Transcriptional Control of Pre-B Cell Development and Leukemia Prevention

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Abstract The differentiation of early B cell progenitors is controlled by multiple transcriptional regulators and growth-factor receptors. The triad of DNA-binding proteins, E2A, EBF1, and PAX5 is critical for both the early specification and commitment of B cell progenitors, while a larger number of secondary determinants, such as members of the Ikaros, ETS, Runx, and IRF families have more direct roles in promoting stage-specific pre-B gene-expression program. Importantly, it is now apparent that mutations in many of these transcription factors are associated with the progression to acute lymphoblastic leukemia. In this review, we focus on recent studies that have shed light on the transcriptional hierarchy that controls efficient B cell commitment and differentiation as well as focus on the oncogenic consequences of the loss of many of the same factors.

Contents

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Current Topics in Microbiology and Immunology (2014) 381: 189–213
DOI: 10.1007/82_2014_377

Published Online: 16 May 2014

1 Introduction to Early B Cell Development

Early B cell development initiates with the gradual stepwise differentiation of multipotent hematopoietic stem cells (HSCs) to early B cell progenitors in the bone marrow (BM). The lymphoid-primed multipotent progenitors (LMPPs) are the first lymphoid specified progenitor cells downstream of the HSC that retain the full lympho-myeloid lineage potential but give rise to little or no megakaryocyte and erythrocyte progenitors (MEPs) (Adolfsson et al. 2001, 2005). LMPPs include lymphoid-biased progenitors such as early lymphoid progenitors (ELPs) that are defined by the expression of the *Rag1* gene (Igarashi et al. 2002). ELPs are the precursors of common lymphoid progenitors (CLPs) in which critical transcriptional regulated B-cell specification and commitment occur (Karsunky et al. 2008; Mansson et al. 2008, 2010; Kondo et al. 1997). CLPs have recently been further split, based on the expression of the cell surface receptor, Ly6D, into all lymphoid progenitors (ALPs) that show pan-lymphocyte developmental potential and B cell-biased lymphoid progenitors (BLPs) (Inlay et al. 2009).

BLPs give rise to pre-pro-B-cells (also known as Fraction A), which can be identified by the expression of the B cell-associated marker B220 (CD45R) (Rumfelt et al. 2006; Gounari et al. 2002; Li et al. 1996). Commitment to the B cell lineage occurs at the pro-B cell stage (Fraction B/C cells) (Rumfelt et al. 2006). Committed pro-B cells can be identified by their expression of CD19 and their lack of fms-like tyrosine kinase FLT3 (also known as Flk2 or CD135 (Rumfelt et al. 2006; Holmes et al. 2006)). Committed pro-B cells express high levels of *Rag1/2* and recombine their variable (V) gene segments to previously rearranged D_H-J_H segments at the *Immunoglobulin heavy chain* (*Igh*) locus (ten Boekel et al. 1995; Li et al. 1993). Successful rearrangement of the *Igh* locus leads to the expression of IgH at the cell surface in association with the surrogate light chain ($\lambda 5$, VpreB) and accessory signaling molecules (Igα, Igβ) to form the pre-B cell receptor (pre-BCR) (Karasuyama et al. 1990, 1994). Signaling through the pre-BCR transiently down regulates expression of *Rag1/2* (Grawunder et al. 1995), induces a proliferative burst (Rolink et al. 1994) and triggers differentiation to the

small pre-B stage (Fraction D cells) (Kitamura et al. 1991, 1992). Small pre-B cells re-express *Rag1/2* and undergo rearrangement of their Ig light chain (*Igl*) locus (ten Boekel et al. 1995; Grawunder et al. 1995). Productive *Igl* rearrangement results in the expression of the B cell receptor (BCR) and progression to the immature B cell stage (Fraction E cells); these cells exit the BM and complete their development in the periphery (Loder et al. 1999).

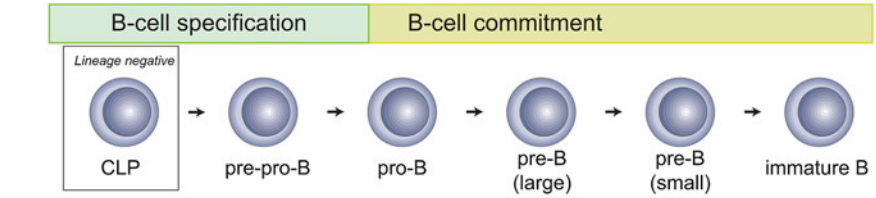
In addition to the productive rearrangements required to produce the pre-BCR, pre-B cell development in the mouse also requires signaling through the IL-7R, as mice lacking signaling components of the IL-7 receptor have few pre-B cells (Peschon et al. 1994; Clark et al. 2014). Similarly, absence of the signaling cascade components that follow IL-7R activation such as STAT5 (A and B) (Goetz et al. 2004; Yao et al. 2006), cyclin D3 (Cooper et al. 2006), and phosphoinositide 3-kinase (PI3K) (Ramadani et al. 2010; Fruman et al. 1999; Suzuki et al. 1999; Clayton et al. 2002; Jou et al. 2002; Okkenhaug et al. 2002) greatly attenuate the proliferation and survival of pre-B cells. Interestingly, mutation in ATP11c, a P4 ATPases (flippase) that is required for IL-7 signaling, results in a progressive loss of pro- and pre-B cells (Clark 2011; Pang and Nutt 2011; Siggs et al. 2011; Yabas et al. 2011). Clearly, efficient pre-B cell differentiation requires the coordination of the intrinsic cell differentiation program, appropriate recombination of the *Igh* and *Igl* genes, and responsiveness to extrinsic signals provided by cytokines (IL-7 in the mouse). In this review we highlight the key transcriptional regulators that control this process and discuss their deregulation in leukemia.

2 Transcriptional Regulation of Early B Cell Development

It is well known that the initiation of B cell development from the lymphoid progenitors relies on a transcriptional network consisting of three main transcription factors E2A (encoded by *Tcfe2a*), Ebf1 (*Ebf1*), and Pax5 (*Pax5*). These factors in turn activate a number of secondary factors that directly drive pre-B cell development. As E2A, Ebf1 and Pax5 have been studied in great detail and are the subject of numerous reviews (Cobaleda et al. 2007; Mandel and Grosschedl 2010; Murre 2007; Singh et al. 2007; Nutt and Kee 2007), here we provide only a brief introduction to these factors and concentrate on factors that have more recently been shown to be important in pre-B cell development (Fig. 1).

2.1 E2A

E2A is a basic helix-loop-helix (bHLH) transcription factor. The bHLH domain of E2A mediates dimerization and binding to the E-box motif in DNA. Alternative splicing of the E2A gene (*Tcfe2a*) gives rise to two isoforms, E12 and E47, which differ only in their bHLH domain. Mice deficient in *Tcfe2a* completely lack mature



	CLP	pre-pro-B	pro-B	pre-B (large)	pre-B (small)	immature B
PU.1	+	+	+	+	+	+
IRF8	++	+	+	+	+	+
Ikaros	+	+	+	++	+++	++
c-Myb	+	+	+	+	++	-
Runx1	+	+	+	+	+	+
Gfi1	+	+	++	+++	++	+
Gfi1b	+	+	+	+	+	+
Miz-1	++	+++	++	++	++	++
Spi-B	-	-	+	+	++	+++
IRF4	-	-	+	++	+++	+
Aiolos	-	-	+	+	++	+++
Foxo1	-	+	++	+	+++	+++
Bach2	-	-	+	+	+++	++
BLNK	-	++	+++	++	+++	++

Fig. 1 B cell development in the bone marrow of adult mice. Important progenitor stages are depicted from common lymphoid progenitor (CLP) to immature B cell, along with the expression of key transcription factors and proteins. Progenitors within the lineage-negative fractions of bone marrow are contained within the box. Expression levels are shown on an arbitrary scale; −, no or very low expression; +, ++, +++ low, intermediate or high relative expression respectively. Expression levels were derived from the Immunological Genome project (www.immgen.org) (Nutt et al. 2005; Vassen et al. 2007) and S.L.N. unpublished

B cells, indicating that E2A is essential for B cell development (Bain et al. 1994; Zhuang et al. 1994). Expression of *Tcfe2a* is upregulated at the CLP stage of development and remains high in pro-B, pre-B and immature B cells in the bone marrow (Kwon et al. 2008). Lymphoid defects in *Tcfe2a*$^{-/-}$ mice are already apparent at the LMPP and CLP stages of development, which are modestly reduced in number and display decreased priming of lymphoid gene expression (Borghesi et al. 2005; Dias et al. 2005, 2008). While E2A appears essential for the priming of the expression of many lymphoid transcripts (Mercer et al. 2011), the major development block occurs at the pre-pro-B cell stage due to a failure to upregulate a number of B cell-specific genes including *Ebf1* and *Pax5* (Bain et al. 1994; Seet et al. 2004). The progenitors from these mice do not express *Rag1* and consequently cannot undergo *Ig* gene rearrangement (Bain et al. 1994; Borghesi et al. 2005). Similarly, conditional deletion of *Tcfe2a* after B cell commitment leads to a breakdown of B cell gene expression and the loss of committed pro-B

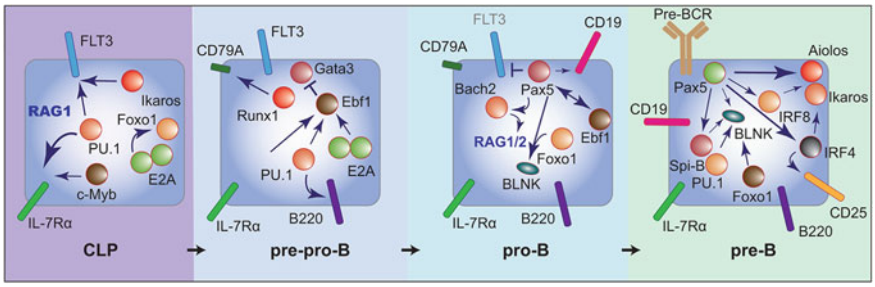


Fig. 2 Model for the transcription regulation of early B cell development. Key stages in early B cell differentiation are depicted, including the important transcription factors and cell surface receptors at each stage. *Arrows* indicate positive regulation, ⊥ indicates inhibition. *CLP* common lymphoid progenitors. Models are adapted from earlier work by (Medina et al. 2004; Nutt and Kee 2007; Singh et al. 2005)

cells (Kwon et al. 2008). E2A activity regulates a large number of genes in B cells, often in conjunction with Ebf1 and Pax5 (Lin et al. 2010). One important E2A target is Foxo1 that in turn then acts with E2A and HeLa E-box binding protein (HEB) to support B cell programming (Welinder et al. 2011) (Fig. 2). The role of Foxo1 will be discussed in further detail below.

2.2 Ebf1

Ebf1 is a zinc finger helix-loop-helix transcription factor that collaborates with E2A to initiate B cell gene expression. *Ebf1* is expressed at a low level in CLPs, but is greatly upregulated in pro-B cells (Mansson et al. 2008; Vilagos et al. 2012). Despite the phenotypic resemblance between the B cell developmental blocks of E2A and Ebf1-deficient mice (Bain et al. 1994; Lin and Grosschedl 1995), CLPs can develop in Ebf1-deficient mice but are unable to undergo B cell differentiation due to the reduced expression of B cell associated genes. Ebf1 regulates expressions of many genes that encode proteins required for B cell development including, Igα, VpreB, λ5 and Pax5 (Treiber et al. 2010; Mandel and Grosschedl 2010), while also repressing genes associated with alternative lineage fates (Nechanitzky et al. 2013). Conditional inactivation after the formation of pro-B cells confirmed the intrinsic requirement of Ebf1 in the earliest stages of B cell development (Gyory et al. 2012; Vilagos et al. 2012).

Overexpression of Ebf1 restricts the developmental potential of hematopoietic progenitors (Zhang et al. 2003; Pongubala et al. 2008) and partially rescues B cell development in the absence of E2A, PU.1, Ikaros and IL-7Rα (Dias et al. 2005; Medina et al. 2004; Reynaud et al. 2008; Seet et al. 2004). Careful investigation of the *Ebf1* promoters has revealed a complex regulatory network that acts to stabilize B cell expression (Roessler et al. 2007). Indeed, from a recent study, Ebf1

directly represses Gata3 by binding to the promoter region of *Gata3* locus, and induces recruitment of silencing modification proximal to the locus (Fig. 2). This highlights a new role of Ebf1 in suppressing T cell differentiation while allowing B cell differentiation in the presence of Pax5 (Banerjee et al. 2013).

2.3 Pax5

Unlike E2A and Ebf1, Pax5 is not required for the initial stages of B cell specification (Nutt et al. 1997, 1999). Pax5 is a member of the paired-box transcription factor family and is expressed in constant level throughout all B cell stages from the pro-B cell stage onwards until it is down regulated in plasma cells (Fuxa and Busslinger 2007). In the absence of *Pax5*, B cell development is arrested at the early pro-B cell stage of differentiation (Nutt et al. 1997). Strikingly, $Pax5^{-/-}$ pro-B cells are unable to differentiate into mature B cells but instead are capable of differentiating into a broad range of other hematopoietic cell types (Nutt et al. 1999; Rolink et al. 1999). Similar results were demonstrated by conditionally inactivating *Pax5* in pro-B cells (Mikkola et al. 2002). This was probably because Pax5 plays a crucial role in repressing genes, such as *Flt3*, *Notch1* and *Mcsfr* (*Csf1r*) that are associated with signaling in multipotent progenitors or non-B cell lineages (Delogu et al. 2006; Holmes et al. 2006, 2008; Souabni et al. 2002; Tagoh et al. 2006; Nutt et al. 1999; Pridans et al. 2008). Notably, a similar capacity for multilineage differentiation was reported for E2A and Ebf1-deficient lymphoid cell lines (Ikawa et al. 2004; Nechanitzky et al. 2013). This is because these cells lack high expression of markers of B cell specification as well as Pax5 (Ikawa et al. 2004).

Pax5 binds to thousands of genes in the B cell genome and plays an active role in regulating B cell chromatin (McManus et al. 2011; Revilla et al. 2012; Tagoh et al. 2006). The consequence of this copious DNA-binding is the direct regulation, both activation and repression, or hundreds of transcripts many of which are of central importance in B cell differentiation and function (Delogu et al. 2006; Pridans et al. 2008; Schebesta et al. 2007). Interestingly, a major function of Pax5 appear to be the activation of the expression of a suite of transcriptional regulators, including IRF4, IRF8, Spi-B, Aiolos and Bach2 (Fig. 2) that have important functions at the pre-B cell stage of development (Holmes et al. 2008; Pridans et al. 2008; Schebesta et al. 2007).

3 Other Players in Early B Cell Development

Besides the conventional co-transcriptional regulatory circuit of E2A, Ebf1, and Pax5 that functions to lock in progenitor cells to B cell fate, a number of transcription factors have been identified as playing important role in pro- and pre-B

cell development (see Fig. 1). This review highlights some of the new players involved in early B cell differentiation and malignancy.

3.1 PU.1 and Spi-B

Two members of the ETS domain transcription factor family, PU.1 and Spi-B have been implicated in early B cell development. PU.1 is encoded by the gene *Sfpi1* and is a critical regulator of hematopoiesis (reviewed by (Dakic et al. 2007)). Both germ line deletion and conditional inactivation of PU.1 in adult HSCs have demonstrated that PU.1 is required for the production of B cell progenitors from HSCs (Dakic et al. 2005; Scott et al. 1994). PU.1 is dynamically expressed throughout hematopoiesis with myeloid cell being characterized by high PU.1 expression, while B cells are uniformly PU.1 low (Back et al. 2005; Dakic et al. 2005). The relatively low expression of PU.1 is an essential requirement for B cell development, as high PU.1 diverts hematopoietic progenitors along the myeloid pathway (DeKoter and Singh 2000; Kueh et al. 2013).

PU.1 regulates FLT3 and IL-7Rα, two key cytokine receptors that are expressed by early lymphoid progenitors (Fig. 2) (DeKoter et al. 2002; Carotta et al. 2010). This regulation is likely to be important as mice lacking both receptors do not generate any B cell progenitors (Vosshenrich et al. 2003). In addition, CD45R, which encodes a common marker for B cells (B220) that is initially expressed in pre-pro B cells, is a direct target of PU.1 (Anderson et al. 2001). Despite the above-mentioned studies showing the regulatory roles of PU.1 in early B cell development, disruption of this transcription factor in CLPs demonstrated no effect (Iwasaki et al. 2005). In support of this observation, two other studies using specific deletion of PU.1 under the promoter of CD19 showed that PU.1 is not strictly essential beyond the pre-B cells in the bone marrow (Polli et al. 2005; Ye et al. 2005).

Spi-B, the ETS protein that is most closely related to PU.1 is also expressed in B cells, where it is under the control of Pax5 (Pridans et al. 2008; Schebesta et al. 2007). Absence of Spi-B only affects the maturation and the function of peripheral B cells while the early B cell development remains undisturbed (Su et al. 1997). The identical DNA binding specificity of Spi-B and PU.1 suggests that the loss of PU.1 function in B cells could be compensated for by Spi-B. In support of this idea, deficiencies in both PU.1 and Spi-B, results in a developmental block at the pre-B cells (Xu et al. 2012; Sokalski et al. 2011). While one identified target of PU.1 and Spi-B is the adaptor molecule BLNK (SLP65) that is important in pre-BCR signaling (Xu et al. 2012), the mechanism by which PU.1 and Spi-B fit into the network of B cell-specific transcription factor remains poorly understood.

3.2 *IRF4 and IRF8*

Interferon regulatory factor (IRF) 4 (also known as Pip, LSIRF, LCSAT, NF-EM5, and MUM1) is part of the IRF family of transcription factors. IRF4 plays a fundamental role in late B cell differentiation to promote *Ig* class switch recombination, germinal center formation and plasma cell differentiation (Mittrücker et al. 1997; Ochiai et al. 2013; Sciammas et al. 2006, 2011; Willis et al. 2014). Additionally, IRF4 has been demonstrated to be important for *Igκ* recombination and the attenuation of the IL-7 signaling pathway, thus promoting the transition from the pre-B to B cell stages of maturation (Clark et al. 2014; Johnson et al. 2008). The interaction between IRF4 and E2A enhances the binding affinity of E2A for the 3' *Igκ* enhancer region (Eκ3'). The knockdown of IRF4 in pre-B cells also reduces the histone acetylation at both *Eκ3'* and the intronic enhancer (*Eκi*), suggesting an important role of IRF4 in early B cell development (Lazorchak et al. 2006). IRF4 is also important for receptor editing in immature B cell stage to establish B cell tolerance (Pathak et al. 2008).

IRF8 (also known as ICSBP) is another IRF family transcription factor family member that is highly homologous to IRF4. Deficiency in IRF8 results in a reduction in CLP, which later accounted for the significant reduction in pre-pro-B cells (Wang et al. 2008). The decreased commitment of CLPs to pre-pro-B cells was found to be associated with the reduced expression of B cell-specific transcription factors such as E2A, Ebf1, and Pax5. Interestingly, IRF8 and PU.1 have been shown to synergistically regulate *Ebf1* expression (Wang et al. 2008).

IRF4 and 8 bind very weakly to DNA containing only IRF sites, but are recruited to their binding sites via interaction with other transcription factors. In particular, PU.1 and Spi-B have been shown to recruit IRF4 or IRF8 to ETS-IRF composite elements (EICE) located in the *Eκ3'* and *Igλ* enhancers (Brass et al. 1999; Eisenbeis et al. 1995; Escalante et al. 2002; Pongubala et al. 1992). Due to their extensive homology, IRF4 and IRF8 were suggested to function redundantly. Indeed, double deficiencies in IRF4 and IRF8 resulted in a development arrest at the pre-B cell stage (Lu et al. 2003). The pre-B cells in the bone marrow of these double mutant mice are hyperproliferative and express high level of pre-BCR. Interestingly, these cells are also defective in *Igl* gene rearrangement and transcription, and restoration of either IRF could rescue the early development of B cells (Ma et al. 2008; Lu et al. 2003). In keeping with the molecular interaction between the IRFs and PU.1/Spi-B, we have found that B cell development in IRF4 and PU.1 double deficient mice also blocks at the pre-B cell stage (S.H.M.P., S.C., and S.L.N. submitted). Interestingly, IRF4 and 8 have been shown to induce the expression of two closely related transcription factors, Ikaros and Aiolos, that promoted the expansion of the pre-B cell numbers (see below; Ma et al. 2008).

3.3 Ikaros and Aiolos

The zinc finger transcription factors, Ikaros (encoded by *Ikzf1*) and Aiolos (*Ikzf3*) are transcriptional regulators that play multiple roles in B cell development and function (John and Ward 2011). The absence of pre-pro-B cells in Ikaros-deficient mice and the reduction of these B cell progenitors in mice bearing hypomorphic alleles of Ikaros suggested a defect in lymphoid priming (Kirstetter et al. 2002; Wang et al. 1996). Indeed, LMPP of Ikaros-deficient mice exhibited lower levels of *Il7r* and *Rag1* expression, which is important for B cell priming and specification (Yoshida et al. 2006). Strikingly, similar to $Pax5^{-/-}$ pro-B cells, $Ikzf1^{-/-}$ pro-B cells (rescued by ectopic expression of Ebf1) are able to differentiate into myeloid cells indicating that Ikaros is restricting lymphoid progenitors to the B cell fate (Reynaud et al. 2008).

Ikaros binds to a number of genes required for pre-BCR signaling, *Ig* gene recombination, cell growth, adhesion and proliferation (Ferreiros-Vidal et al. 2013; Schwickert et al. 2014). Strikingly, by using specific deletion of Ikaros in pro/pre-B cells, Ikaros activates a transcriptional event essential for BCR signaling by attenuating IL-7 signals for B cell differentiation (Heizmann et al. 2013; Schwickert et al. 2014). Ikaros was also found, using a slightly different model, to be critical in pre-B cells during the transition from stroma-adherent proliferative stage to non-adherent differentiation stage. Loss of Ikaros locks pre-B cells with enhanced integrin signaling and highly proliferative stage (Joshi et al. 2014). Similarly, Ikaros also promotes the migration of pro-B cells and simultaneously prevents cell adhesion in early B cell development (Schwickert et al. 2014).

Aiolos is expressed throughout B cell development from the pre-pro-B cell stage where it is under the control of Pax5 (Fig. 1) (Pridans et al. 2008; Schebesta et al. 2007). Aiolos-deficient mice have relatively normal B cell development, however Aiolos has been shown to play roles in the silencing of the *Igll1* gene (encoding $\lambda 5$) in pre-B cells after pre-BCR signaling (Thompson et al. 2007; Karnowski et al. 2008). Indeed, this mechanism correlates with the expression of *Ikzf3* being highly upregulated in response to pre-BCR signals (Ferreiros-Vidal et al. 2013). Ikaros and Aiolos can form both hetero- and homodimers and in keeping with this, genome wide studies revealed that Ikaros and Aiolos share many target genes, including the B cell associated genes such as *Cd79b*, *Foxo1, Blnk* and *Syk* (Ferreiros-Vidal et al. 2013) implicated in pre-BCR signaling, cell cycle regulation and somatic rearrangement of *Ig* genes. Interestingly, there is significant enrichment of Ikaros binding sites within regulatory regions that also bind Ebf1, E2A, Pax5 and Foxo1, further reinforcing the notion that B cell development is initiated and stabilized by a combinatorial transcriptional network (Ferreiros-Vidal et al. 2013; Lin et al. 2010; Revilla et al. 2012).

3.4 c-Myb, Gfi1 and Miz-1; Regulators of IL-7 Signaling

Signaling through the IL-7R is essential for early B-lymphopoiesis in the mouse, although the mechanisms by which this signal is regulated are complex and only partially understood. In addition to the previously discussed roles of PU.1 in activating *Il7r* expression (DeKoter et al. 2002) and STAT5A/B (Malin et al. 2010) in transducing the signal three other transcription factors, c-Myb, Gfi1, and Miz-1 are implicated.

c-Myb has long been known to be essential for hematopoiesis, however, its function in B cell development has only been appreciated more recently (Greig et al. 2008). Mice bearing hypomorphic *c-Myb* alleles displayed profound reduction in the B cell compartment (Emambokus et al. 2003; Carpinelli et al. 2004; Sandberg et al. 2005; Xiao et al. 2007). In addition, conditional deletion of c-Myb specifically in the B cell lineage demonstrating a direct requirement of c-Myb in developing pro-B cells (Fahl et al. 2009; Greig et al. 2010). c-Myb was further demonstrated to be required for lymphoid priming before the CLP stage, and also to maintain normal expression of IL-7R in pro-B cells (Greig et al. 2010). A recent study has suggested that both Ebf1 and c-Myb repress *Rag1/2* transcription by negatively regulating the binding of Foxo1 to the *Rag* locus during the transition between large pre-B to small pre-B cells (Timblin and Schlissel 2013). While the role of c-Myb in early B cell development has been slowly elucidated, its collaborating role with other transcription factors such as PU.1, E2A, Ikaros, Runx1—to mention a few, remains poorly understood. Nevertheless, c-Myb has been shown to synergise with PU.1 to activate *Il7r* transcription (Greig et al. 2010).

Gfi1 is a zinc finger containing repressor that plays an important role in early lymphopoiesis (Moroy and Khandanpour 2011). Gfi1-deficient mice have a reduced CLP compartment and few pre-pro-B and pro-B cells, a phenotype that resembles both mice lacking the IL-7R or harboring c-Myb hypomorphic alleles (Moroy and Khandanpour 2011). Interestingly, Gfi1 has been shown to inhibit PU.1 activity in hematopoietic progenitors capable of both lymphoid and myeloid differentiation, thus promoting the B cell fate (Spooner et al. 2009). As Ikaros is thought to be upstream of *Gfi1*, this finding provides a mechanism by which Ikaros promotes lymphopoiesis. The highly related gene *Gfi1b* is also expressed in developing B cells and while the degree of redundancy of these factors remains to be fully determined, mice lacking both factors have a more severe block in early B cell development than that observed for Gfi1 knockouts alone (Schulz et al. 2012). Gfi1b has been implicated in pre-B cell differentiation where it represses *Rag1/2* expression through both direct binding to the shared *Rag* enhancer and indirect repression of *Foxo1* (Schulz et al. 2012).

Miz-1 is a BTB/POZ domain transcription factor that has been implicated in cell cycle control and the inhibition of apoptosis (Moroy et al. 2011). Miz-1 is an important regulator of IL-7 signaling, with B-lymphopoiesis blocked at the pre-pro-B cell stage in its absence. Miz-1-deficient CLPs express normal amounts of the IL-7R but fail to adequately transduce the required survival and proliferation signals

(Kosan et al. 2010). Interestingly, Miz1-deficient progenitors have increased SOCS1 and decreased Bcl2, potentially explaining the high apoptotic rate and inability to respond to IL-7. Ebf1 may also act downstream of Miz1, as ectopic Ebf1 and Bcl2 can partially rescue B cell development in the absence of Miz-1 (Kosan et al. 2010).

3.5 Runx1

Runx1 (also known as acute myeloid leukemia 1 (AML1)) encodes a transcription factor belonging to the highly conserved family of DNA-binding proteins that contain a *Runt* homology domain. It forms a heterodimeric complex with a core non-DNA-binding factor (Cbf) which is essential for hematopoiesis (Speck and Gilliland 2002). The expression of Runx1 remains constant throughout B cell development (Lorsbach et al. 2004). By using a conditional knockout model, Runx1 was shown to be indispensable in generating CLPs (Growney et al. 2005). The function of Runx1 was further analyzed using a conditional deletion of Runx1 specifically in B cells, pinpointing its role during the transition of pre-pro-B cells to pro-B cells (Seo et al. 2012). Interestingly, expression of Ebf1 was able to partially rescue the phenotype, indicating that Runx1 serves as an upstream regulator of *Ebf1* activation. (Seo et al. 2012) Together with this, it was also shown that Runx1 cooperates with Ebf1 and Pax5 to synergistically activate *mb1* expression (encoding Igα), thus allowing pre-B cell signaling to occur (Maier et al. 2004).

A recent study further elucidated the role of Runx1 in early B cell progenitors (Niebuhr et al. 2013a). This study suggested that Runx1 has no role in B cell specification but rather their survival or subsequent development. Strikingly, overexpression of Bcl2 rescued the survival of the B cell progenitors. *Lyn*, *Spib* and *Aiolos* were identified as target genes (with the two latter being upregulated), suggesting that the Runx1 regulation of *Lyn* was critical for IL-7 and pre-BCR stimulation in pre-B cells (Niebuhr et al. 2013b). Spi-B and Runx1 share several target genes, suggesting these two transcription factors may cooperate together to regulate the genes necessary for pre-B cell transition. Aiolos, on the other hand, is required to silence *Igll1* gene in pre-B cells after pre-BCR signaling (Thompson et al. 2007), demonstrating the importance of Runx1 repression of Aiolos during the pre-B cell transition (Niebuhr et al. 2013a).

3.6 Foxo1

Foxo1 is part of the forkhead O (Foxo) transcription factor family that acts downstream of phosphatidylinositol-3-OH kinase [PI(3)K] pathway, which is critical in both B cell development and the maturation and function of peripheral B

cells. Phosphorylation of the Foxo proteins by Akt induces their nuclear export and consequent inactivation of the transcriptional activity (Calnan and Brunet 2008), which is important for subsequent early B cell differentiation (Herzog et al. 2009). Deficiency of Foxo1 in B cells revealed a partial developmental arrest at the pro-B cells. The pro-B cells exhibited reduced expressions of *Il7r* that led to apoptosis, and of *Rag1* and *Rag2,* which led to impaired *Igh* rearrangement (Dengler et al. 2008). It has been suggested that Foxo1 and another Forkhead P transcription family member, FoxP1, both regulate *Rag1/2* expression (Amin and Schlissel 2008; Dengler et al. 2008; Herzog et al. 2009; Hu et al. 2006). It has also been recently shown that attenuation of IL-7 signaling results in induction of Foxo1, which in turns activates the expression of Blnk and Syk thus enabling the differentiation signaling functions of the pre-BCR (Ochiai et al. 2012). This suggest a feedforward mechanism whereby Blnk inhibits IL-7 signaling (Herzog et al. 2009), thereby promoting its own expression via Foxo1, Pax5 (Ochiai et al. 2012) and possibly PU.1/Spi-B (Xu et al. 2012).

3.7 Bcl6 and Bach2

Bcl6 is a transcriptional repressor that is well known for its essential role in germinal center B cells. One primary function of Bcl6 in germinal centers is to protect the cells from the pro-apoptotic effects of the DNA damage response to allow somatic hypermutation and class switch recombination (Basso and Dalla-Favera 2012). Recently, Bcl6 has also been shown to play a similar role in pre-B cells. *Bcl6* expression in the bone marrow was repressed by IL-7R signaling, but activated by successful pre-BCR recombination and signaling (Duy et al. 2010). Bcl6 then functions to protect the pre-B cells from the DNA damage induced apoptosis associated with Igκ gene recombination, as well as to promote pre-B cell quiescence (Nahar et al. 2011). In keeping with this finding, Bcl6-deficient mice showed a reduction in both the number and clonal diversity of pre-B cells.

BTB and CNC homology 2 (Bach2) is a B cell-specific transcription factor, which also is required for class switch recombination and somatic hypermutation in B cells as well as for efficient formation of germinal centers (Muto et al. 2004). Pax5 activates *Bach2* expression in developing B cells (Schebesta et al. 2007) where it plays an important role in regulating the pre-BCR checkpoint (Swaminathan et al. 2013b). Bach2 is crucial for negative selection of pre-B cells that failed to productively rearrange VDJ gene segments of the *Igh* by directly regulating the transcription of *Rag1/2* (Swaminathan et al. 2013b). Recent molecular studies demonstrate that Bach and Bcl6 have competing and opposing functions in the pre-BCR checkpoint and suggest that this interaction is important to prevent leukemogenesis (Swaminathan et al. 2013a).

4 Transcription Factors and Their Association with B Cell Acute Lymphoblastic Leukemia

Given their high proliferative potential and sequential *Ig* gene rearrangements requiring Rag1/2 activity, it is not surprising that pre-B cells are the source of one of the most common human leukemias, precursor-B/B cell acute lymphoblastic leukemia (collectively termed here B-ALL). Even though bone marrow B cell differentiation has been extensively studied for three decades, it has only more recently become apparent that mutations in the transcriptional regulators of pre-B cell development are also major players in pre-B cell malignancies.

The involvement of pre-B cell transcriptional regulators in B-ALL pathogenesis is highlighted by the finding that factors such as *PAX5, IKZF1, IKZF3, TCF3 (E2A), LEF1* and *EBF1* are commonly mutated in B-ALL (Mullighan et al. 2007). *PAX5,* for example, is mutated in 30–50 % of B-ALL cohorts through a variety of mechanisms including deletions, translocations, and point mutations. More recently, a *PAX5* mutation has also been shown to be associated with familial B-ALL (Shah et al. 2013). While the *PAX5* mutations are thought to be pivotal to the initial leukemogenesis, deletions and point mutations in *IKZF1* and *IKZF3* account for 10–15 and 2 % of the B-ALL cases respectively (Kuiper et al. 2007; Mullighan et al. 2007). Notably, genetic alterations in *IKZF1* are associated with a poor clinical outcome and act as a strong predictor of relapse (Mullighan et al. 2009; Kuiper et al. 2010). Similarly, deletions of *BACH2* have been found in 32 % of B-ALL cases (Merup et al. 1998), and lower-than-median expression levels of *BACH2* define patients with the worse clinical outcome (Swaminathan et al. 2013b). On the other hand, mutations in *FOXO1* are often associated with diffuse large B cell lymphoma (DLBCL), but have not been implicated in B-ALL (Trinh et al. 2013). Interestingly, the mutations of essentially all these factors appear to occur only on one allele, suggesting that these transcription factors are haploinsufficient tumor suppressors.

While the human studies have clearly shown that the major transcriptional regulators of pre-B cell development are also tumor suppressors, it has proven difficult to gain a molecular understanding of the process. One difficulty is that mice heterozygous for any of these factors do not spontaneously develop B-ALL, suggesting that other cooperating mutations are required. This possibility has been supported by the finding that mice that are heterozygous for either *Pax5* or *Ebf1* develop B-ALL only when they also harbor a constitutively active form of STAT5 (Heltemes-Harris et al. 2011).

The interaction between the ETS family transcription factors, PU.1 and Spi-B and the IRF family members IRF4 and IRF8 are also implicated in B-ALL. For example the compound loss of both PU.1 and Spi-B in B cell progenitors results in a developmental arrest at the pre-B cell stage. This block eventually leads to leukemia at a high frequency that closely resembles B-ALL in humans (Sokalski et al. 2011). Interestingly, *Blnk* was identified as a downstream target of both PU.1 and Spi-B (Sokalski et al. 2011; Xu et al. 2012), an important finding as Blnk-

deficiency is sufficient to induce B-ALL in mice (Jumaa et al. 2003), and mutation or aberrant mRNA splicing of *BLNK* is associated with B-ALL (Mullighan et al. 2007, 2009).

In keeping with the interaction of ETS and IRF family members during lymphopoiesis, it has been similarly demonstrated by the deficiencies of IRF4 and IRF8, which produce a developmental block at the pre-B cell stage (Lu et al. 2003), result in B-ALL at a high frequency (Jo et al. 2010). Moreover, while *Irf4*$^{-/-}$ mice do not develop B-ALL, IRF4 deficiency cooperates with oncogenes such as BCR-Abl and c-Myc to promote leukemogenesis in mouse models (Acquaviva et al. 2008; Pathak et al. 2008). We have recently extended these findings to show that mice deficient in either PU.1 and IRF4 or PU.1 and IRF8 develop B-ALL at high frequency. These B-ALLs show low expression of *Blnk*, *Spib* and *Ikaros*, suggesting that the ETS/IRF complexes function as tumor suppressors by regulating these important target genes (S.H.M.P., S.C. and S.L.N. submitted).

The importance of the ETS/IRF interaction in human B-ALL is only now emerging. Rare mutations in *SPI1* (*PU.1*) and *IRF8* have been found in human B-ALL (Mullighan et al. 2011; Zhang et al. 2011) and DLBCL (Bouamar et al. 2013), while *SPIB* expression is reduced in pre-B-ALL carrying the t(12;21) *ETV6-RUNX1* translocation (Niebuhr et al. 2013a). IRF4 has been implicated in several B cell malignancies, including chronic lymphocytic leukemia (Shukla et al. 2013) and multiple myeloma (Shaffer et al. 2008). *IRF4* was also recently reported to be 2-fold overexpressed in pediatric B-ALL compared to unfractionated healthy BM (Adamaki et al. 2013), a finding that contrasts with our own analysis of a large cohort of B-ALL samples that suggests that *IRF4*, as well as *SPI-B* expression, is uniformly reduced in B-ALL (S.H.M.P., S.C. and S.L.N. submitted).

RUNX1 is also a major target for mutation in B-ALL through translocation. The most prevalent translocation involving *RUNX1* is the *ETV6-RUNX1* (encoding the TEL-AML1 protein) that represents the most common genetic subtype in B-ALL (25 %). Genome-wide studies have identified additional genetic alterations in this subtype of ALL, including B cell-specific transcription factors, *PAX5* and *EBF1*, and deletion of second copy of *ETV6* (Mullighan et al. 2007; Parker et al. 2008; Kuiper et al. 2007). Recent analysis of gene expression in a large number of cases of B-ALL showed that reduced *IKZF3* and *SPI-B* correlated with the *ETV6-RUNX1* translocation (Niebuhr et al. 2013a).

5 Conclusions

While our understanding of the mechanisms by which the transcription factor triad of E2A/EBF1/PAX5 acts to specify the earliest stages of B cell development from hematopoietic progenitors is relatively advanced, less is known about how

committed progenitors subsequently differentiate down the B cell developmental pathway. Recent advances show that a complex mix of secondary factors, including members of the Ikaros, ETS, Runx and IRF families act often downstream of E2A, EBF1 or PAX5 to coordinate the differentiation process (Fig. 2). Further genome wide studies of the binding sites for these secondary determinants, as well as studies of the alterations in nuclear structure and the epigenetic landscape will aid in developing a robust model of the gene regulatory network for early B cell differentiation. Interestingly, for most of the past three decades research into the transcriptional controls of pre-B cell differentiation and that investigating acute leukemia formation showed little overlap, however the explosion in cancer genome information has demonstrated that mutations in most of the transcriptional regulator of pre-B cell development are key drivers of the oncogenic process. Thus the promotion of normal differentiation and tumor suppression are intimately linked at the pre-B cell stage of B-lymphopoiesis.

Acknowledgments This work was supported by a National Health and Medical Research Council (NHRMC) of Australia program grant (575500 to S.L.N). S.H.M.P. was supported by the Leukaemia Foundation of Australia, S.L.N. by an Australian Research Council Future Fellowship and S.C. by an NHMRC Career Development Fellowship. This work was made possible through Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRIIS.

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