

B Lymphocyte Lineage Specification, Commitment and Epigenetic Control of Transcription by Early B Cell Factor 1

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Abstract Early B cell factor 1 (EBF1) is a transcription factor that is critical for both B lymphopoiesis and B cell function. EBF1 is a requisite component of the B lymphocyte transcriptional network and is essential for B lineage specification. Recent studies revealed roles for EBF1 in B cell commitment. EBF1 binds its target genes via a DNA-binding domain including a unique ‘zinc knuckle’, which mediates a novel mode of DNA recognition. Chromatin immunoprecipitation of EBF1 in pro-B cells defined hundreds of new, as well as previously identified, target genes. Notably, expression of the pre-B cell receptor (pre-BCR), BCR and PI3K/Akt/mTOR signaling pathways is controlled by EBF1. In this review, we highlight these current developments and explore how EBF1 functions as a tissue-specific regulator of chromatin structure at B cell-specific genes.

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1 Prologue

Twenty years ago, Early B cell factor 1 (EBF1; first identified as EBF) was detected as a novel DNA-binding activity specific for the *Cd79a* (*mb-1*) promoter (Hagman et al. 1991; Feldhaus et al. 1992). This activity was restricted to nuclear extracts from B cells. The discovery of EBF1 generated much excitement because it was identified as a potential regulator of B lymphocyte lineage specification and commitment. These roles of EBF1 were supported eventually by extensive data. A series of seminal observations concerning EBF1's structure, functions and mechanisms of action in B lymphopoiesis were made in the past 3 years. Here, we review the recent literature concerning EBF1 and its roles in the production of B cells, the antibody-producing arm of the immune system.

2 Introduction

B lymphocytes produce antibodies in response to antigenic challenges. In the bone marrow, these cells are generated from multi-potent progenitors (MPPs) that have the ability to become a variety of hematopoietic cells. A key event during B cell differentiation is the expression of Early B cell Factor 1 (EBF1), which drives the specification of B lineage cells in concert with other DNA-binding proteins including E2A and Pax5. How EBF1 accomplishes these functions has been a mystery. Until very recently, even the structure of EBF1 and its mode of DNA binding were unknown. Structural determinations have resolved many of these issues and are discussed here in detail.

The ability of EBF1 to direct the differentiation of uncommitted progenitors is a function of two of its intrinsic properties: EBF1 (1) activates transcription of B cell specific genes including *Pax5*, which encodes a B lineage commitment factor (O'Riordan and Grosschedl 1999) and (2) enforces commitment by repressing the expression of drivers of alternative lineages (such as *C/EBP α* and *Id2*) (Pongubala et al. 2008; Thal et al. 2009). Prior to lineage commitment of hematopoietic progenitor cells, EBF1 directs expression of the B cell program and represses other programs. Recent data suggest that B lineage specification and commitment are each affected by the dosage of EBF1.

In the past, biochemical methods identified a small number of potential gene targets of EBF1. More recently, chromatin immunoprecipitation (ChIP) was used to isolate DNA occupied by EBF1 in pro-B cells (Lin et al. 2010; Treiber et al. 2010b). These studies enabled the characterization of sequences bound by EBF1 in vivo. The experiments revealed an unexpectedly high number of promoter, enhancer and intergenic sites bound by EBF1. These DNA sequences were often clustered with binding sites of other regulators within the B lineage network (E2A, Runx1 and FOXO1). These studies also revealed epigenetic signatures of activated and repressed genes in pro-B cells. An important conclusion of these reports is that EBF1 is essential for initiating epigenetic changes in target genes during early B cell differentiation. However, these activities require prior modifications of chromatin and/or other factors, which may be responsible for lineage priming that precedes B lymphopoiesis. The nature of these signals and their origins is unknown and is a focus of speculation below.

An important distinction between EBF1 and other transcription factors is its ability to activate the B cell program by epigenetic remodeling of chromatin in early B cell progenitors. In this regard, EBF1 may interact directly with co-activators and SWI/SNF chromatin remodeling complexes. At the early B cell-specific *Cd79a* promoter, binding of EBF1 results in increased chromatin accessibility and decreased DNA methylation (Gao et al. 2009). Recent genomic analyses of histone modifications suggest a model of progressive gene activation predicated upon modifications initiated prior to the expression of EBF1, as well as those that are critically dependent on EBF1 itself.

3 Early B Cell Factor 1: Protein Structure and Function

3.1 Early Studies of EBF1

EBF1 (also known as EBF, O/E-1 and COE1) is a member of the EBF family of transcription factors. Early studies detected EBF1 binding to a functionally important palindromic site within the early B cell-specific *Cd79a* promoter (Hagman et al. 1991; Feldhaus et al. 1992). The *Cd79a* promoter drives expression of Ig- α , a transmembrane protein that is essential for display of the pre-B cell receptor (pre-BCR) and the BCR on the B cell plasma membrane as well as for signaling functions (Hombach et al. 1990; Campbell et al. 1991; Gold et al. 1991). Biochemical studies of EBF1 revealed that it assembles stable homodimers in the absence of DNA (Travis et al. 1993). Cloning and sequencing of cDNAs encoding EBF1 revealed its novel protein sequence (Fig. 1a) (Hagman et al. 1993; Wang and Reed, 1993). The major isoform of EBF1 is 591 amino acids, which includes a ~215 residue DNA-binding domain (DBD) and an atypical helical region comprising the helix-loop-helix-loop-helix (HLHLH) domain.

Biochemical and mutational studies confirmed the function of the DBD. The amino acid sequence alignment of the DBD with those of other known DBDs

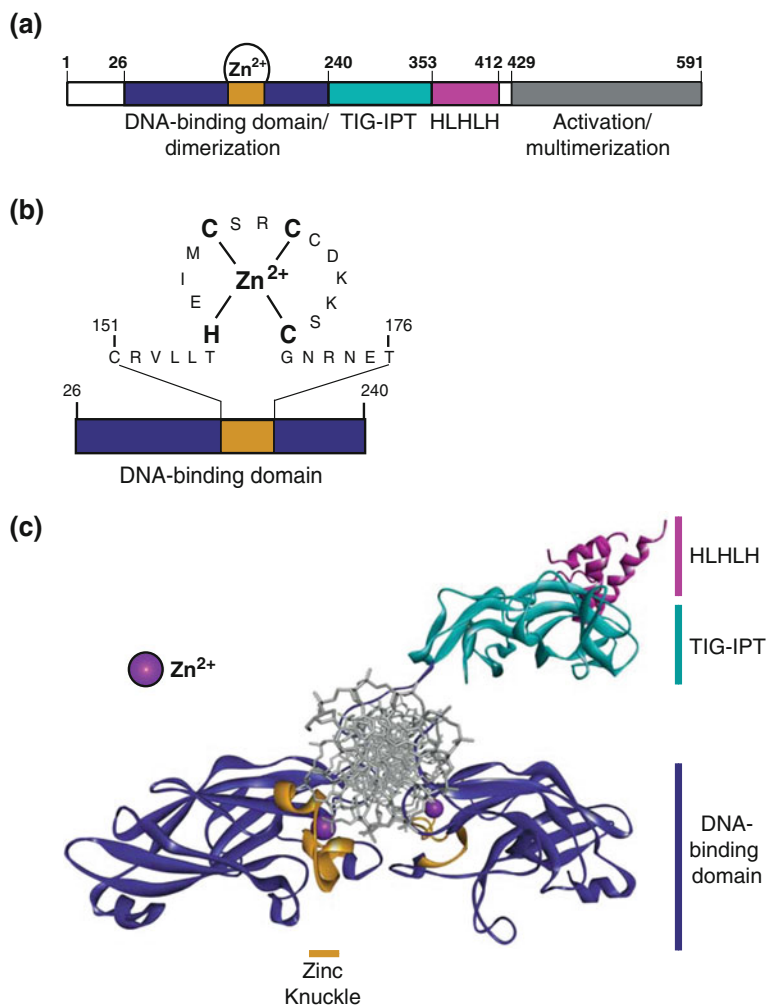


Fig. 1 The structure of Early B cell factor 1 (EBF1). The domains of EBF are labeled and colored consistently throughout. **a** A schematic representation of the domains in EBF₁₋₅₉₁ includes the DNA-binding domain's (DBD) unique zinc knuckle (gold). The DBD (blue), TIG/IPT (teal) and HLHLH (magenta) domains all participate in EBF dimerization. The carboxyl terminus includes the Ser/Thr/Pro-rich activation domain. The amino acids demarcating each domain are numbered. **b** An expanded view of the zinc knuckle highlights the histidine and three cysteine residues that coordinate the zinc ion required for DNA binding. **c** The structure of an EBF₁₂₆₋₄₂₂ dimer bound to DNA (grey). The perspective is parallel to the helical axis of the DNA molecule. The visible portion of the HLHLH domain, the TIG/IPT domain, the DBD domains and the zinc knuckle motifs are indicated. The zinc ions are depicted as purple spheres. The structure was generated using PDB file ID 3MLP (Treiber et al. 2010a) and was modeled using Discovery Studio Visualizer 3.0, Accelrys Inc., San Diego, CA

detected only very limited sequence identity (14% with the p65 subunit of NF- κ B; (Siponen et al. 2010). EBF1 homodimers bind efficiently to inverted repeat DNA sequences consisting of two half-sites that are separated by a two base pair spacer. In vitro measurements suggested that the optimal nucleotide target sequence of EBF1 is 5'-ATTCCCNNGGGAAT-3' (Hagman et al. 1995). Although it lacks consensus zinc fingers, the ability of EBF1 to bind DNA is dependent on its incorporation of zinc ions. Mutagenesis studies suggested that the metal ion is coordinated by a single histidine and three cysteines within a fourteen residue motif. The motif is termed the zinc knuckle and is required for DNA binding (Fig. 1b) (Hagman et al. 1995; Fields et al. 2008).

Dimerization of EBF1 is essential for its function. The HLHLH domain of EBF1 was predicted to include three putative α -helical motifs (H1, H2A and H2B) similar to those identified in basic-HLH family proteins (such as MyoD1); however, these proteins only have H1 and H2 (Hagman et al. 1993). Homodimerization of EBF1 requires contributions of the DBD, HLHLH and the Transcription factor Immunoglobulin (Ig)/Ig Plexin-like fold in Transcription factors (TIG/IPT) domain between the DBD and HLHLH (Hagman et al. 1993; Hagman et al. 1995; Aravind and Koonin 1999). The carboxy-terminal region of EBF1 is enriched with serine, threonine and proline residues and potently activates transcription when appended to a heterologous DBD (Hagman et al. 1995). Together, these studies helped delineate functionally important sequences in EBF1, but they did little to reveal how the protein folds, binds DNA or functions in vivo.

3.2 X-ray Crystallographic Analysis of EBF1 Structure

Recent structural determinations confirmed that EBF1 is the founding member of a distinct family of DNA-binding proteins. Structural characterization of the EBF1 DBD revealed a 'pseudoimmunoglobulin' fold similar to those of Rel family proteins (Siponen et al. 2010; Treiber et al. 2010a). The overall fold includes a core consisting of an anti-parallel β -barrel that contains nine β -strands arranged in two interacting sheets. An amino-terminal α -helix packs against the bottom of this structure. A series of loops that extend from the Rel-like core are among the distinct features of the EBF1 DBD. Protruding from the rest of the DBD, the zinc knuckle coordinates zinc ions using three short α -helices within the His-X₃-Cys-X₂-Cys-X₅-Cys motif. This configuration, which is one of the smallest independently-folding protein domains, is different from other types of zinc fingers (reviewed in Klug 2010).

EBF1 (residues 26–240 or 26–422) crystallized as a dimer bound to an optimal palindromic DNA sequence (Fig. 1c) (Treiber et al. 2010a). The structure revealed much concerning DNA recognition by EBF1 and of other closely related family members (e.g. EBF3). The complex has several novel features. Three distinct motifs within each subunit of the paired EBF1 homodimer make contacts with the major and minor grooves of the palindromic site. A highly unusual feature is the

recognition of bases within both half-sites by each EBF1 monomer. Residues within an extended loop between the β -strands of the DBD's amino-terminus and a carboxyl-terminal loop recognize the invariant bases within the major groove of one half-site. The zinc knuckle makes contacts with the minor groove of the other half-site. Thus, contacts made by each monomer within the homodimer assemble a symmetric clamp that extends across both half-sites of the palindrome. This configuration explains the requirement for a two base pair spacer between the two half-sites recognized by EBF1 (Travis et al. 1993). Mutagenesis of contact residues confirmed their importance for DNA binding (Fields et al. 2008; Siponen et al. 2010; Treiber et al. 2010a).

The DNA sequence used for crystallization with EBF1 (including 5'-ATT-CCCATGGAAT-3') and the majority of EBF1 binding sites identified using ChIP and bioinformatics are highly palindromic. In contrast, the EBF1 binding site of the *Cd79a* promoters (5'-AGACTCAAGGAAT-3') is less symmetric. Thus, the clamping mode of DNA binding may be critical for the ability of EBF1 to activate promoters with less than optimal binding sites.

3.3 Structures of Other Domains in EBF1

Regions of EBF1 involved in homodimerization were crystallized both as individual domains (Siponen et al. 2010) and in the context of dimers of residues 26–422 bound to DNA (Treiber et al. 2010a). Folding of the TIG/IPT domain in an Ig-like structure similar to Rel family members was confirmed. Some similarities were noted between the TIG/IPT domain and human calmodulin-binding transcription activator 1 (CAMTA1), a member of a family of proteins that includes highly conserved DBDs (CG-1) (Finkler et al. 2007). Packing of the TIG/IPT domain of EBF3 in crystals was used to model interactions between interfaces of the homologous domains in EBF1, suggesting that they contribute to the formation of multimers in solution (Siponen et al. 2010). The HLHLH domain was defined only weakly in the context of the DNA-bound EBF1 complex (26–422), with only one helix-loop-helix motif visible in the complex (Treiber et al. 2010a). However, comparisons of EBF1 with related b-HLH proteins (i.e., homodimers of E47; (Ellenberger et al. 1994) approximates how the HLHLH may mediate homodimerization. More studies are needed to reveal contributions of these domains to the DNA binding and function of EBF1.

4 Control of B Lymphopoiesis Requires a Network of Proteins Including EBF1

Hematopoietic stem cells (HSCs) are the source of all major blood cell lineages. B cells develop from HSCs following a series of progressively restricted rounds of differentiation in the bone marrow (Fig. 2) (reviewed in Kondo 2010). Expansion

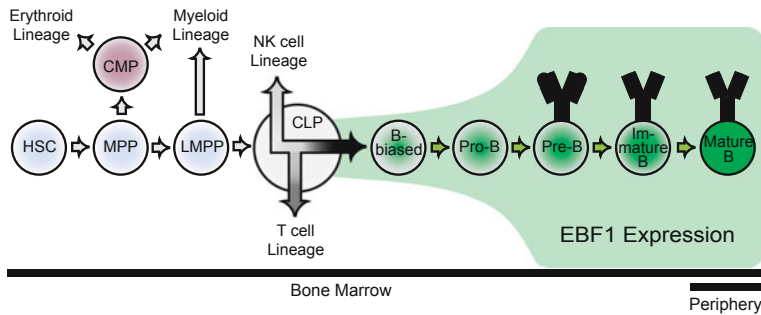


Fig. 2 *EBF1* expression in the context of hematopoiesis. Beginning with HSCs (hematopoietic stem cells), progressive steps of cell-fate restriction result in the designated cell types and lineages. MPPs (multi-potent progenitors) give rise to CMPs (common-myeloid progenitors), which possess myeloid and lymphoid potential. Additionally, MPPs yield LMPPs (lymphoid-primed multi-potent progenitors), which possess myeloid, lymphoid and NK cell potential. Common-lymphoid progenitors (CLPs), can be trisected into **a** $\text{Rag1}^{\text{hi}} \lambda 5^{-}$ cells which are confined to the NK lineage, **b** $\text{Rag1}^{\text{hi}} \lambda 5^{-}$ cells which can develop into NK cells or T lymphocytes and **c** $\text{Rag1}^{\text{hi}} \lambda 5^{+}$ cells which retain NK, T and B cell potential. Pre-B cells express the pre-B cell receptor (pre-BCR), which is composed of Ig heavy chains and the surrogate light chain proteins VpreB1 and $\lambda 5$. Immature and mature B cells express mature BCRs comprised of Ig heavy and light chains. The relative levels of EBF1 expression in CLPs through mature B cells are represented by the size of the green shaded block. Developmental steps that occur in the bone marrow versus the periphery are indicated

of short-term HSCs is followed by the production of MPPs, which have the ability to produce all hematopoietic lineages, but which lack self-renewing capacity. Among the descendants of these cells, lymphoid-primed multi-potent progenitors (LMPPs) are the earliest lineage cells to express receptors for Interleukin-7 (IL-7) and maintain the ability to generate both myeloid and lymphoid cells (Adolfsson et al. 2005). LMPPs differentiate into common lymphoid progenitors (CLPs), which were first identified as $\text{Lin}^{-}\text{IL-7R}\alpha^{+}\text{c-Kit}^{\text{Lo}}\text{Sca1}^{+}$ cells in bone marrow (Kondo 1997). It is now understood that CLPs constitute heterogeneous populations that produce B, T or natural killer (NK) cells (reviewed in Ichii et al. 2010).

Generation of LMPPs requires transcription factors including PU.1 (*Sfpi1*), Gfi-1 (*Gfi1*), Ikaros (*Ikaros*) and E2A (multiple proteins expressed by the *Tcf2a* gene) (Reviewed in Ramirez et al. 2010), which regulate cells' decisions to attain myeloid versus lymphoid fates. The concentration, or dosage, of regulatory factors helps determine the priming of cell differentiation (lineage priming) and subsequent fate decisions. For example, the control of alternative myeloid or B lymphoid fates of MPPs is a function of high or low concentrations of PU.1, respectively (Dekoter and Singh 2000). Levels of PU.1 expression are specified by the zinc finger protein Gfi-1, which promotes the development of B cells by repressing *Sfpi1*, the gene that encodes PU.1 (Spooner et al. 2009). Ikaros directs hematopoietic progenitors by promoting B cell-specific gene expression and *Ig heavy chain* gene rearrangements (Reynaud et al. 2008). Lineage priming is one of the mechanisms by which Ikaros directs cell fates toward lymphopoiesis

(Ng et al. 2009). This activity of Ikaros is essential for B and T cell development. These processes are promoted directly by Ikaros' binding to gene targets and indirectly via its restriction of other transcriptional programs including those of early progenitors and non-lymphoid lineages. Lineage priming by Ikaros may be a consequence of its establishment of epigenetic modifications in chromatin at genes that will be expressed in a tissue-specific manner following lineage commitment.

E2A also received attention recently as an essential primer of B lymphopoiesis. Although expressed in both B and T cells, early studies showed that ablation of E2A expression in mice resulted in the complete loss of B lineage cells (Bain et al. 1994; Zhuang et al. 1994). The generation of hematopoietic cells including CLPs, and subsequently, lymphoid cells, was dependent on the expression of E2A proteins in HSCs (Dias et al. 2008; Semerad et al. 2009). E2A proteins also promoted lymphoid development and suppressed myeloid fates in a dose-dependent fashion. E2A-dependent lineage priming of lymphocyte-specific genes may promote lymphoid fates. One of the proteins encoded by *Tcf2a*, E12, drives B cell development in *Tcf2a*^{-/-} MPPs (Bhalla et al. 2008). A consequence of E12 expression is the restoration of EBF1 expression, which is lost in the absence of E2A. These activities of E2A proteins, together with co-occupancy of regulatory modules with EBF1, suggest the importance of functional collaborations between the two factors in B lymphopoiesis.

4.1 EBF1 and the Basis of B Lineage Fate Decisions

Many studies have implicated EBF1 as a primary determinant of B cell lineage specification. The lack of EBF1 in *Ebf1* knockout mice results in complete developmental arrest at a CLP-like stage of development (Lin and Grosschedl 1995). Having lost a key regulator of B cell development, it is not surprising that these mice exhibit: (1) loss of B cell-specific gene expression including key proteins required for differentiation (*Cd79a*, *B29/Cd79b*, *Vpreb1*, *Igll1*(λ 5) and *Rag1*) and (2) a complete absence of V(D)J recombination, which is necessary to assemble functional *Ig* genes. In contrast, enforced expression of EBF1 in murine HSCs drives B cell development at the expense of other hematopoietic lineages (Zhang et al. 2003). Moreover, in non-lymphoid cells, EBF1 can activate at least part of the B cell program in the absence of other upstream regulators (Kee and Murre 1998; Romanow et al. 2000; Goebel et al. 2001; Medina et al. 2004; Pongubala et al. 2008).

The central role of EBF1 in the B cell-specific network of transcription factors was confirmed by Medina et al. (2004). The differentiation of PU.1-deficient progenitor cells is arrested completely at the MPP stage. In these mutant cells, enforced expression of EBF1 rescued B cell development, resulting in pro-B cells that expressed key B cell-specific genes (e.g. the genes that fail to be expressed in EBF1-deficient mice) and activated V(D)J recombination. While *Pax5* genes were turned on by EBF1, enforced expression of *Pax5* alone did not rescue B cell development similarly.

During normal hematopoiesis, EBF1 is first expressed at low levels in CLPs (Dias et al. 2005; Roessler et al. 2007). Comparisons of CLPs derived from wild-type versus EBF1-deficient mice revealed important roles of EBF1 in B cell specification and commitment (Zandi et al. 2008). In the absence of EBF1, populations of CLPs failed to express transcripts of key genes required for functional B cells, including *Cd79a*, *B29/Cd79b* and *Igll1*. Additionally, EBF1 was required for the activation of transcription factor genes *Pax5*, *Pou2af1* (OCA-B/BOB-1/OBF1) and *Foxo1*, which play important roles at later stages of B cell development (Kim et al. 1996; Schubart et al. 1996; Nutt et al. 1999; Hess et al. 2001; Dengler et al. 2008; Herzog et al. 2008; Srinivasan et al. 2009). EBF1-deficient CLPs also failed to initiate *Ig* heavy chain gene *D-J* recombination. Together, these data provide evidence for lineage priming of B cell-specific genes by EBF1 in CLPs.

More recently, CLPs were sub-divided into three populations that possess different lineage potentials related to their expression of EBF1 (Månsson et al. 2010). CLPs that expressed low levels of both EBF1 and a GFP reporter of *Rag1* expression gave rise to NK, B and T cells. Increased *Rag1*/GFP expression correlated with increased *Ebf1* expression and restriction of lineage potential to B and T lymphocytes. The mice used in these studies also possessed a *Igll1* promoter:human CD25 transgene, which served as a reporter for $\lambda 5$ surrogate light chain expression. Single-cell multiplex PCR analysis confirmed that *Rag1*^{hi} $\lambda 5$ ⁺ CLPs correlated with the highest frequency of *Ebf1* expression, expressed *Pax5* transcripts and were restricted solely to the B lineage (similar to Hardy fraction A cells). The authors concluded that EBF1 plays a crucial role in CLPs to direct B cell lineage commitment (together with Pax5 and Ikaros; Nutt et al. 1999; Reynaud et al. 2008).

How EBF1 is activated in a subset of B cell progenitors is an open question. Expression of EBF1 is likely driven by E2A proteins. Regulation of EBF1 may also involve FOXO1 because FOXO1 binding sites have been found in regulatory regions across the *Ebf1* locus (Kee and Murre 1998; Smith et al. 2002; Lin et al. 2010). *Ebf1* transcription may be regulated by IL-7/STAT5 signaling as well (Purohit et al. 2003; Dias et al. 2005; Kikuchi et al. 2005; Roessler et al. 2007). However, a recent report demonstrated that EBF1 is expressed in STAT5-deficient pro-B cells that have been rescued by Bcl-2 (Malin et al. 2010). Activation of *Ebf1* genes may involve a stochastic mechanism in which activation of a single allele is a consequence of low levels of activating signals in a subset of CLPs. Additionally, EBF1 may promote its own expression by repressing Id2 and Id3 expression, which in turn suppress E2A activity (Pongubala et al. 2008; Thal et al. 2009). Once activated by EBF1, expression of Pax5 increases EBF1 expression via a positive feedback loop (Roessler et al. 2007). Moreover, EBF1 has been reported to upregulate its own expression (Smith et al. 2002). The data are consistent with a cascade of factors that drive cells toward increasing production of EBF1 by direct positive regulation (Rothenberg 2007), which promotes the B cell fate while limiting other lineage choices.

4.2 Effects of Changes in *Ebf1* Gene Dosage on B Cell Development

As described above, transcription of *Ebf1* genes begins in CLPs, where the factor biases cells toward B lymphopoiesis. As cells progress from CLPs to functional B cells, they pass through multiple intermediate stages (i.e. Hardy fractions) as identified by the sequential expression of cell surface markers and V(D)J recombination (reviewed in Hardy et al. 2007) (Fig. 2). Progression through the various stages is characterized by the expression of increasing amounts of EBF1, which is generated by complex regulation of the *Ebf1* gene's two promoters (Roessler et al. 2007). In a process that is linked with upregulation of Pax5, levels of *Ebf1* transcripts increase significantly between 'B-biased' cells (Hardy fraction A) and pro-B cells (fraction B). *Ebf1* transcripts are upregulated (>five-fold) in the transition of pro-B cells to pre-B cells (fractions C' and D; Roessler et al. 2007; H. Lei and J.H., unpublished data). The basis of the upregulation in fractions C' and D is unknown. However, expression of different concentrations of EBF1 as development progresses suggests that its activities are, in part, dosage-dependent.

Dosage-dependent effects of EBF1 have been confirmed in heterozygous knock-out mice with single functional *Ebf1* alleles. Numbers of B cell progenitors and mature B cells were reduced by half in fetal livers, bone marrow and spleens of *Ebf1*^{+/-} mice (Lin and Grosschedl 1995; O'Riordan and Grosschedl 1999; Lukin et al. 2010). This effect was compounded by combining the *Ebf1*^{+/-} genotype with haplo-insufficiency of *Tcf2a* or *Runx1* genes, which encode transcription factors that bind DNA cooperatively with EBF1. EBF1:E2A (E47) complexes assemble on promoters including *Igll1* (Sigvardsson et al. 1997). Combined haplo-insufficient mice with single functional *Ebf1* and *Tcf2a* alleles (*Ebf1*^{+/-}*Tcf2a*^{+/-}) displayed defective development of pro-B cells together with reduced expression of B cell-specific transcripts including *Pax5*, *Rag1*, *Rag2*, *Vpreb1*, *Igll1* and *Cd79a*. These studies also included the first observations that EBF1 directly regulates *Pax5* promoter transcription. Together, these studies indicated the importance of collaborative interactions between EBF1 and E2A proteins.

EBF1 also binds DNA cooperatively with the Runx1 transcription factor, which was first described at the *Cd79a* promoter (Maier et al. 2004). Bone marrow B cell development exhibited a striking compound phenotype in *Ebf1*^{+/-}*Runx1*^{+/-}, or *ER*^{het} mice (Lukin et al. 2010). Most effects were apparent at the pro-B-pre-B boundary and at subsequent stages. This disruption occurs later than effects observed in *Ebf1*^{+/-}*Tcf2a*^{+/-} mice. Reduced levels of EBF1 alone decreased the frequency of *Igλ* light chain gene rearrangements significantly. Other effects of the compound genotype included: (1) delayed shut off of early progenitor-specific genes (*c-Kit*, *Vpreb1* and *Igll1*), (2) delayed activation of stage-specific markers (*Irf3*, *Cd25* and *Cd2*) and (3) loss of most pre-B, immature and mature B cells, which normally express the highest levels of EBF1. The data are consistent with stochastic mechanisms requiring increased levels of EBF1 at the pro-B to pre-B cell boundary.

It is notable that *Ebf1*^{+/-}*Tcf2a*^{+/-} and *Ebf1*^{+/-}*Runx1*^{+/-} compound haplo-insufficient mice have defective B cell development, while other combinations of heterozygous alleles (*Pax5*^{+/-}*Runx1*^{+/-}, *Ebf1*^{+/-}*Irf1*^{+/-} and *Ebf1*^{+/-}*Gfi1*^{+/-}) do not exhibit compound effects in B cells (K.L. and J.H., unpublished data; H. Xu and H. Singh, privileged communication). In mammals, the expression patterns of most genes are not affected by haploinsufficiency (Qian and Zhang, 2008). Thus, it can be concluded that cooperative interactions between factors at nearby sites may provide special conditions resulting in reduced transcription in mice with compound genotypes. An exception to this model was observed at the *Cd79a* promoter, which binds EBF1 and Runx1 with robust cooperativity. Expression of *Cd79a* transcripts was not affected in *Ebf1*^{+/-}*Runx1*^{+/-} compound haplo-insufficient mice. A high frequency of co-occupancy of promoters and enhancers by EBF1 and E2A, or by EBF1 and Runx1, has now been confirmed by genomic analysis at a wide range of loci in developing B cells.

4.3 EBF1 and B Cell Lineage Commitment

Lineage commitment is defined as the fixation of cells in a single lineage or fate with the loss of potential to generate cells of other lineages. In higher eukaryotes, mechanisms governing lineage commitment are best understood in B cells due to the discovery that Pax5 regulates this process (Nutt et al. 1999; Rolink et al. 1999; Mikkola et al. 2002). Pax5 contributes to B cell development in three major ways: Pax5 (1) regulates B cell-specific gene expression in concert with EBF1, E2A and other factors (reviewed in Ramirez et al. 2010), (2) positively regulates the expression of *Ebf1* genes (Roessler et al. 2007) and (3) represses the expression of a large spectrum of non-B cell specific genes (Delogu et al. 2006). Together, these mechanisms help activate the B cell program while restricting the expression of other programs in committed B cells.

Recent work by Pongubala and colleagues elucidated a role for EBF1 in regulating B cell commitment (Pongubala et al. 2008). Lymphoid progenitors of *Ebf1*^{-/-} mice are unable to differentiate into B cells and possess increased potential to generate myeloid, dendritic and NK cells in reconstituted mice. These cells also produced CD4⁺CD8⁺ double-positive and single-positive T cells in RAG-deficient mice. Increased expression of EBF1 in MPPs induced B cell development at the expense of myeloid development in vitro. Importantly, EBF1 inhibited the ability of Pax5-deficient cells to attain alternative fates. These data suggest that the expression of EBF1 is a commitment signal to the B lineage in the absence of Pax5.

Clues as to how EBF1 promotes the B cell fate were derived from the analysis of gene expression with and without EBF1. In transduced cells, EBF1 antagonized expression of both C/EBP α and PU.1, major determinants of myeloid differentiation. EBF1 also repressed expression of Id2 and Id3, which inhibit the functional activities of E2A proteins (Pongubala et al. 2008; Thal et al. 2009). *Id2*, *Id3* and *Cebpa* genes (encoding C/EBP α) are targets of direct regulation by EBF1.

The ability of EBF1 to repress genes characteristic of earlier progenitors and of other lineages is also dosage-dependent. Pro-B cells of *Ebf1*^{+/-} mice expressed significantly higher levels of *Ly6a/Sca1* than cells of wild-type mice (Lukin et al. 2011). The haplo-insufficient pro-B cells also expressed multiple NK cell-specific genes, including *Cd244* (2B4). These effects were more apparent in these pro-B cells and occurred despite expression of Pax5. Enforced expression of EBF1 in *Ebf1*^{+/-}*Runx1*^{+/-} pro-B cells increased expression of B cell markers including *Igll1* and repressed expression of NK-lineage genes including *Cd244*, *Cd160*, *Klrblc* (NK1.1) and *Nfil3* (E4BP4), a transcription factor that contributes to NK cell development (Gascoyne et al. 2009). The data suggest that normal levels of EBF1 are required for B cell identity, which EBF1 maintains by repressing the expression of non-B lineage-specific genes.

5 Identification of EBF1 Target Genes Using Genome-Wide Analysis

The full extent of EBF1's control of the B cell transcriptome was made apparent recently in two extensive studies (Lin et al. 2010; Treiber et al. 2010b). These publications made use of ChIP-based technologies to assess the breadth of EBF1 binding to regulatory modules in the chromatin of pro-B cells. Common themes arising from these experiments included the extent of EBF1 DNA binding, which was detected at >500 genes. These sites were localized within sets of genes involved in a host of biological processes including pre-BCR, BCR and PI3K/Akt/mTOR signaling, B cell adhesion, cell cycle control and migration. The studies also correlated EBF1 DNA binding with the status of epigenetic marks on histones. Notably, EBF1 binding correlated with histone modifications necessary for appropriate transcriptional activation and repression characteristic of the B cell program. This suggests that EBF1 plays a primary role in the epigenetic regulation of B cell development.

5.1 Binding of EBF1 to a Vast Array of Sites in Pro-B Cells

Treiber et al. (2010b) used multiple methods to identify functionally important binding sites of EBF1 in vivo. First, a 'ChIP-on-chip' approach was employed to estimate the number of genes that are occupied by EBF1 in pro-B cells. Immunoprecipitated fragments were used to probe DNA tiling arrays consisting of 17,000 DNA fragments surrounding transcriptional start sites (TSS) of promoter regions. As a result, 228 potential target genes were identified. The functional importance of EBF1 binding to these genes was confirmed by comparing patterns of gene expression in *Ebf1*^{-/-} pre-pro-B cells without and with expression of EBF1. A high percentage of potential target genes were activated by enforced

EBF1 expression in EBF1-deficient cells, including the canonical EBF1 targets *Igll1* and *Vpreb1*. A second set of experiments examined consequences of the loss of EBF1 following Cre-mediated deletion of floxed *Ebf1* alleles in gene-targeted mice. The loss of EBF1 due to Cre resulted in down-regulation of genes identified in the ChIP-on-chip experiments. Overlap between the datasets of gain-of-function and loss-of-function experiments confirmed the regulation of a subset of genes by EBF1, including *Hes1* (Notch pathway), *Sox4* and *Pou2af1*. The experiments confirmed the previous identification of *Ceacam1* and the immunomodulatory adaptor gene *Dok3* as targets of EBF1 regulation (Månsson et al. 2007; Ng et al. 2007). The list of genes repressed by EBF1 also included *Pdcd1* and *Ctla4* (members of the CD28 family), *Icosl* (the ligand of the Icos receptor) and *Hlx* (a homeobox factor that regulates Th1 differentiation). Overall, the patterns of activated and repressed genes suggest a positive role for EBF1 in regulating B cell signaling, including BCR, CD19 and the PI3K pathways, while repressing signaling pathways that are important in other cell types. It is noteworthy that many EBF1-regulated genes involved in pre-BCR and BCR signaling are co-regulated by Pax5, underscoring the importance of the EBF1-Pax5 axis in the B cell program.

To extend the analysis of EBF1 DNA binding to regions outside known promoters, Treiber et al. sequenced libraries of DNA generated following immunoprecipitation using anti-EBF1 antibodies or control input DNA. This deep sequencing approach identified ~4,500 binding sites occupied by EBF1 within 100 kb of annotated genes (corresponding to 5,025 genes). The data displayed a high degree of concordance with previous ChIP-on-chip data, as over 94% of previously identified sites were also detected using deep sequencing. Interestingly, the sites were restricted to genes that are expressed specifically in B cells. Binding was not observed on genes that are thought to bind EBF1 in other cell types (i.e., forebrain neurons, adipocytes and osteoblasts). Therefore, EBF1 binding is restricted to genes of the B cell program in B cells.

5.2 Co-occupancy of Genes by EBF1 and E2A

Lin and colleagues (Lin et al. 2010) used a ChIP-seq approach not only to detect the presence of EBF1 at promoter, enhancer and intragenic regions of genes in murine pro-B cells, but also focused extensively on co-occupancy by other factors that contribute to the B cell fate (Lin et al. 2010). The compiled sequences were used to generate the consensus 5'-G/A/TC/GTCCCT/C/AA/G/TGGGA-3', which is very similar to the optimized EBF1 site identified previously using binding site selection in vitro (5'-ATTCCCNNGGAAT-3')(Travis et al. 1993). In addition to detection of EBF1 DNA binding, ChIP-seq was used to localize binding sites for E2A and the Forkhead protein FOXO1 in pre-pro-B and pro-B cells. While comparative analysis detected only rare evidence of co-occupancy of sites (within 150 base pairs) by EBF1 and FOXO1. Nine percent of the 1,753 genes upregulated

in pro-B cells were co-occupied by EBF1 and E2A. Interestingly, sites occupied by E2A shifted significantly between pre-pro-B cells in the absence of EBF1 and pro-B cells that express EBF1. Sites common to both E2A and EBF1 binding corresponded to genes involved in processes required for B cell development, including transcriptional regulators, cell differentiation, control of the cell cycle and signaling via the PI3K pathway, overlapping significantly with the list of (Treiber et al. 2010b). Together, these data confirm the importance of synergistic interactions between EBF1 and E2A.

5.3 Epigenetic Regulation of Genes by EBF1

DNA binding by EBF1 has been linked with changes in epigenetic marks at target genes. Lin and colleagues (Lin et al. 2010) correlated the binding of EBF1 and other factors including E2A with a series of histone modifications associated with transcriptional activation or repression. Interestingly, the presence of the E2A protein E47 correlated with histone H3K4 monomethylation (H3K4me1) at target genes, which included those co-regulated by EBF1. H3K4me1 likely constitutes a mark of ‘poised’ chromatin, an intermediate state that facilitates additional epigenetic modifications necessary for transcription (Robertson et al. 2008). However, at co-regulated genes, increased acquisition of the active trimethylated mark H3K4me3 and subsequent H3 acetylation was observed following DNA binding by EBF1. These data imply that active transcription requires cooperation between EBF1 and E2A.

Treiber et al. also noted differences in epigenetic marks in the absence or presence of EBF1 in B cell progenitors (Treiber et al. 2010b). Three classes of B cell-specific genes were identified that bind EBF1 at their promoters: activated, repressed and poised. At activated promoters, chromatin in the vicinity of EBF1 binding sites gained the H3K4me2 mark during the transition between pre-pro-B and pro-B cells, which may be concurrent with expression of E2A. H3K4me3 and H3 acetylation were noted in pro-B cells that expressed high levels of *Ebf1* transcripts. In contrast, promoters of repressed genes possessed variable degrees of H3K4 methylation, but were more likely to maintain negative H3K27me3 marks. Poised genes represent a group in which EBF1 modifications of chromatin, including H3K4me2, are induced at early stages and in the absence of transcription. However, EBF1-dependent transcription of these genes occurs only at later stages (mature B cells). H3K4me2 was identified previously as a mark of developmental ‘poising’ at hematopoietic genes (Orford et al. 2008). Transcription of these poised genes (including *Cd40*) in mature B cells is ostensibly due to the need for other factors, which require the modifications implemented by EBF1 to activate transcription at later stages of development. Interestingly, one report suggests that factors (i.e. Sox4) induce histone modifications (including H3K4me2) in the chromatin of EBF1 target genes (*Igll1-Vpreb1*) as early as blood-forming hemangioblasts (Liber et al. 2010). Together, these data have led to a revision of

the characterization of EBF1 as a pioneer factor (Hagman and Lukin, 2005) because EBF1 mediates changes in chromatin and facilitates the binding of other proteins in a ‘hematopoietic context’ (Treiber et al. 2010b).

5.4 Multiple Mechanisms Activate *Cd79a* Transcription in Early B Cells

For many years, the B cell-specific *Cd79a* promoter has served as a useful model for defining requirements for gene activation in early progenitors, and thus, B lineage specification. When fully activated, the TATA-less promoter (localized to ~200 base pairs) binds multiple lineage-restricted DNA-binding proteins including EBF1, Runx1 (and its obligate partner CBF β), E2A proteins and Pax5, which recruits Ets family proteins to bind a composite site (Fig. 3) (Hagman et al. 1991; Travis et al. 1991; Feldhaus et al. 1992; Fitzsimmons et al. 1996; Sigvardsson et al. 2002; Maier et al. 2004). Based on recent publications, it is possible to propose a new model that incorporates factor binding, histone modifications, nucleosome remodeling and DNA demethylation in the activation of *Cd79a* transcription.

Prior to their activation, *Cd79a* promoters are maintained in a highly-condensed state that is relatively inaccessible (Gao et al. 2009). Analysis of histone modifications in non-B cells and pre-pro-B cells lacking EBF1 suggests that a ‘hematopoietic’ state is maintained in *Cd79a* promoter chromatin prior to the expression of EBF1 (Treiber et al. 2010a, b). Key features of this state may include: (1) repression by Polycomb group proteins, which have been linked with repression of the *Pax5* gene prior to its activation by EBF1 (Decker et al. 2009), (2) epigenetic modifications that facilitate EBF1 binding (Heinz et al. 2010; Liber et al. 2010), and/or (3) interactions with E2A, Runx1 or other transcription factors. Recent data demonstrated that E2A, which is expressed as early as HSCs, is sufficient for modifications of histones at *Cd79a* promoters that include H3K4me1 (Lin et al. 2010). An additional modification that has received less attention is DNA methylation, which modifies CpG dinucleotides with 5-methylcytosine (reviewed in Jaenisch and Bird, 2003; Bonasio et al. 2010). Prior to the expression of EBF1, *Cd79a* promoters possess only methylated CpGs, which are generally associated with inactive promoters. In turn, hypermethylated chromatin with inactive histone modifications provides a substrate for Mi-2/Nucleosome Remodeling and Deacetylase (Mi-2/NuRD) complexes. Mi-2/NuRD includes subunits that bind methylated DNA, deacetylate or demethylate histones and mobilize nucleosomes to assemble and maintain compact chromatin (reviewed in Ramírez and Hagman, 2009).

Biochemical experiments suggest that, following its expression in CLPs/pre-pro-B cells, EBF1 assembles complexes on *Cd79a* promoters with Runx1/CBF β and E2A proteins. Experiments in murine EBF1-deficient fetal liver progenitors and plasmacytoma cells demonstrated that EBF1 is sufficient to initiate DNA demethylation and chromatin remodeling. These modifications increase local

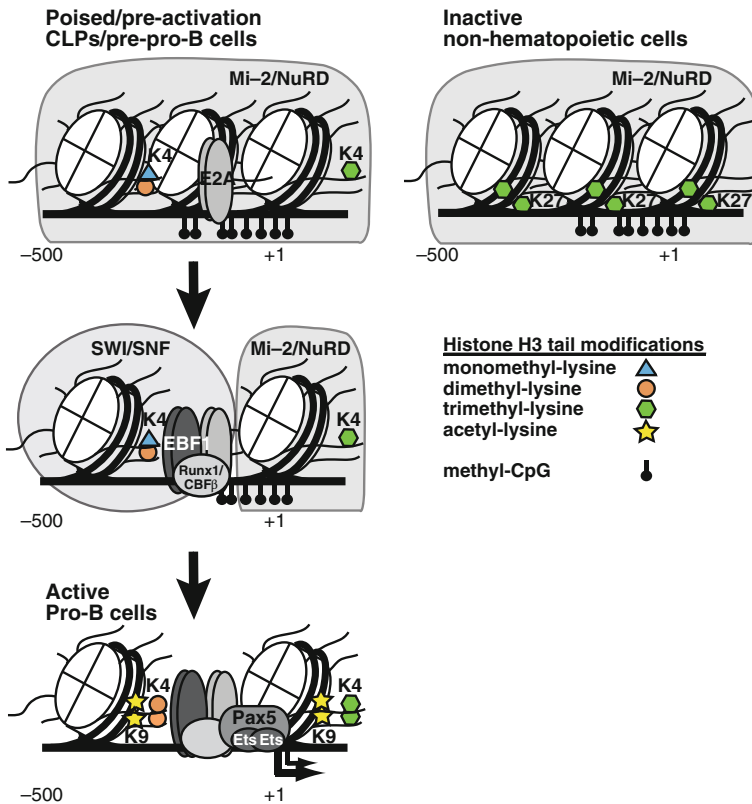


Fig. 3 A model of stepwise epigenetic modifications of *Cd79a* (*mb-1*) promoters during early *B* cell development. Transcriptional activation of the *Cd79a* gene is necessary for progression to the pre-B cell stage. Inactive *Cd79a* promoters in non-hematopoietic cells (right) are characterized by histone H3K27 trimethylation, a mark of heterochromatin and repression. Chromatin at these promoters is likely maintained in an inactive state by repressive Mi-2/NuRD chromatin remodeling complexes (CRCs). During pre-activation of *Cd79a* genes in common lymphoid progenitors (CLPs) and pre-pro-B cells (top left), H3K4 mono- and di-methylation is correlated with binding of the transcription factor E2A to *Cd79a* promoters. Poised promoters generally display low levels of mono-, di- and tri-methylated H3K4 and do not display significant levels of H3K27 trimethylation. Binding of EBF1 initiates demethylation of promoter DNA and recruits activating SWI/SNF CRCs, which result in nucleosome displacement (middle left). Activation of *Cd79a* expression in pro-B cells involves the subsequent recruitment of other transcription factors including Pax5 and Ets proteins and correlates with **a** displacement of repressive Mi-2/NuRD complexes, **b** complete demethylation of promoter DNA, **c** increased H3K4 di- and trimethylation with loss of monomethylation and **d** high levels of H3K9 acetylation (bottom left). This model is based, in part, on data from Gao et al. (2009), Lin et al. (2010), Treiber et al. (2010b) and unpublished data (J.R. and J.H.)

chromatin accessibility, but they do not lead to transcription in the absence of other transcription factors (Gao et al. 2009). In both of these cellular models, expression of Pax5 was important for the propagation of DNA demethylation and subsequent

transcription. These processes are dependent on SWI/SNF chromatin remodeling complexes, which are recruited to the promoter following DNA binding by EBF1. The activity of SWI/SNF is opposed functionally by Mi-2/NuRD. shRNA-mediated depletion of Mi-2 β (*Chd4*), a core catalytic subunit of Mi-2/NuRD, greatly enhances activation of *Cd79a* promoters by EBF1 and Pax5 in plasmacytoma cells. Depleting Mi-2 β facilitates the propagation of DNA demethylation and allows for the assembly of Pax5:Ets ternary complexes on unmethylated CpGs (Maier et al. 2003; Maier et al. 2004). The mechanism by which EBF1, Pax5 and SWI/SNF exclude Mi-2/NuRD from the *Cd79a* promoter is unknown. A dynamic competition likely exists between EBF1 and other transcription factors that promote DNA demethylation versus the recruitment of Mi-2/NuRD to methylated CpGs.

Following the binding of *Cd79a* promoters by EBF1, Pax5 and other proteins, additional changes in histone modifications take place. These activities may be mediated by histone acetyltransferase (HAT) domains of the p300 co-activator, which are recruited by EBF1 (Zhao et al. 2003; He et al. 2011). Interestingly, p300 can acetylate Pax5 directly, which increases its activity in some contexts (He et al. 2011). Efficient transcription is associated with a reduction in nucleosome density across the promoter region (Gao et al. 2009; J.R. and J.H., data not shown) and an increase in histone H3K4 trimethylation and H3 acetylation at flanking nucleosomes (Lin et al. 2010; Treiber et al. 2010b). Although much has been revealed recently, additional experiments are needed to determine the identities and define the temporal progression of factors that mediate the changes in chromatin structure necessary for *Cd79a* transcription.

6 Conclusions

Initial investigations concerning EBF1 confirmed its regulation of key genes necessary for early B cell development. These observations led to the hypothesis that EBF1 participates in the specification and commitment of B cell progenitors. It is now established that EBF1 instructs this lineage choice. In collaboration with a hierarchy of partner proteins, including E2A, Runx1 and Pax5, EBF1 activates the B cell transcriptome and represses programs of alternate hematopoietic lineages.

The elucidation of much of the structure of EBF1 provides important information concerning its DNA binding, and provides clues to how it may interact with other proteins and activate transcription. The structures provide a basis for addressing aspects of its regulation, including the potential for modulating its activity and integration with signaling pathways. Additional structural and functional studies are needed to elucidate requirements for the three α -helices in the HLHLH domain of EBF1, while only two α -helices suffice for functions of b-HLH proteins. The role of the TIG/IPT domain also remains an enigma. Does it enhance dimerization or does it have other functions?

Collaborative networks of transcription factors direct lineage specification in all known developmental programs. Data confirms the essential role of EBF1 in the B

cell network. As early as the CLP stage, EBF1 directs the expression of genes of the B cell program. Failure to express sufficient levels of EBF1 results in reduced ability to maintain commitment and limits production of B cells. Although it has been determined that EBF1 regulates hundreds of genes in early B cells, many questions remain. At early stages of development, how are different outcomes of transcriptional activation or repression achieved by EBF1? EBF1 activates genes largely in concert with E2A. Do interactions with other factors convert EBF1 into a repressor? Does EBF1 regulate V(D)J recombination directly?

Recent studies have established multiple roles for EBF1 in modulating chromatin structure. However, the underlying mechanisms are yet to be revealed. Does EBF1 recruit histone modifying enzymes and/or chromatin remodeling complexes directly? How does EBF1 facilitate the demethylation of DNA? Are Mi-2/NuRD complexes excluded from promoters by EBF1?

Regulation of early B cell development by EBF1 has provided a paradigm for the control of transcription and cellular differentiation. EBF1 has also proven to be an important regulator of signaling pathways in B cells. Future investigations should address the functions of EBF1 in B cell responses to antigens.

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References

- Adolfsson J, Mansson R, Buza-Vidas N, Hultquist A, Liuba K, Jensen CT, Bryder D, Yang L, Borge OJ, Thoren LA, Anderson K, Sitnicka E, Sasaki Y, Sigvardsson M, Jacobsen SE (2005) Identification of Flt3 + lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell* 121:295–306
- Aravind L, Koonin E (1999) Gleaning non-trivial structural, functional and evolutionary information about proteins by iterative database searches. *J Mol Biol* 287:1023–1040
- Bain G, Maandag EC, Izon DJ, Amsen D, Kruisbeek AM, Weintraub BC, Krop I, Schlissel MS, Feeney AJ, van Roon M, van der Valk M, te Reile HPJ, Berns A, Murre C (1994) E2A proteins are required for proper B cell development and initiation of immunoglobulin gene rearrangements. *Cell* 79:885–892
- Bhalla S, Spaulding C, Brumbaugh RL, Zagort DE, Massari ME, Murre C, Kee BL (2008) differential roles for the E2A activation domains in B lymphocytes and macrophages. *J Immunol* 180:1694–1703
- Bonasio R, Tu S, Reinberg D (2010) Molecular signals of epigenetic states. *Science* 330:612–616
- Campbell KS, Hager EJ, Friedrich RJ, Cambier JC (1991) IgM antigen receptor complex contains phosphoprotein products of B29 and mb-1 genes. *Proc Natl Acad Sci USA* 88:3982–3986
- Decker T, Pasca di Magliano M, McManus S, Sun Q, Bonifer C, Tagoh H, Busslinger M (2009) Stepwise activation of enhancer and promoter regions of the B cell commitment gene Pax5 in early lymphopoiesis. *Immunity* 30:508–520
- DeKoter RP, Singh H (2000) Regulation of B lymphocyte and macrophage development by graded expression of PU.1. *Science* 288:1439–1441
- Delogu A, Schebesta A, Sun Q, Aschenbrenner K, Perlot T, Busslinger M (2006) Gene repression by Pax5 in B cells is essential for blood cell homeostasis and is reversed in plasma cells. *Immunity* 24:269–281

- Dengler HS, Baracho GV, Omori SA, Bruckner S, Arden KC, Castrillon DH, DePinho RA, Rickert RC (2008) Distinct functions for the transcription factor Foxo1 at various stages of B cell differentiation. *Nat Immunol* 9:1388–1398
- Dias S, Silva H Jr, Cumano A, Viera P (2005) Interleukin-7 is necessary to maintain the B cell potential in common lymphoid progenitors. *J Exp Med* 201:971–979
- Dias S, Mansson R, Gurbuxani S, Sigvardsson M, Kee BL (2008) E2A proteins promote development of lymphoid-primed multipotent progenitors. *Immunity* 29:217–227
- Ellenberger T, Fass D, Arnaud M, Harrison SC (1994) Crystal structure of transcription factor E47: E box recognition by a basic region helix-loop-helix dimer. *Genes Dev* 8:970–980
- Feldhaus A, Mbangkollo D, Arvin K, Klug C, Singh H (1992) BlyF, a novel cell-type- and stage-specific regulator of the B-lymphocyte gene *mb-1*. *Mol Cell Biol* 12:1126–1133
- Fields S, Ternyak K, Gao H, Ostraat R, Akerlund J, Hagman J (2008) The ‘zinc knuckle’ motif of Early B cell Factor is required for transcriptional activation of B cell-specific genes. *Mol Immunol* 45:3786–3796
- Finkler A, Ashery-Padan R, Fromm H (2007) CAMTAs: calmodulin-binding transcription activators from plants to human. *FEBS Lett* 581:3893–3898
- Fitzsimmons D, Hodsdon W, Wheat W, Maira S-M, Wasyluk B, Hagman J (1996) Pax-5 (BSAP) recruits Ets proto-oncogene family proteins to form functional ternary complexes on a B-cell-specific promoter. *Genes Dev* 10:2198–2211
- Gao H, Lukin K, Ramírez J, Fields S, Lopez D, Hagman J (2009) Opposing effects of SWI/SNF and Mi-2/NuRD chromatin remodeling complexes on epigenetic reprogramming by EBF and Pax5. *Proc Natl Acad Sci USA* 106:11258–11263
- Gascoyne DM, Long E, Veiga-Fernandes H, de Boer J, Williams O, Seddon B, Coles M, Kioussis D, Brady HJ (2009) The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. *Nat Immunol* 10:1118–1124
- Goebel P, Janney N, Valenzuela JR, Romanow WJ, Murre C, Feeney AJ (2001) 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* 194:645–656
- Gold MR, Matsuuchi L, Kelly RB, DeFranco AL (1991) Tyrosine phosphorylation of components of the B-cell antigen receptors following receptor crosslinking. *Proc Natl Acad Sci USA* 88:3436–3440
- Hagman J, Lukin K (2005) Early B cell factor ‘pioneers’ the way to B cell development. *Trends Immunol* 26:455–461
- Hagman J, Travis A, Grosschedl R (1991) A novel lineage-specific nuclear factor regulates mb-1 gene transcription at the early stages of B cell differentiation. *EMBO J* 10:3409–3417
- Hagman J, Belanger C, Travis A, Turck CW, Grosschedl R (1993) Cloning and functional characterization of early B-cell factor, a regulator of lymphocyte-specific gene expression. *Genes Dev* 7:760–773
- Hagman J, Gutch MJ, Lin H, Grosschedl R (1995) EBF contains a novel zinc coordination motif and multiple dimerization and transcriptional activation domains. *EMBO J* 14:2907–2916
- Hardy RR, Kincade PW, Dorshkind K (2007) The protean nature of cells in the B lymphocyte lineage. *Immunity* 26:703–714
- He T, Hong SY, Huang L, Xue W, Yu Z, Kwon H, Kirk M, Ding SJ, Su K, Zhang Z (2011) Histone acetyltransferase p300 acetylates Pax5 and strongly enhances Pax5-mediated transcriptional activity. *J Biol Chem* 286:14137–14145
- Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, Cheng JX, Murre C, Singh H, Glass CK (2010) Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 38:576–589
- Herzog S, Hug E, Meixlsperger S, Paik JH, DePinho RA, Reth M, Jumaa H (2008) SLP-65 regulates immunoglobulin light chain gene recombination through the PI(3)K-PKB-Foxo pathway. *Nat Immunol* 9:623–631
- Hess J, Nielsen PJ, Fischer KD, Bujard H, Wirth T (2001) The B lymphocyte-specific coactivator BOB.1/OBF.1 is required at multiple stages of B-cell development. *Mol Cell Biol* 21:1531–1539

- Hombach J, Lottspeich F, Reth M (1990) Identification of the genes encoding the IgM-alpha and Ig-beta components of the IgM antigen receptor complex by amino-terminal sequencing. *Eur J Immunol* 20:2795–2799
- Ichii M, Shimazu T, Welner RS, Garrett KP, Zhang Q, Esplin BL, Kincade PW (2010) Functional diversity of stem and progenitor cells with B-lymphopoietic potential. *Immunol Rev* 237:10–21
- Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 33(Suppl):245–254
- Kee BL, Murre C (1998) Induction of early B cell factor (EBF) and multiple B lineage genes by the basic helix-loop-helix transcription factor E12. *J Exp Med* 188:699–713
- Kikuchi K, Lai AY, Hsu C-L, Kondo M (2005) IL-7 receptor signaling is necessary for stage transition in adult B cell development through up-regulation of EBF. *J Exp Med* 201:1197–1203
- Kim U, Qin X-F, Gong S, Stevens S, Luo Y, Nussenzweig M, Roeder RG (1996) The B-cell-specific transcription coactivator OCA-B/OBF-1/Bob-1 is essential for normal production of immunoglobulin isotypes. *Nature* 383:542–547
- Klug A (2010) The discovery of zinc fingers and their applications in gene regulation and genome manipulation. *Annu Rev Biochem* 79:213–231
- Kondo M (1997) Identification of clonogenic lymphoid progenitors in mouse bone marrow. *Cell* 91:661–672
- Kondo M (2010) Lymphoid and myeloid lineage commitment in multipotent hematopoietic progenitors. *Immunol Rev* 238:37–46
- Liber D, Domaschenz R, Holmqvist PH, Mazzarella L, Georgiou A, Leleu M, Fisher AG, Labosky PA, Dillon N (2010) Epigenetic priming of a pre-B cell-specific enhancer through binding of Sox2 and Foxd3 at the ESC stage. *Cell Stem Cell* 7:114–126
- Lin H, Grosschedl R (1995) Failure of B-cell differentiation in mice lacking the transcription factor EBF. *Nature* 376:263–267
- Lin YC, Jhunjhunwala S, Benner C, Heinz S, Welinder E, Månsson R, Sigvardsson M, Hagman J, Espinoza CA, Dutkowski J, Ideker T, Glass CK, Murre C (2010) A global network of transcription factors, involving E2A, EBF1 and Foxo1, that orchestrates B cell fate. *Nat Immunol* 11:635–643
- Lukin K, Fields S, Lopez D, Cherrier M, Ternyak K, Ramirez J, Feeney AJ, Hagman J (2010) Compound haploinsufficiencies of Ebf1 and Runx1 genes impede B cell lineage progression. *Proc Natl Acad Sci USA* 107:7869–7874
- Lukin K, Fields S, Guerretaz L, Straign D, Rodriguez V, Zandi S, Mansson R, Cambier JC, Sigvardsson M, Hagman J (2011) A dose-dependent role for EBF1 in repressing non-B-cell-specific genes. *Eur J Immunol* 41:1787–1793
- Maier H, Colbert J, Fitzsimmons D, Clark DR, Hagman J (2003) Activation of the early B cell-specific mb-1 (Ig-a) gene by Pax-5 is dependent on an unmethylated Ets binding site. *Mol Cell Biol* 23:1946–1960
- Maier H, Ostraat R, Gao H, Fields S, Shinton SA, Medina KL, Ikawa T, Murre C, Singh H, Hardy RR, Hagman J (2004) Early B cell factor cooperates with Runx1 and mediates epigenetic changes associated with *mb-1* transcription. *Nat Immunol* 5:1069–1077
- Malin S, McManus S, Cobaleda C, Novatchkova M, Delogu A, Bouillet P, Strasser A, Busslinger M (2010) Role of STAT5 in controlling cell survival and immunoglobulin gene recombination during pro-B cell development. *Nat Immunol* 11:171–179
- Mansson R, Zandi S, Welinder E, Tsapogas P, Sakaguchi N, Bryder D, Sigvardsson M (2010) Single-cell analysis of the common lymphoid progenitor compartment reveals functional and molecular heterogeneity. *Blood* 115:2601–2609
- Månsson R, Lagergren A, Hansson F, Smith E, Sigvardsson M (2007) The CD53 and CEACAM-1 genes are genetic targets for early B cell factor. *Eur J Immunol* 37:1365–1376
- Medina KL, Pongubala JMR, Reddy KL, Lancki DW, DeKoter RP, Kieslinger M, Grosschedl R, Singh H (2004) Defining a regulatory network for specification of the B cell fate. *Dev Cell* 7:607–617

- Mikkola I, Heavey B, Horcher M, Busslinger M (2002) Reversion of B cell commitment upon loss of Pax5 expression. *Science* 297:110–113
- Ng CH, Xu S, Lam KP (2007) Dok-3 plays a nonredundant role in negative regulation of B-cell activation. *Blood* 110:259–266
- Ng SY, Yoshida T, Zhang J, Georgopoulos K (2009) Genome-wide lineage-specific transcriptional networks underscore Ikaros-dependent lymphoid priming in hematopoietic stem cells. *Immunity* 30:493–507
- Nutt SL, Heavey B, Rolink AG, Busslinger M (1999) Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature* 401:556–562
- O’Riordan M, Grosschedl R (1999) Coordinate regulation of B cell differentiation by the transcription factors EBF and E2A. *Immunity* 11:21–31
- Orford K, Kharchenko P, Lai W, Dao MC, Worhunsky DJ, Ferro A, Janzen V, Park PJ, Scadden DT (2008) Differential H3K4 methylation identifies developmentally poised hematopoietic genes. *Dev Cell* 14:798–809
- Pongubala JM, Northrup DL, Lancki DW, Medina KL, Treiber T, Bertolino E, Thomas M, Grosschedl R, Allman D, Singh H (2008) Transcription factor EBF restricts alternative lineage options and promotes B cell fate commitment independently of Pax5. *Nat Immunol* 9:203–215
- Purohit SJ, Stephan RP, Kim HG, Herrin BR, Gartland L, Klug CA (2003) Determination of lymphoid cell fate is dependent on the expression status of the IL-7 receptor. *EMBO J* 22: 5511–5521
- Qian W, Zhang J (2008) Gene dosage and gene duplicability. *Genetics* 179:2319–2324
- Ramirez J, Hagman J (2009) The Mi-2/NuRD complex: a critical epigenetic regulator of hematopoietic development, differentiation and cancer. *Epigenetics* 4:532–536
- Ramirez J, Lukin K, Hagman J (2010) From hematopoietic progenitors to B cells: mechanisms of lineage restriction and commitment. *Curr Opin Immunol* 22:177–184
- Reynaud D, Demarco IA, Reddy KL, Schjerven H, Bertolino E, Chen Z, Smale ST, Winandy S, Singh H (2008) Regulation of B cell fate commitment and immunoglobulin heavy-chain gene rearrangements by Ikaros. *Nat Immunol* 9:927–936
- Robertson AG, Bilenky M, Tam A, Zhao Y, Zeng T, Thiessen N, Cezard T, Fejes AP, Wederell ED, Cullum R, Euskirchen G, Krzywinski M, Birol I, Snyder M, Hoodless PA, Hirst M, Marra MA, Jones SJ (2008) Genome-wide relationship between histone H3 lysine 4 mono- and trimethylation and transcription factor binding. *Genome Res* 18:1906–1917
- Roessler S, Györy I, Imhof S, Spivakov M, Williams RR, Busslinger M, Fisher AG, Grosschedl R (2007) Distinct promoters mediate the regulation of Ebf1 gene expression by interleukin-7 and Pax5. *Mol Cell Biol* 27:579–594
- Rolink AG, Nutt SL, Melchers F, Busslinger M (1999) Long-term in vivo reconstitution of T-cell development by Pax5-deficient B-cell progenitors. *Nature* 401:603–606
- Romanow WJ, Langerak AW, Goebel P, Wolvers-Tettero ILM, van Dongen JJM, Feeney AJ, Murre C (2000) E2A and EBF act in synergy with the V(D)J recombinase to generate a diverse immunoglobulin repertoire in nonlymphoid cells. *Mol Cell* 5:343–353
- Rothenberg EV (2007) Cell lineage regulators in B and T cell development. *Nat Immunol* 8:441–444
- Schubart DB, Rolink A, Kosco-Vilbois MH, Botteri F, Matthias P (1996) B-cell-specific coactivator OBF-1/OCA-B/Bob1 required for immune response and germinal centre formation. *Nature* 383:538–542
- Semerad CL, Mercer EM, Inlay MA, Weissman IL, Murre C (2009) E2A proteins maintain the hematopoietic stem cell pool and promote the maturation of myelolymphoid and myeloerythroid progenitors. *Proc Natl Acad Sci USA* 106:1930–1935
- Sigvardsson M, O’Riordan M, Grosschedl R (1997) EBF and E47 collaborate to induce expression of the endogenous immunoglobulin surrogate light chain genes. *Immunity* 7:25–36
- Sigvardsson M, Clark DR, Fitzsimmons D, Doyle M, Åkerblad P, Breslin T, Bilke S, Liu Y-F, Yeaman C, Zhang G, Hagman J (2002) Early B cell Factor, E2A, and Pax-5 cooperate to activate the early B cell-specific *mb-1* promoter. *Mol Cell Biol* 22:8539–8551

- Siponen MI, Wisniewska M, Lehtio L, Johansson I, Svensson L, Raszewski G, Nilsson L, Sigvardsson M, Berglund H (2010) Structural determination of functional domains in early B-cell factor (EBF) family of transcription factors reveals similarities to Rel DNA-binding proteins and a novel dimerization motif. *J Biol Chem* 285:25875–25879
- Smith EMK, Gisler R, Sigvardsson M (2002) 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* 169:261–270
- Spooner CJ, Cheng JX, Pujadas E, Laslo P, Singh H (2009) A recurrent network involving the transcription factors PU.1 and Gfi1 orchestrates innate and adaptive immune cell fates. *Immunity* 31:576–586
- Srinivasan L, Sasaki Y, Calado DP, Zhang B, Paik JH, DePinho RA, Kutok JL, Kearney JF, Otipoby KL, Rajewsky K (2009) PI3 kinase signals BCR-dependent mature B cell survival. *Cell* 139:573–586
- Thal M, Carvalho TL, He T, Kim HG, Gao H, Hagman J, Klug CA (2009) Ebf1-mediated down-regulation of Id2 and Id3 is essential for specification of the B cell lineage. *Proc Natl Acad Sci USA* 106:552–557
- Travis A, Hagman J, Grosschedl R (1991) Heterogeneously initiated transcription from the pre-B- and B-cell-specific *mb-1* promoter: analysis of the requirement for upstream factor-binding sites and initiation site sequences. *Mol Cell Biol* 11:5756–5766
- Travis A, Hagman J, Hwang L, Grosschedl R (1993) Purification of early-B-cell factor and characterization of its DNA-binding specificity. *Mol Cell Biol* 13:3392–3400
- Treiber N, Treiber T, Zocher G, Grosschedl R (2010a) Structure of an Ebf1:DNA complex reveals unusual DNA recognition and structural homology with Rel proteins. *Genes Dev* 24:2270–2275
- Treiber T, Mandel EM, Pott S, Györy I, Firner S, Liu ET, Grosschedl R (2010b) Early B cell factor 1 regulates B cell gene networks by activation, repression, and transcription-independent poising of chromatin. *Immunity* 32:714–725
- Wang MM, Reed RR (1993) Molecular cloning of the olfactory neuronal transcription factor OLF-1 by genetic selection in yeast. *Nature* 364:121–126
- Zandi S, Mansson R, Tsapogas P, Zetterblad J, Bryder D, Sigvardsson M (2008) EBF1 is essential for B-lineage priming and establishment of a transcription factor network in common lymphoid progenitors. *J Immunol* 181:3364–3372
- Zhang Z, Cotta CV, Stephan RP, deGuzman CG, Klug CA (2003) Enforced expression of EBF in hematopoietic stem cells restricts lymphopoiesis to the B cell lineage. *EMBO J* 22:4759–4769
- Zhao F, McCarrick-Walmsley R, Akerblad P, Sigvardsson M, Kadesch T (2003) Inhibition of p300/CBP by early B-cell factor. *Mol Cell Biol* 23:3837–3846
- Zhuang Y, Soriano P, Weintraub H (1994) The helix-loop-helix gene E2A is required for B cell formation. *Cell* 79:875–884

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