is the only member of this family identified in B lineage cells. EBF is expressed at all stages of B lineage differentiation, with the exception of terminally differentiated plasma cells [28]. EBF-deficient mice have no mature B lineage cells and few, if any, B lineage progenitors. Few B-lineage-associated genes can be detected in the bone marrow of these mice, with the exception of germline transcripts for *Ig μ* and IL-7R, leading Lin and Grosschedl [29] to propose that the developmental arrest is after commitment to the B lineage.

In light of recent data regarding the role of Pax5 in B lineage commitment and the absence of Pax5 transcripts in EBF-deficient mice, however, the level of B lineage commitment in these mice remains in question.

EBF binds the palindromic sequence 5'-ATTCCCN-NGGGAAT-3' through an amino-terminal cysteine-rich DNA-binding domain that contains a zinc coordination motif [28,30,31]. EBF binds DNA as a homodimer, and dimerization is mediated by a domain in the carboxy-terminal portion of the protein that shows some similarity to the helix-loop-helix domain [28]. Surprisingly, however, the DNA-binding domain itself can mediate dimerization on properly spaced half-sites and also has some inherent transactivation capacity [31]. EBF was originally cloned on the basis of its ability to interact with sites in the mb-1/Ig α promoter, which drives the expression of a component of the signal transducing machinery of IgH [32]. Functional EBF-binding sites have also been identified in the promoters for Pax5, B29, λ 5 and Ig κ [25^{*},26,33]. Consistent with the presence of EBF-binding sites in some Ig κ promoters, Romanow *et al.* [21^{*}] have found that EBF is able to induce V-J rearrangement of certain Ig λ genes when transfected into BOSC cells (a human kidney epithelial cell line) along with the *Rag* gene products Rag-1 and Rag-2.

The development of early B lineage progenitors is under the coordinate control of E2A and EBF. The latter is a likely target of E2A, and mice that lack one copy of E2A and EBF (E2A^{+/-}EBF^{+/-}) show a substantial decrease in B lymphopoiesis at a slightly later stage than that caused by deletion of either gene [25^{*}]. E2A^{+/-}EBF^{+/-} mice have a decreased number of B220⁺CD43⁺ cells in fetal liver at day 17.5 of gestation, almost no BP-1⁺ cells and a three-fold reduction in CD19-expressing cells, indicating a decreased number of late pro-B cells. Interestingly, a decrease in a number of B-lineage-associated genes was detected, however, single-cell PCR indicates that for many genes it is the frequency of expressing cells that is decreased rather than the level of expression per cell. Therefore, the main defect in E2A^{+/-}EBF^{+/-} mice may be at the level of cell survival or proliferation rather than at the level of differentiation.

B-cell-specific activating protein/Pax5

BSAP is the product of the Pax5 gene, a member of the Pax family of transcription factors, and is expressed in all B lineage cells with the exception of plasma cells ([34]; for a review, see [35]). Mice that carry a targeted deletion of the Pax5 gene have no mature B lymphocytes, and B cell development in the bone marrow is arrested at an early developmental stage [36]. In the absence of Pax5, B lineage progenitors are able to rearrange the D-J segment of the *IgH* locus but there are no detectable V-DJ rearrangements [36,37]. These mice lack CD19⁺ cells and cells fail to proliferate in the presence of IL-7 alone although stromal cells plus IL-7-responsive progenitors are present [37]. In addition, most B-lineage-associated genes are expressed normally with the exception of CD19, *N-myc*, mb-1/Ig α

and LEF-1, which have all been shown to be Pax5-responsive target genes [38]. The defect in Pax5-deficient mice is not solely the result of the inability to rearrange and express IgH, because IgH-deficient mice show an arrest at a later developmental stage, and expression of an IgH transgene in Pax5-deficient pro-B lymphocytes does not rescue B cell development [39]. Interestingly, B lymphopoiesis in Pax5-deficient mice is arrested before the initiation of D-JH rearrangement in FL. This earlier developmental arrest appears to be due to a defect in the hematopoietic microenvironment, rather than being intrinsic to FL-derived progenitors, as transplantation of Pax5-deficient FL-derived progenitors into adult recipients results in B lymphopoiesis that is arrested after the D-JH rearrangement stage [37].

Wallin *et al.* [40^{**}] have shown that, in addition to being a transcriptional activator, BSAP/Pax5 can function as a transcriptional repressor. The repressor and activator functions are dependent on the concentration of Pax5 protein and the context of the Pax5-binding site. Such sites that activate gene expression, such as those found in the CD19 and Ige promoters, have higher affinity for Pax5 than repressive sites, such as the site in the J chain promoter. Therefore, Pax5 activator functions predominate at low concentrations of Pax5 protein, and Pax5 will function as a repressor only at higher concentrations. This is an important observation given the central role of Pax5 repressor activity in the commitment of multipotential progenitors to the B lineage.

Busslinger and co-workers [41^{**},42^{**}] have shown that in the absence of Pax5, progenitors do not become committed to the B lineage, even though they have the phenotype of committed B lineage progenitors. An examination of gene-expression patterns in Pax5-deficient pro-B lymphocytes showed that in the absence of Pax5, these cells continued to express a number of genes associated with non-B lineage cells such as M-CSF, GM-CSF, myeloperoxidase, perforin, pre-T α and GATA-1 [41^{**}]. Therefore, Pax5 appears to have an essential role in the commitment process by repressing the expression of genes that function in the differentiation of alternative cell lineages. Consistent with role of BSAP in B lineage commitment, the Pax5 gene is expressed mono-allelically [43^{*},44].

Lymphoid enhancer-binding factor

LEF-1 is a member of the T-cell-specific transcription factor (TCF)/LEF family of high mobility group box-binding proteins, which includes TCF-1, TCF-3 and TCF-4 [45]. LEF has very little transactivation capacity on its own but can act as an architectural protein, causing increased bending in DNA such as that observed in the TCR- α enhancer [46]. Recently, however, LEF-1 has been shown to be the target of the Wnt signaling pathway. Signal transduction in response to Wnt binding to its receptor, Frizzled, leads the stabilization of cytosolic β -catenin, which then accumulates in the nucleus and functions as a co-activator for LEF-1 transcriptional responses [47-49].

This signal transduction pathway has proved to be very important in a number of developmental processes and in many forms of cancer [50]. Wnt signaling may also be involved in the development of some forms of pre-B acute lymphoblastic leukemia (ALL), given that Wnt16 was identified as a target of the E2A-Pbx1 oncoprotein which is associated with a subset of pre-B ALL [51].

LEF is expressed in pro-B and pre-B cells, but not immature or mature B lymphocytes [52*]. In the absence of LEF-1, B lymphocyte differentiation appears to be normal although fewer pro-B and pre-B lymphocytes develop. This decrease in B lineage cell numbers is the result of a decrease in proliferation and survival of early B lineage progenitors. LEF-1-deficient pro-B lymphocytes have an increased level of mRNA encoding Fas and *c-myc*, both of which could potentially lead to an increase in apoptosis [52*]. Surprisingly, however, the frequency of progenitors that respond to IL-7 — a growth and survival factor required at this stage — is normal [52*,53]. LEF-1-deficient pro-B cells do show a reduced response to Wnt3A, which can induce DNA synthesis in this population *in vitro* [52*]. These data suggest that Wnt signaling may be important for the development of B lineage progenitors.

Conclusions

Recent studies from gene-ablated mice have led to significant insights into the mechanism by which transcriptional regulatory proteins control B lineage commitment and differentiation. Early B lineage transcription factors clearly function in a transcriptional hierarchy, leading from E2A to induction of EBF followed by induction of Pax5 and LEF-1 [18,54]. It remains to be determined whether PU.1 might function in the activation of E2A in this transcriptional cascade.

Of these transcription factors, the role of Pax5 in B lineage commitment has been studied most thoroughly owing, in part, to the ability to expand Pax5-deficient B lineage cells *in vitro* in the presence of IL-7 [37]. Pax5 has been shown to be critical for the full commitment to the B lineage, and the absence of Pax5 transcripts in PU.1-, E2A- and EBF-deficient mice indicates that each of these mutations may be affecting the ability of progenitors to commit to the B lineage. Future studies will be directed toward the identification of the critical target genes that regulate the growth and survival of B lineage progenitors and establish the committed state.

Acknowledgements

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