Published in final edited form as:
Semin Immunol. 2008 August ; 20(4): 221–227. doi:10.1016/j.smim.2008.07.004.

Early B cell Factor: regulator of B lineage specification and commitment

Kara Lukin, **Scott Fields**, **Jacqueline Hartley**, and **James Hagman***
Integrated Department of Immunology, National Jewish Medical and Research Center, Denver, CO 80206, USA

Abstract

B lymphocytes are generated from hematopoietic stem cells in a series of steps controlled by transcription factors. One of the most important regulators of this process is Early B cell Factor (EBF). Multiple lines of evidence indicate that expression of EBF is a principle determinant of the B cell fate. In the absence of EBF, progenitor cells fail to express classical markers of B cells, including immunoglobulins. EBF drives B cell differentiation by activating the *Pax5* gene and other genes required for the pre-B and B cell receptors. New evidence suggests that expression of EBF in common lymphoid progenitors directs B cell fate decisions. Specification and commitment of cells to the B cell lineage are further established by Pax5, which increases expression of EBF. Recently, it was demonstrated that *both* EBF and Pax5 contribute to the commitment of cells to the B lineage. Together, these studies confirm that EBF is a keystone in a regulatory network that coordinates B cell lineage specification and commitment.

1. Introduction

The development of leukocytes from hematopoietic stem cells (HSCs) is characterized by the expression of distinct sets of genes at discrete stages of differentiation (Fig. 1). To generate early lymphoid progenitors (ELPs), HSCs with long- or short-term repopulating activities (LTRC and STRC) differentiate through intermediate stages that possess progressively restricted developmental potential. ELPs seed the thymus and may generate early T cell progenitors (ETPs; reviewed in [1]). ELPs are also the precursors of common lymphoid progenitors (CLPs), which express interleukin-7 receptors (IL-7R) and engender B and T lymphocytes, natural killer (NK) cells and dendritic cells, but lack the ability to produce other hematopoietic lineages (i.e. myeloid cells). Although this process is incompletely understood, it is proposed that the maturation of progenitor cells is a tightly controlled process governed by a select set of transcriptional regulators. These regulators activate successive developmental programs while progressively limiting potential cell fates.

Differentiation of progenitors to B cells is heralded by the expression of cell surface markers including B220, CD43 and IL-7Rα (encoded by the *Il7ra* gene). Expression of these genes precedes the commencement of V(D)J recombination which results in immunoglobulin (Ig) gene rearrangements. In addition to *Ig* genes, the expression of accessory proteins is required for display of the pre-B and B cell receptors (pre-BCR and BCR) on the plasma membranes of pre- or immature B cells, respectively. Competent BCR complexes mediate the selection of functional B cells. These cells migrate from the bone marrow to peripheral lymphoid organs including the spleen, lymph nodes and gut-associated lymphoid tissues. At these secondary

*Corresponding author. Address: 1400 Jackson Street, K516B, National Jewish Medical and Research Center, Denver, CO 80206 USA, Phone: (303)398-1398, Fax: (303)398-1396, E-mail address: hagmanj@njc.org (James Hagman).

sites, stimulation of B cells by antigens results in antibody production by plasma cells. Activated B cells also generate memory cells that facilitate rapid immune responses to repeated challenges by the same antigens.

The earliest definable stages of B cell development are characterized by expression of transcriptional regulatory proteins, which initiate the B cell-specific program, or 'transcriptome,' via targeted gene activation and repression [2,3]. Recent research has provided new insights as to how these proteins (including PU.1, Ikaros, EBF, E2A, and Pax5) function within an interactive network of regulators. Here, we focus on the roles of EBF, which has recently gained additional significance as a driver of both B lineage determination and commitment.

2. EBF structure, DNA binding and functions

Given its proposed role as a key determinant of the B cell fate, the biochemistry of EBF is of considerable interest. EBF and closely related proteins (EBF2, EBF3, EBF4, Collier/Knot and Unc-3) constitute a novel transcription factor family (here, termed the EBF family; also referred to as the O/E or COE family). All members of the EBF family possess a highly conserved DNA-binding domain (DBD) that is distinct from that of other known DNA-binding proteins (Fig. 2). The DBD of murine EBF comprises residues 35–251 [4,5]. EBF binds to sequences of promoters that loosely fit the consensus 5′-CCCNNGGG-3′; however, the DBD can recognize 14 basepairs centered over this sequence [6]. Although the three dimensional structure of the EBF DBD has not been determined, a notable feature of this domain is an atypical zinc-binding motif: HEIMCSRCCDKKSC (bold residues coordinate zinc; Fig. 2B) [5]. Recent studies demonstrated the importance of this motif, termed the 'zinc knuckle', for binding to divergent, target-specific nucleotide sequences [7]. These studies also revealed the complexity of amino acid requirements for DNA recognition by EBF. Enforced expression of EBF (together with Pax5) in terminally differentiated 558LμM plasmacytoma cells restored expression of endogenous *Cd79a* (*mb-1*) and *Vpreb1* genes. *Cd79a* (*mb-1*/Igα) and *Vpreb1* encode components of the pre-BCR. Igα is also a component of the BCR. Activation of each of these genes required residues involved in zinc coordination; however, other residues within and flanking the zinc knuckle were differentially required for activating *mb-1* vs. *Vpreb1* transcription. These results suggest that, similar to the flexible recognition of DNA by Pax5 [8], EBF utilizes different sets of residues to bind a degenerate set of DNA sequences.

EBF persists as homodimers in solution and when bound to DNA. Homodimerization is a function of the helix-loop-helix (HLH) domains of EBF similar to those of the basic-HLH proteins c-Myc and MyoD1 [4,9]. Dimerization is also facilitated by sequences within the DBD that are as yet unidentified. A dimerization function was also proposed for the 'transcription factor-Ig-like' (TIG) or immunoglobulin-plexin transcription factor (IPT) domain. The TIG/IPT domain links the DBD and HLH domains of EBF [10]; however, functions of this domain have not been determined. EBF also includes a C-terminal activation domain [5]. This domain is not required for transcription from the *Cd79a* or *Vpreb1* promoters [7].

Recent studies determined that EBF functions as an epigenetic pioneer factor, which is defined as a DNA-binding protein that can direct modifications of chromatin that are permissive for transcriptional activation [11,12]. Hypermethylation of *Cd79a* promoters was detected in precursor cells ex vivo, but these promoters were completely unmethylated in committed B cells [13]. Interestingly, promoters in B cell progenitors lacking EBF failed to become unmethylated. The ability of EBF to initiate DNA demethylation and enhance chromatin accessibility of *Cd79a* promoters was demonstrated in 558LμM plasmacytoma cells. In this context, the opening of chromatin by EBF was necessary for its cooperation with Pax5. In turn, Pax5 recruited Ets family transcription factors necessary for efficient *Cd79a* transcription.

Thus, EBF is necessary for both the expression and function of Pax5. In more recent experiments, we confirmed that *Cd79a* promoter chromatin is progressively 'opened' in response to EBF (and Pax5) in vivo (Hua Gao, J.H., unpublished data). The pioneer effects of EBF are likely to be important for activating many genes of the B cell-specific program including the rearrangement and expression of *Ig* genes.

3. Temporal regulation and control of EBF expression

Because EBF is a critical determinant of B cell fate decisions, it is important to determine when EBF is first expressed during B lymphopoiesis. Using RAG1/GFP 'knock-in' mice, Igarashi and colleagues [14] detected *Ebf1* transcripts at high levels in $Lin^{-}CD27^{+}c\text{-}kit^{Lo}Sca\text{-}1^{Lo}GFP^{+}$ bone marrow cells representing CLPs and subsequent B cell progenitors. These cells exhibited B lymphoid and NK cell potential, but had greatly reduced myeloid potential. Detection of *Ebf1* transcripts was coincident with *IL-7ra* transcripts. Earlier stage $Lin^{-}CD27^{+}c\text{-}kit^{Hi}Sca\text{-}1^{Hi}$ GFP^{+} cells expressed barely detectable levels of *Ebf1* transcripts and gave rise to T lineage cells in addition to other cell types. These populations suggest the presence of different (or divergent) pathways to B (and NK) cells vs. T cells, with high levels of EBF promoting the B cell pathway.

Other studies have demonstrated the linkage between expression of EBF in CLPs and IL-7 signaling. CLPs from IL-7-deficient mice expressed lower amounts of *Ebf1* transcripts together with greatly reduced B cell potential [15]. Restoration of EBF expression in these mice restored B cell potential in colony forming assays. Similar results were obtained using IL-7- or IL-7Rα-deficient mice in which *Ebf1* transcript levels were similarly decreased in the absence of IL-7 signaling [16]. Pre-pro-B cells of IL-7-deficient mice expressed only low levels of *Ebf1* transcripts. These transcripts were upregulated following the addition of IL-7 in vitro. Moreover, effects of IL-7 signaling could be mimicked by enforced expression of active STAT5 (the nuclear mediator of IL-7 signaling). Enforced expression of EBF in pre-pro-B cells of IL-7Rα-deficient mice partially rescued B cell development in the absence of IL-7 signaling. Together, these results suggest that EBF is first expressed at significant levels in early progenitors (CLPs) in response to IL-7 signaling. This hypothesis was further supported by microarray and real time PCR studies that detected *Ebf1* transcripts at low levels in CLPs and at increasingly higher levels in progressively later stages of B cell development. The induction of *Ebf1* transcripts was coincident with the loss of other developmental potentials [17]. Microarray studies of purified human bone marrow cell populations suggested similar patterns of EBF expression [18]. The expression of EBF in CLPs suggests that it begins the process of B lineage specification in these cells. This model is supported by recent observations suggesting that EBF drives 'lineage priming' in CLPs, which results in expression of B cell markers (e.g. *CD79b*) [17,19,20]. Interestingly, EBF-deficient mice (first described in [21]) retained the ability to generate CLPs and cells with pre-pro-B cell characteristics ($B220^{+}CD43^{+}AA4.1^{+}$), but lacked expression of B lineage markers [19]. Thus, although EBF is detected in normal CLPs, it is not required for their development.

While the *Ebf1* gene is transcribed in multiple tissues including progenitor and mature B cells, adipocytes, forebrain neurons, plasmacytoid dendritic cells, bone marrow stromal cells and developing limb buds, the pattern of expression of *Ebf1* is tightly regulated [22-27]. Roessler and colleagues [28] identified two functional promoters within the *Ebf1* gene, termed *Ebf1α* (−5113 to −4027) and *Ebf1β* (−1671 to +1), that function in B cells. These promoters produce distinct transcripts encoding two isoforms of EBF, EBF1α and EBF1β, which differ in their first 14 amino acids. Transactivation assays suggested that the two EBF isoforms have similar abilities to activate transcription; however, the two *Ebf1* promoters are differentially regulated. Transcription from the *Ebf1β* promoter is dominant at each stage of normal B cell development. Experiments suggest that the *Ebf1* gene's responsiveness to cytokines is a property of the

Ebf1α promoter, but not of the *Ebf1β* promoter. In Ba/F3 cells, enforced expression of constitutively active STAT5 or stimulation with IL-3 (which also functions via STAT5) increased expression of the endogenous *Ebf1* gene by 9-fold and 66-fold, respectively. However, evidence was not obtained for direct binding of STAT5 to either *Ebf1* promoter. This suggests that STAT5 may regulate *Ebf1* gene transcription indirectly by enhancing the expression of another activator. The *Ebf1α* promoter was also activated by E47. E47 synergistically activated this promoter together with EBF (EBF1β) as part of a potential autoregulatory loop. These data are supported by previous studies linking E2A (E47) and EBF with the control of *Ebf1* transcription [29]. More recently, Kwon and colleagues demonstrated that *Ebf1* transcription requires E2A for both its initiation and maintenance [30].

Other data suggest that *Ebf1β* promoter transcription is regulated by a positive feedback loop involving PU.1, Pax5 and Ets proteins [28,31]. Medina and colleagues demonstrated that the absence of PU.1 results in greatly reduced numbers of $Flk2^{+}/Flt3^{+}IL\text{-}7R^{+}$ lymphoid progenitors and impaired expression of EBF [31]. Restoration of PU.1 expression induced expression of EBF. Furthermore, a functional PU.1 binding site was localized in the *Ebf1* gene downstream of the *Ebf1β* promoter. Interestingly, the *Ebf1β*, but not the *Ebf1α* promoter is activated by PU. 1 [28]. The *Ebf1β* promoter was also synergistically activated by co-expression of Pax5 and the winged helix protein Ets-1. Unlike the cooperative interactions observed between Pax5 and Ets-1 on the *mb-1* promoter [32], Ets-1 bound the *Ebf1β* promoter independently of Pax5 in vitro. In further support of Pax5's role in the regulation of *Ebf1* transcription, *Ebf1* transcripts are expressed at peak levels in pro-B and pre-B cells coincidently with the onset of Pax5 expression [17,28]. Because EBF directly regulates Pax5 transcription, these data suggest a role for Pax5 in a positive feedback mechanism. In mice, levels of *Ebf1* transcripts are reduced in Pax5-deficient proB cells [28,33]. Pax5-dependent activation of the *Ebf1* gene may be due in part to an epigenetic mechanism because expression of Pax5 directed the early replication of the *Ebf1* locus in S phase [28]. The early replication of genes reflects the presence of epigenetic modifications indicative of a 'transcription permissible' chromatin structure.

The cascade of positive regulators of *Ebf1* transcription would suggest that once EBF is made in CLPs, the cells proceed unabatedly towards the B cell fate. However, pathways that act to silence EBF expression would consign cells to other lineage fates (e.g. NK or T lymphoid fates). In this regard, the *Ebf1* gene has been identified as a target of negative regulation by Notch1 signaling. The ability of Notch1 signaling to drive T cell development at the expense of B cell development is well documented (reviewed in [34]). As a possible mechanism to account for its inhibition of B cell development, expression of intracellular Notch1 in a pre-B cell line reduced both DNA binding by EBF and promoter activity of EBF-target genes [35]. EBF counters these effects through its activation of Pax5 expression, which in turn represses transcription of *Notch1* [36]. The importance of this regulatory circuit is made clear by studies in Pax5-deficient mice. In the absence of continuous Pax5 expression, pro-B cells down-regulated B cell-specific genes in response to Notch signaling [37]. Furthermore, transient expression of Notch1 increased the promiscuous differentiation of Pax5-deficient pro-B cells to NK cells [38,39]. Thus, B lineage specification is dependent on the expression of EBF, which is supported by IL-7 signaling, E2A and Pax5 in early B cell progenitors.

4. EBF drives the B cell-specific transcriptional program

The ability of EBF to initiate activation of B cell-specific genes has been explored in vivo and in vitro using EBF-deficient progenitor cells. As described above, the lack of EBF results in the early arrest of B cell development [21]. Enforced expression of EBF in HSCs prior to their transfer into irradiated mice resulted in biased developmental outcomes, as evidenced by overproduction of B cells (some myeloid cells were generated as well) [40]. In other experiments, enforced expression of EBF in PU.1-deficient multipotent progenitors activated

the expression of transcripts indicative of B lineage commitment (*Pax5* and *Cd19*)[31]. In addition, retroviral expression of EBF in $B220^{+}IL\text{-}7R^{+}$ progenitors of EBF-deficient mice similarly induced markers of active B lymphopoiesis and commitment. In contrast, enforced expression of Pax5 in such systems did not restore B lymphopoiesis. Although EBF functions cooperatively with E2A, B lymphopoiesis was largely restored following enforced expression of EBF in E2A-deficient progenitor cells [41]. Thus, EBF is critical for the generation of B cell precursors. Interestingly, a recent study demonstrated EBF's ability to restore B cell development in LSK cells lacking Ikaros (*Ikzf1*$^{-/-}$) [42]. However, the resulting cells were not committed, suggesting that EBF (and Pax5, which was co-expressed) could not fully activate the entire B cell program in this context.

EBF's ability to restore the B cell program is due partially to the fact that EBF target genes encode proteins required for survival signals that mediate developmental checkpoints (i.e. components of the pre-BCR and BCR). Biochemical studies identified functionally important EBF binding sites in promoters of the *Cd79a* (mb-*1*;Igα), *Cd79b* (*B29*; Igβ), *B lymphoid kinase* (*Blk*), *Vpreb1*, *Igll1* (γ*5*) and *Cd19* genes [43–48]. Many of these genes were not expressed in *Ebf1* knockout mice [21]. Other identified targets of EBF included *Pax5* [49]. More recently, microarray and biochemical analysis of Ba/F3 cells transduced to express EBF identified the *Cd53* and *carcinoembryonic antigen-related cell adhesion molecule-1* genes as targets of EBF in B cells [50]. Microarray analysis of EBF-deficient CLPs before and after reconstitution to express EBF revealed additional EBF targets including *Pou2af1* (encoding the OcaB transactivator) and *FoxO1* [19], a forkhead family factor involved in the control of B cell signaling and proliferation.

One of the hallmarks of EBF function is its cooperation with other regulators of B cell development. In particular, functional interactions between EBF and E2A (E47) constitute a well-documented regulatory pathway. The two proteins bind together on promoters of the *Cd79a*, *Vpreb1* and *Igll1* genes [47,51–53]. EBF and E47 also function together in the activation of V(D)J recombination. Here, they increase the local accessibility of *Ig* gene segments [54,55]. In other partnerships, EBF activates transcription of the *mb-1* gene synergistically with E2A, Runx1 and Pax5 [13,51]. A similar constellation of factors activates the *Cd19* promoter together with EBF [56]. A recent report suggested that EBF regulates transcription of the *Igll1* promoter by binding alternately with Ikaros family zinc finger proteins [57]. In this model, EBF appears to compete with Ikaros (*Ikzf1*) for *Igll1* promoter binding in early pre-B cells. Silencing of the promoter is thought to occur in late pre-B cells following the up-regulation of a second Ikaros family member, Aiolos (*Izkf3*), in response to pre-BCR signaling. This model suggests an interesting new paradigm in which transcriptional activation or repression results from competition between multiple transcription factors for binding sites during B cell differentiation.

5. Control of B lineage commitment by EBF and Pax5

A substantial body of literature defines Pax5 as the B cell lineage commitment factor. This is largely due to the highly significant studies of the phenotype of Pax5-deficient mice. In these mice, B cell development is arrested at a pro-B cell-like stage. Interestingly, Pax5-deficient pro-B cells exhibit promiscuous gene expression and a lack of lineage commitment [58,59]. In these studies, Pax5-deficient pro-B cells were shown to have the capacity to differentiate into a variety of non-B cell lineages, including NK cells, macrophage/myeloid cells, granulocytes, osteoclasts and T cells. Further studies revealed that continuous Pax5 expression is required to maintain B cell identity [60–62].

Previously, it was believed that the role of EBF in B lineage commitment was limited to its activation of Pax5 transcription. However, a recent study [33] revealed a new complexity of

cell fate decisions during B lymphopoiesis by suggesting that EBF and Pax5 function together to mediate B cell lineage commitment (Fig. 3). In appropriate culture conditions, *Ebf1*-deficient lymphoid progenitor cells can be induced to differentiate into granulocytes and macrophages in vitro. Reconstitution of lethally irradiated mice with *Ebf1*-deficient lymphoid progenitor cells resulted in production of myeloid cells, NK cells, dendritic cells and T lineage cells in vivo. Expression of EBF blocked promiscuous differentiation in a dosage-dependent manner. This is similar to the responses of multipotent progenitors to graded levels of PU.1 [63]. Limiting dilution analysis of progenitors transduced to express EBF or Pax5 demonstrated a relatively greater ability of EBF to drive the B cell fate at the expense of the myeloid cell fate. Importantly, EBF possesses the ability to reprogram myeloid progenitors. This activity may be due to the attenuation of C/EBPα (*Cebpa*) and PU.1 (*Sfpi1*) [33], which synergistically activate myeloid differentiation [64–66]. High levels of EBF resulted in the expression of B cell-specific genes, including *Igll1*, *Vpreb1*, *Cd79a* and *Cd79b* [33]. Enforced expression of E47 or Pax5 did not restore B cell-specific gene expression efficiently in *Ebf1*-deficient lymphoid progenitors. Furthermore, in pre-pro-B cells of *IL-7*$^{-/-}$ mice, the B cell program was established in a dose-dependent fashion by EBF [67]. In this study it was concluded that IL-7 is responsible for maintaining sufficient levels of EBF for B cell lineage specification.

EBF can drive B cell differentiation, but does it actively mediate B cell lineage commitment? Recent experiments demonstrated that EBF promotes B cell fates in the absence of Pax5 [33]. Enforced expression of EBF in Pax5-deficient pro-B cells blocked promiscuous differentiation under conditions that favor myeloid cell development in vitro. Thus, EBF enforced B cell specification and blocked alternative differentiation pathways. This is likely due to EBF's abilities to 1) activate expression of critical genes in the B cell-specific lineage program and 2) repress other lineage programs. Concerning the latter function, it was proposed that EBF directly represses transcription of genes, including *Id2* and *Cebpa*, that promote alternative fates in Pax5-deficient pro-B cells. DNA binding of the *Id2* promoter by EBF, which may result in its silencing, was detected in a B cell line. However, details of gene silencing in response to EBF are currently unknown. It will be important to determine whether EBF, like Pax5 [68,69], has the ability to activate or repress transcription in different contexts. Together, these data demonstrate the unique functions of EBF in B lineage specification and commitment; however, other data suggest that EBF (and Pax5) may be insufficient for commitment in the absence of other factors (e.g. Ikaros; [42].

6. EBF and origins of disease

The reduction of EBF expression may result in the development of disease in B lineage cells. For example, Hodgkin lymphoma Reed-Sternberg cells have a B cell origin [70]. These tumor cells have silenced the expression of many markers of B cells, including *CD79a*, *CD79b*, *Blk*, *CD53* and *Pou2af1* [71]. Recent data suggest that the phenotype of Hodgkin lymphomas is the result of aberrant Notch signaling [72]. Consequences of the over-expression of Notch1 may include antagonism of EBF (and E2A), which in turn interferes with normal B cell identity.

Another potential link between EBF and disease was indicated by studies of the core-binding factor fusion protein Cbfβ-SMMHC, which results from a chromosomal inversion (creating a *Cbfb-MYH11* fusion gene) associated with acute myeloid leukemia (AML). A conditional knock-in allele in mice that resulted in expression of Cbfβ-SMMHC in myeloid cells created an abnormal myeloid progenitor population [73]; however, Cbfβ-SMMHC also significantly reduced the production of B cells [20]. Expression of EBF target genes including *Cd79a*, *Igll1*, *Vpreb1* and *Blk* were reduced in CLPs from these mice. One explanation for these effects was the reduction of *Ebf1* transcripts by 60–80% in the mutant CLPs. Importantly, restoration of EBF expression, but not that of other factors in CLPs of Cbfβ-SMMHC-expressing mice restored B cell development. These data suggest that Cbfβ-SMMHC represses expression of

EBF. The mechanism of this repression is unknown, but it may involve regulation of the *Ebf1* gene by Runx family factors (Cbfβ is a co-factor of Runx proteins).

The development of leukemia may be a consequence of the loss of EBF expression. In a landmark study, eight cases of B cell progenitor acute lymphoblastic leukemia (ALL) demonstrated monoallelic deletions of *EBF1* genes in tumor cells [74]. The loss of *EBF1* genes was acquired somatically. In addition, the complete loss of *EBF1* genes was observed in some leukemic cells. These data suggest that EBF may function as a tumor suppressor. Other recent observations identified the *Ebf1* gene as a target of the activation-induced deaminase (AID) [75], which generates DNA breaks associated with translocations and lymphomagenesis [76]. These data suggest the importance of maintaining EBF expression in B cells and the potential for tumorigenesis when expression of EBF is impaired or lost.

7. Conclusions

Together, the studies described above suggest a model of B lineage specification that exploits a dynamic equilibrium between EBF and other factors in CLPs and early B lymphoid progenitors. The process begins in CLPs with the expression of low levels of EBF in response to PU.1 and IL-7 signaling. At these early stages of differentiation *Ebf1* may be repressed by Notch1 signaling and/or decreased IL-7 signaling. This would turn the cell towards other, non-B cell fates. Alternatively, in a permissive environment (in the absence of Notch signaling), EBF may amplify its own expression, which is enhanced further by E47 and by Pax5 following its activation in pro-B cells. In turn, expression of Pax5 negatively regulates Notch signaling by repressing expression of the Notch1 gene itself.

Many questions concerning EBF and its functions remain to be answered. What are the details of B cell lineage specification? Which genes are activated or repressed in response to EBF? Is EBF a direct repressor of transcription, or does it activate other transcriptional repressors? What are the details of epigenetic mechanisms that are modulated by EBF? Concerning the basis of lineage decisions, what is the role of the local microenvironment within the bone marrow? If a non-B cell fate is adopted by a CLP, how is the expression of EBF extinguished? What are the roles of other transcription factors (e.g. Bcl11a/Evi9, NF-κB2/p100 and Foxp1), which are required for normal *Ebf1* expression at early stages of development [77–79]? Have we only scratched the surface of relationships between EBF and human diseases? These and other questions will be addressed in the near future using a combination of biochemical and molecular methods, together with new mouse models that will enable the manipulation of EBF expression during early B cell development.

Acknowledgements

The authors wish to than Glen McConville for advice concerning figures.

References

1. Bhandoola A, von Boehmer H, Petrie HT, Zúñiga-Pflücker JC. Commitment and developmental potential of extrathymic and intrathymic T cell precursors: plenty to choose from. Immunity 2007;26 (6):678–89. [PubMed: 17582341]
2. Hagman J, Lukin K. Transcription factors drive B cell development. Curr Opin Immunol 2006;18(2): 127–34. [PubMed: 16464566]
3. Roessler S, Grosschedl R. Role of transcription factors in commitment and differentiation of early B lymphoid cells. Seminars Immunol 2006;18(1):12–9.
4. Hagman J, Belanger C, Travis A, Turck CW, Grosschedl R. Cloning and functional characterization of Early B-cell Factor, a regulator of lymphocyte-specific gene expression. Genes Dev 1993;7(5):760–73. [PubMed: 8491377]

5. Hagman J, Gutch MJ, Lin H, Grosschedl R. EBF contains a novel zinc coordination motif and multiple dimerization and transcriptional activation domains. EMBO J 1995;14(12):2907–16. [PubMed: 7796816]
6. Travis A, Hagman J, Hwang L, Grosschedl R. Purification of Early-B-cell Factor and characterization of its DNA- binding specificity. Mol Cell Biol 1993;13(6):3392–400. [PubMed: 8497258]
7. Fields A, Ternyak K, Ostraat R, Akerlund J, Hagman J. The 'zinc knuckle' motif of Early B cell Factor is required for transcriptional activation of B cell-specific genes. Mol Immunol. 2008in press
8. Czerny T, Schaffner G, Busslinger M. DNA sequence recognition by Pax proteins: bipartite structure of the paired domain and its binding site. Genes Dev 1993;7(10):2048–61. [PubMed: 8406007]
9. Wang MM, Reed RR. Molecular cloning of the olfactory neuronal transcription factor OLF-1 by genetic selection in yeast. Nature 1993;364(6433):121–6. [PubMed: 8321284]
10. Aravind L, Koonin E. Gleaning non-trivial structural, functional and evolutionary information about proteins by iterative database searches. J Mol Biol 1999;287(5):1023–1040. [PubMed: 10222208]
11. Cirillo LA, Zaret KS. An early developmental transcription factor complex that is more stable on nucleosome core particles than on free DNA. Molec Cell 1999;4(6):961–969. [PubMed: 10635321]
12. Xu J, Pope SD, Jazirehi AR, Attema JL, Papathanasiou P, Watts JA, Zaret KS, Weissman IL, Smale ST. Pioneer factor interactions and unmethylated CpG dinucleotides mark silent tissue-specific enhancers in embryonic stem cells. Proc Natl Acad Sci USA 2007;104(30):12377–82. [PubMed: 17640912]
13. Maier H, Ostraat R, Gao H, Fields S, Shinton SA, Medina KL, Ikawa T, Murre C, Singh H, Hardy RR, Hagman J. Early B cell factor cooperates with Runx1 and mediates epigenetic changes associated with *mb-1* transcription. Nat Immun 2004;5(10):1069–77.
14. Igarashi H, Gregory SC, Yokota T, Sakaguchi N, Kincade PW. Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. Immunity 2002;17(2):117–30. [PubMed: 12196284]
15. Dias S, Silva H Jr, Cumano A, Viera P. Interleukin-7 is necessary to maintain the B cell potential in common lymphoid progenitors. J Exp Med 2005;201(6):971–9. [PubMed: 15767371]
16. Kikuchi K, Lai AY, Hsu C-L, Kondo M. IL-7 receptor signaling is necessary for stage transition in adult B cell development through up-regulation of EBF. J Exp Med 2005;201(8):1197–203. [PubMed: 15837809]
17. Rumfelt LL, Zhou Y, Rowley BM, Shinton SA, Hardy RR. Lineage specification and plasticity in CD19- early B cell precursors. J Exp Med 2006;203(3):675–87. [PubMed: 16505143]
18. Hystad ME, Myklebust JH, Bø TH, Sivertsen EA, Rian E, Forfang L, Munthe E, Rosenwald A, Chiorazzi M, Jonassen I, Staudt LM, Smeland EB. Characterization of early stages of human B cell development by gene expression profiling. J Immunol 2007;179(6):3662–71. [PubMed: 17785802]
19. Zandi S, Månsson R, Tsapogas P, Zetterblad J, Bryder D, Sigvardsson M. EBF1 is essential for B-lineage priming and establishment of a transcription factor network in common lymphoid progenitors. Blood. 2008in press
20. Kuo YH, Gerstein RM, Castilla LH. Cbfbeta-SMMHC impairs differentiation of common lymphoid progenitors and reveals an essential role for RUNX in early B-cell development. Blood 2008;111(3): 1543–51. [PubMed: 17940206]
21. Lin H, Grosschedl R. Failure of B-cell differentiation in mice lacking the transcription factor EBF. Nature 1995;376(6537):263–7. [PubMed: 7542362]
22. Åkerblad P, Lind U, Liberg D, Bamberg K, Sigvardsson M. Early B-cell factor (O/E-1) is a promoter of adipogenesis and involved in control of genes important for terminal adipocyte differentiation. Mol Cell Biol 2002;22(22):8015–25. [PubMed: 12391167]
23. Dowell P, Cooke DW. Olf-1/Early B cell Factor is a regulator of glut4 gene expression in 3T3-L1 adipocytes. J Biol Chem 2002;277(3):1712–8. [PubMed: 11696544]
24. Garel S, Marin F, Mattéi MG, Vesque C, Charnay P. Ebf1 controls early cell differentiation in the embryonic striatum. Development 1999;126(23):5285–94. [PubMed: 10556054]
25. Pelayo R, Hirose J, Huang J, Garrett KP, Delogu A, Busslinger M, Kincade PW. Derivation of 2 categories of plasmacytoid dendritic cells in murine bone marrow. Blood 2005;105(11):4407–15. [PubMed: 15728131]

26. Lagergren A, Månsson R, Zetterblad J, Smith E, Basta B, Bryder D, Åkerblad P, Sigvardsson M. The Cxcl12, periostin, and Ccl9 genes are direct targets for Early B-cell Factor in OP-9 stroma cells. J Biol Chem 2007;282(19):14454–62. [PubMed: 17374609]
27. Mella S, Soula C, Morello D, Crozatier M, Vincent A. Expression patterns of the coe/ebf transcription factor genes during chicken and mouse limb development. Gene Expr Patterns 2004;4(5):537–42. [PubMed: 15261831]
28. Roessler S, György I, Imhof S, Spivakov M, Williams RR, Busslinger M, Fisher AG, Grosschedl R. Distinct promoters mediate the regulation of Ebf1 gene expression by interleukin-7 and Pax5. Mol Cell Biol 2007;27(2):579–94. [PubMed: 17101802]
29. Smith EMK, Gisler R, Sigvardsson M. Cloning and characterization of a promoter flanking the Early B cell Factor (EBF) gene indicates roles for E-proteins and autoregulation in the control of EBF expression. J Immunol 2002;169(1):261–70. [PubMed: 12077253]
30. Kwon K, Hutter C, Sun Q, Bilic I, Cobaleda C, Malin S, Busslinger M. Instructive role of the transcription factor E2A in early B lymphopoiesis and germinal center B cell development. Immunity 2008;28(6):751–62. [PubMed: 18538592]
31. Medina KL, Pongubala JMR, Reddy KL, Lancki DW, DeKoter RP, Kieslinger M, Grosschedl R, Singh H. Defining a regulatory network for specification of the B cell fate. Dev Cell 2004;7(4):607–17. [PubMed: 15469848]
32. Fitzsimmons D, Hodsdon W, Wheat W, Maira S-M, Wasylyk B, Hagman J. Pax-5 (BSAP) recruits Ets proto-oncogene family proteins to form functional ternary complexes on a B-cell-specific promoter. Genes Dev 1996;10(17):2198–211. [PubMed: 8804314]
33. Pongubala JM, Northrup DL, Lancki DW, Medina KL, Treiber T, Bertolino E, Thomas M, Grosschedl R, Allman D, Singh H. Transcription factor EBF restricts alternative lineage options and promotes B cell fate commitment independently of Pax5. Nature Immunol 2008;9(2):203–15. [PubMed: 18176567]
34. Ye M, Graf T. Early decisions in lymphoid development. Curr Opin Immunol 2007;19(2):123–8. [PubMed: 17306518]
35. Smith EM, Åkerblad P, Kadesch T, Axelson H, Sigvardsson M. Inhibition of EBF function by active Notch signaling reveals a novel regulatory pathway in early B-cell development. Blood 2005;106(6):1995–2001. [PubMed: 15920012]
36. Souabni A, Cobaleda C, Schebesta M, Busslinger M. Pax5 promotes B lymphopoiesis and blocks T cell development by repressing *Notch1*. Immunity 2002;17(6):781–93. [PubMed: 12479824]
37. Höflinger S, Kesavan K, Fuxa M, Hutter C, Heavey B, Radtke F, Busslinger M. Analysis of Notch1 function by in vitro T cell differentiation of Pax5 mutant lymphoid progenitors. J Immunol 2004;173(6):3935–44. [PubMed: 15356142]
38. Carotta S, Brady J, Wu L, Nutt SL. Transient Notch signaling induces NK cell potential in Pax5-deficient pro-B cells. Eur J Immunol 2006;36(12):3294–304. [PubMed: 17111353]
39. Rolink AG, Balciunaite G, Demolière C, Ceredig R. The potential involvement of Notch signaling in NK cell development. Immunol Lett 2006;107(1):50–7. [PubMed: 16930724]
40. Zhang Z, Cotta CV, Stephan RP, deGuzman CG, Klug CA. Enforced expression of EBF in hematopoietic stem cells restricts lymphopoiesis to the B cell lineage. EMBO J 2003;22(18):4759–69. [PubMed: 12970188]
41. Seet CS, Brumbaugh RL, Kee BL. Early B cell factor promotes B lymphopoiesis with reduced interleukin-7 responsiveness in the absence of E2A. J Exp Med 2004;199(12):1689–700. [PubMed: 15210745]
42. Reynaud D, Demarco IA, Reddy KL, Schjerven H, Bertolino E, Chen Z, Smale ST, Winandy S, Singh H. Regulation of B cell fate commitment and immunoglobulin heavy-chain gene rearrangements by Ikaros. Nat Immunol. 2008in press
43. Hagman J, Travis A, Grosschedl R. A novel lineage-specific nuclear factor regulates *mb-1* gene transcription at the early stages of B cell differentiation. EMBO J 1991;10(11):3409–17. [PubMed: 1915300]
44. Feldhaus A, Mbangkollo D, Arvin K, Klug C, Singh H. BlyF, a novel cell-type- and stage-specific regulator of the B-lymphocyte gene *mb*-1. Mol Cell Biol 1992;12:1126–33. [PubMed: 1545794]

45. Åkerblad P, Rosberg M, Leanderson T, Sigvardsson M. The B29 (immunoglobulin β-chain) gene is a genetic target for early B cell factor. Mol Cell Biol 1999;19(1):392–401. [PubMed: 9858563]
46. Åkerblad P, Sigvardsson M. Early B cell factor is an activator of the B lymphoid kinase promoter in early B cell development. J Immunol 1999;163(10):5453–61. [PubMed: 10553071]
47. Sigvardsson M, O'Riordan M, Grosschedl R. EBF and E47 collaborate to induce expression of the endogenous immunoglobulin surrogate light chain genes. Immunity 1997;7(1):25–36. [PubMed: 9252117]
48. Gisler R, Åkerblad P, Sigvardsson M. A human early B-cell factor-like protein participates in the regulation of the human CD19 promoter. Mol Immunol 1999;36(15–16):1067–77. [PubMed: 10698309]
49. O'Riordan M, Grosschedl R. Coordinate regulation of B cell differentiation by the transcription factors EBF and E2A. Immunity 1999;11(1):21–31. [PubMed: 10435576]
50. Månsson R, Lagergren A, Hansson F, Smith E, Sigvardsson M. The CD53 and CEACAM-1 genes are genetic targets for early B cell factor. Eur J Immunol 2007;37(5):1365–76. [PubMed: 17429843]
51. Sigvardsson M, Clark DR, Fitzsimmons D, Doyle M, Åkerblad P, Breslin T, Bilke S, Liu Y-F, Yeamans C, Zhang G, Hagman J. Early B cell Factor, E2A, and Pax-5 cooperate to activate the early B cell-specific *mb-1* promoter. Mol Cell Biol 2002;22(24):8539–51. [PubMed: 12446773]
52. Sigvardsson M. Overlapping expression of Early B cell Factor and basic helix-loop-helix proteins as a mechanism to dictate B lineage specific activity of the γ5 promoter. Mol Cell Biol 2000;20(10): 3640–54. [PubMed: 10779354]
53. Gisler R, Sigvardsson M. The human V-preB promoter is a target for coordinated activation by early B cell factor and E47. J Immunol 2002;168(10):5130–8. [PubMed: 11994467]
54. Goebel P, Janney N, Valenzuela JR, Romanow WJ, Murre C, Feeney AJ. Localized gene-specific induction of accessibility to V(D)J recombination induced by E2A and early B cell factor in nonlymphoid cells. J Exp Med 2001;194(5):645–56. [PubMed: 11535632]
55. Romanow WJ, Langerak AW, Goebel P, Wolvers-Tettero ILM, van Dongen JJM, Feeney AJ, Murre C. E2A and EBF act in synergy with the V(D)J recombinase to generate a diverse immunoglobulin repertoire in nonlymphoid cells. Mol Cell 2000;5(2):343–353. [PubMed: 10882075]
56. Walter K, Bonifer C, Tagoh H. Stem cell specific epigenetic priming and B cell specific transcriptional activation at the mouse *Cd19* locus. Blood. 2008in press
57. Thompson EC, Cobb BS, Sabbattini P, Meixlsperger S, Parelho V, Liberg D, Taylor B, Dillon N, Georgopoulos K, Jumaa H, Smale ST, Fisher AG, Merkenschlager M. Ikaros DNA-binding proteins as integral components of B cell developmental-stage-specific regulatory circuits. Immunity 2007;26 (3):335–44. [PubMed: 17363301]
58. Nutt SL, Rolink AG, Busslinger M. The molecular basis of B-cell lineage commitment. Cold Spring Harb Symp Quant Biol 1999;64:51–9. [PubMed: 11232328]
59. Rolink AG, Nutt SL, Melchers F, Busslinger M. Long-term *in vivo* reconstitution of T-cell development by Pax5-deficient B-cell progenitors. Nature 1999;401(6753):603–6. [PubMed: 10524629]
60. Horcher M, Souabni A, Busslinger M. Pax5/BSAP maintains the identity of B cells in late B lymphopoiesis. Immunity 2001;14(6):779–90. [PubMed: 11420047]
61. Mikkola I, Heavey B, Horcher M, Busslinger M. Reversion of B cell commitment upon loss of Pax5 expression. Science 2002;297(5578):110–3. [PubMed: 12098702]
62. Cobaleda C, Jochum W, Busslinger M. Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. Nature 2007;449(7161):473–7. [PubMed: 17851532]
63. DeKoter RP, Singh H. Regulation of B lymphocyte and macrophage development by graded expression of PU1. Science 2000;288(5470):1439–41. [PubMed: 10827957]
64. Xie H, Ye M, Feng R, Graf T. Stepwise reprogramming of B cells into macrophages. Cell 2004;117 (5):663–676. [PubMed: 15163413]
65. Yeamans C, Wang D, Paz-Priel I, Torbett BE, Tenen DG, Friedman AD. C/EBPalpha binds and activates the PU1 distal enhancer to induce monocyte lineage commitment. Blood 2007;110(9): 3136–42. [PubMed: 17671233]

66. Laslo P, Spooner CJ, Warmflash A, Lancki DW, Lee HJ, Sciammas R, Gantner BN, Dinner AR, Singh H. Multilineage transcriptional priming and determination of alternate hematopoietic cell fates. Cell 2006;126(4):755–66. [PubMed: 16923394]

67. Kikuchi K, Kasai H, Watanabe A, Lai AY, Kondo M. IL-7 specifies B cell fate at the common lymphoid progenitor to pre-proB transition stage by maintaining Early B cell Factor expression. J Immunol 2008;181(1):383–92. [PubMed: 18566404]

68. Delogu A, Schebesta A, Sun Q, Aschenbrenner K, Perlot T, Busslinger M. Gene repression by Pax5 in B cells is essential for blood cell homeostasis and is reversed in plasma cells. Immunity 2006;24 (3):269–81. [PubMed: 16546096]

69. Schebesta A, McManus S, Salvagiotto G, Delogu A, Busslinger GA, Busslinger M. Transcription factor Pax5 activates the chromatin of key genes involved in B cell signaling, adhesion, migration, and immune function. Immunity 2007;27(1):49–63. [PubMed: 17658281]

70. Braeuninger A, Küppers R, Strickler JG, Wacker HH, Rajewsky K, Hansmann ML. Hodgkin and Reed-Sternberg cells in lymphocyte predominant Hodgkin disease represent clonal populations of germinal center-derived tumor B cells. Proc Natl Acad Sci USA 1997;94(25):9337–42. [PubMed: 9256483]

71. Schwering I, Bräuninger A, Klein U, Jungnickel B, Tinguely M, Diehl V, Hansmann ML, Dalla-Favera R, Rajewsky K, Küppers R. Loss of the B-lineage-specific gene expression program in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. Blood 2003;101(4):1505–12. [PubMed: 12393731]

72. Jundt F, Acikgöz O, Kwon SH, Schwarzer R, Anagnostopoulos I, Wiesner B, Mathas S, Hummel M, Stein H, Reichardt HM, Dörken B. Aberrant expression of Notch1 interferes with the B-lymphoid phenotype of neoplastic B cells in classical Hodgkin lymphoma. Leukemia. 2008in press

73. Kuo YH, Landrette SF, Heilman SA, Perrat PN, Garrett L, Liu PP, Le Beau MM, Kogan SC, Castilla LH. Cbf beta-SMMHC induces distinct abnormal myeloid progenitors able to develop acute myeloid leukemia. Cancer Cell 2006;9(1):57–68. [PubMed: 16413472]

74. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, Girtman K, Mathew S, Ma J, Pounds SB, Su X, Pui CH, Relling MV, Evans WE, Shurtleff SA, Downing JR. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 2007;446(7137):758–64. [PubMed: 17344859]

75. Liu M, Duke JL, Richter DJ, Vinuesa CG, Goodnow CC, Kleinstein SH, Schatz DG. Two levels of protection for the B cell genome during somatic hypermutation. Nature 2008;451(7180):841–5. [PubMed: 18273020]

76. Pasqualucci L, Bhagat G, Jankovic M, Compagno M, Smith P, Muramatsu M, Honjo T, Morse HC 3rd, Nussenzweig MC, Dalla-Favera R. AID is required for germinal center-derived lymphomagenesis. Nat Genet 2008;40(1):108–12. [PubMed: 18066064]

77. Liu P, Keller JR, Ortiz M, Tessarollo L, Rachel RA, Nakamura T, Jenkins NA, Copeland NG. Bcl11a is essential for normal lymphoid development. Nat Immunol 2003;4(6):525–32. [PubMed: 12717432]

78. Guo F, Tanzer S, Busslinger M, Weih F. Lack of NF-κB2/p100 causes a RelB-dependent block in early B lymphopoiesis. Blood. 2008in press

79. Hu H, Wang B, Borde M, Nardone J, Maika S, Allred L, Tucker PW, Rao A. Foxp1 is an essential transcriptional regulator of B cell development. Nat Immunol 2006;7(8):819–26. [PubMed: 16819554]

80. Hardy RR, Kincade PW, Dorshkind K. The protean nature of cells in the B lymphocyte lineage. Immunity 2007;26(6):703–14. [PubMed: 17582343]

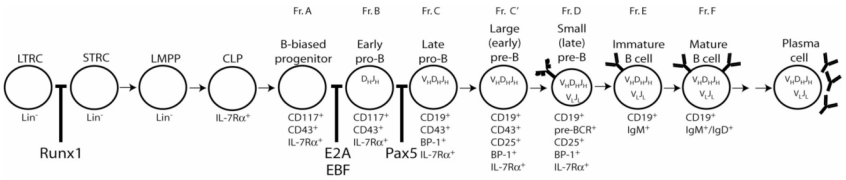

Figure 1.

Schematic of B cell development. Designations of the stages of B lymphopoiesis are indicated above each cell [3,80]. Stages at which various V(D)J rearrangements of *Ig heavy* (H) or *light* (L) *chain* genes occur are indicated within the cells. Select cell surface markers used to discriminate various stages of development are shown below. B lineage cells are $B220^{+}$ at all stages of development (fr.A through plasma cells). CD117 is also known as c-kit. CD43 is expressed on progenitors (HSCs to CLPs) prior to B cell differentiation. Stage specific expression of pre-BCR, B cell receptor (IgM or IgM/IgD) and secreted immunoglobulins are indicated. Stages at which developmental arrest occurs due to loss of transcription factors are indicated.

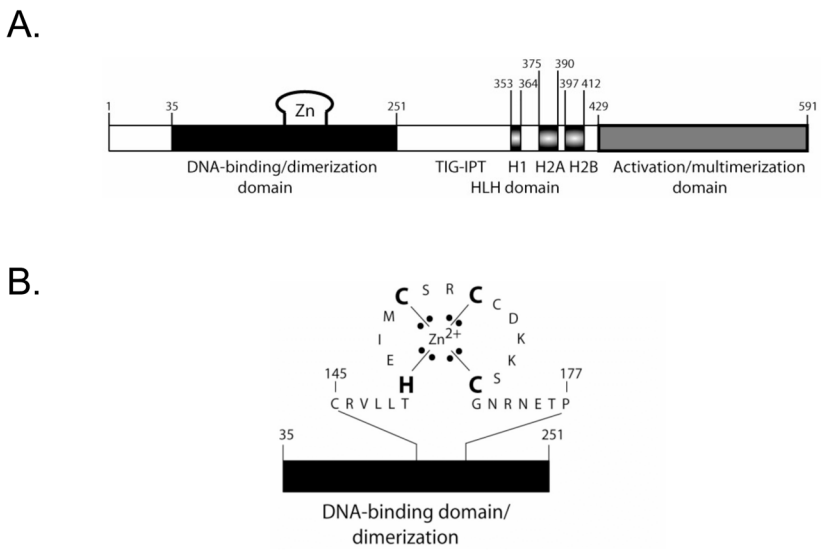

Figure 2.

The structure of EBF. A. Schematic of murine EBF. Functional domains and their positions are indicated by shading and by numbering of boundary residues. The relative position of the zinc-knuckle (Zn), which is detailed in B, is shown above the DBD. The HLH domain contains three putative α-helices indicated by boxes with graded shading. The C-terminal activation domain is not required for activation of all EBF target genes. B. The zinc-knuckle of the DBD includes four residues (bold) that coordinate zinc ions.

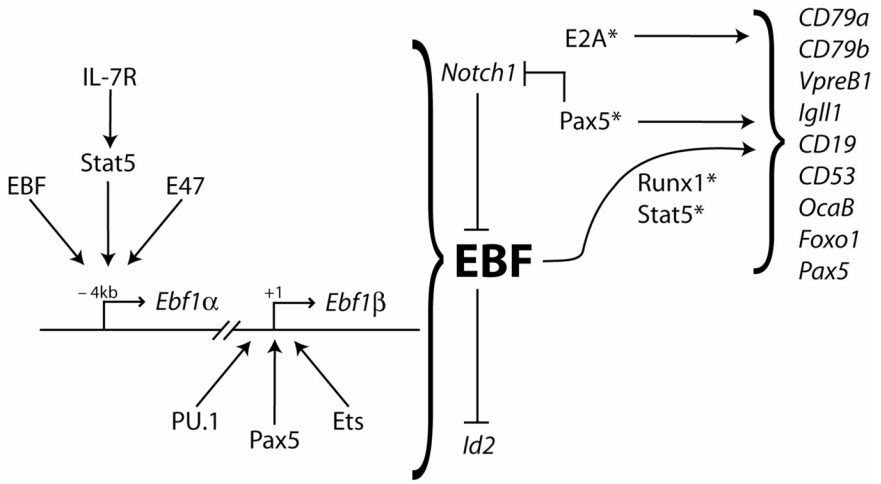

Figure 3.

EBF activates the B cell program and commitment to the B cell lineage. EBF activates a host of genes which encode proteins 1) required for V(D)J rearrangement, 2) components of the pre- and mature BCR, and 3) transducers of BCR signals. After induction of its expression by EBF, Pax5 activates 170 lineage-appropriate genes and represses 110 lineage-inappropriate genes [68,69], resulting in B lineage commitment. EBF also contributes to commitment (see text). EBF synergistically activates transcription of genes with various factors (indicated by *), including Pax5 (e.g. *Cd19* and *Cd79a*), E2A (e.g. *Igll1*, *Vpreb1* and *Cd79a*), Runx1 (*Cd79a* and *Cd19*) and STAT5 (*Pax5*)(see text for references). Similar to Pax5, EBF is hypothesized to silence transcription of lineage-inappropriate genes (e.g. *Id2*). Together, EBF and Pax5 function in an interdependent regulatory loop (reviewed in [30].