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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 are 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

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; Adolfsson et al. 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. 2008b; Mansson et al. 2010; Kondo et al. 1997). CLPs have recently been further split, based 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 (λ5, VpreB) and accessory signaling molecules (Igα, Igβ) to form the pre-B cell receptor (pre-BCR) (Karasuyama et al. 1990; Karasuyama et al. 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; Kitamura et al. 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 will 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 will provide only a brief introduction to these factors and concentrate the factors that have more recently been shown to be important in pre-B cell development (Figure 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 for *Tcfe2a* completely lack mature B cells, indicating that E2A is essential for B cell development (Bain et al. 1994b; 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; Dias et al. 2008).

While E2A appears essential for the priming of the expression of many lymphoid transcripts (Mercer et al. 2011), the major development block, however, occurs at pre-pro-B cell stage due to a failure to up-regulate a number of B cell specific genes including *Ebf1* and *Pax5* (Bain et al. 1994a; Seet et al. 2004). The progenitors from these mice do not express *Rag1* and consequently cannot undergo *Ig* gene rearrangement (Bain et al. 1994a; 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 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 HEB to support B cell programming (Welinder et al. 2011) (Figure 2). The role of Foxo1 will be discussed in further detail.

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. 2008a; Vilagos et al. 2012). Despite the phenotypic resemblance between the B cell developmental blocks of E2A and Ebf1 deficient mice (Bain et al. 1994a; 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. 2004b; Reynaud et al. 2008; Seet et al. 2004). Careful investigation of the *Ebf1* promoters has revealed a complex regulatory network that acts to stabilise 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 (Figure 2). This highlights a new role of Ebf1 in supressing 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; Nutt et al. 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 haematopoietic 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 signalling in multipotent progenitors or non B cell lineages (Delogu et al. 2006; Holmes et al. 2006; Holmes et al. 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 expression (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 (Figure 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 Figure

1). This review summaries 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 IL7Rα, two key cytokine receptors that are expressed by early lymphoid progenitors (Figure 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 signalling (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. 2011; Sciammas et al. 2006; Willis et al. 2014). Additionally, IRF4 has been demonstrated to be important for *Igκ* recombination and the attenuation of the IL-7 signalling 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 signalling, *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 signalling by attenuating IL7 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 signalling 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 (Figure 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 λ5) in pre-B cells after pre-BCR signalling (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 signalling, 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 as a requirement 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 lymphopoeisis. 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). During B cell development Miz-1 is an important regulator of IL-7 signaling, with B-lymphopoiesis blocked at the pre-pro-B cell stage. 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 leukaemia 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 analysed 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 signalling 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 IL7 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 signalling

(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 transcription of Blnk and Syk thus enabling the differentiation signaling functions of the pre-BCR (Ochiai et al. 2012). This suggest a feed-forward 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 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 (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; Mullighan et al. 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 for 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). While a

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).

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 (Figure 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. Interesting, 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.

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References

Acquaviva J, Chen X, Ren R (2008) IRF-4 functions as a tumor suppressor in early B-cell development. Blood 112 (9):3798-3806. doi:10.1182/blood-2007-10-117838

Adamaki M, Lambrou GI, Athanasiadou A, Tzanoudaki M, Vlahopoulos S, Moschovi M (2013) Implication of IRF4 aberrant gene expression in the acute leukemias of childhood. PLoS One 8 (8):e72326. doi:10.1371/journal.pone.0072326

Adolfsson J, Borge OJ, Bryder D, Theilgaard-Monch K, Astrand-Grundstrom I, Sitnicka E, Sasaki Y, Jacobsen SE (2001) Upregulation of Flt3 expression within the bone marrow Lin(-)Sca1(+)c-kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. Immunity 15 (4):659-669

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 (2):295-306. doi:10.1016/j.cell.2005.02.013

Amin RH, Schlissel MS (2008) Foxo1 directly regulates the transcription of recombination-activating genes during B cell development. Nat Immunol 9 (6):613-622. doi:10.1038/ni.1612

Anderson KL, Nelson SL, Perkin HB, Smith KA, Klemsz MJ, Torbett BE (2001) PU.1 is a lineage-specific regulator of tyrosine phosphatase CD45. J Biol Chem 276 (10):7637-7642. doi:10.1074/jbc.M009133200

Back J, Allman D, Chan S, Kastner P (2005) Visualizing PU.1 activity during hematopoiesis. Exp Hematol 33 (4):395-402. doi:10.1016/j.exphem.2004.12.010

Bain G, Maandag EC, Izon DJ, Amsen D, Kruisbeek AM, Weintraub BC, Krop I, Schlissel MS, Feeney AJ, van Roon M (1994a) E2A proteins are required for proper B cell development and initiation of immunoglobulin gene rearrangements. Cell 79 (5):885-892

Bain G, Maandag EC, Izon DJ, Amsen D, Kruisbeek AM, Weintraub BC, Krop I, Schlissel MS, Feeney AJ, van Roon M, et al. (1994b) E2A proteins are required for proper B cell development and initiation of immunoglobulin gene rearrangements. Cell 79 (5):885-892

Banerjee A, Northrup D, Boukarabila H, Jacobsen SE, Allman D (2013) Transcriptional repression of Gata3 is essential for early B cell commitment. Immunity 38 (5):930-942. doi:10.1016/j.immuni.2013.01.014

Basso K, Dalla-Favera R (2012) Roles of BCL6 in normal and transformed germinal center B cells. Immunol Rev 247 (1):172-183. doi:10.1111/j.1600-065X.2012.01112.x

Borghesi L, Aites J, Nelson S, Lefterov P, James P, Gerstein R (2005) E47 is required for V(D)J recombinase activity in common lymphoid progenitors. The Journal of experimental medicine 202 (12):1669-1677. doi:10.1084/jem.20051190

Bouamar H, Abbas S, Lin AP, Wang L, Jiang D, Holder KN, Kinney MC, Hunicke-Smith S, Aguiar RC (2013) A capture-sequencing strategy identifies IRF8, EBF1, and APRIL as novel IGH fusion partners in B-cell lymphoma. Blood 122 (5):726-733. doi:10.1182/blood-2013-04-495804

Brass AL, Zhu AQ, Singh H (1999) Assembly requirements of PU.1-Pip (IRF-4) activator complexes: inhibiting function in vivo using fused dimers. EMBO J 18 (4):977-991. doi:10.1093/emboj/18.4.977

Calnan DR, Brunet A (2008) The FoxO code. Oncogene 27 (16):2276-2288. doi:10.1038/onc.2008.21

Carotta S, Dakic A, D'Amico A, Pang SH, Greig KT, Nutt SL, Wu L (2010) The transcription factor PU.1 controls dendritic cell development and Flt3 cytokine receptor expression in a dose-dependent manner. Immunity 32 (5):628-641. doi:10.1016/j.immuni.2010.05.005

Carpinelli MR, Hilton DJ, Metcalf D, Antonchuk JL, Hyland CD, Mifsud SL, Di Rago L, Hilton AA, Willson TA, Roberts AW, Ramsay RG, Nicola NA, Alexander WS (2004) Suppressor screen in Mpl-/- mice: c-Myb mutation causes supraphysiological production of platelets in the absence of thrombopoietin signaling. Proceedings of the National Academy of Sciences of the United States of America 101 (17):6553-6558. doi:10.1073/pnas.0401496101

Clark MR (2011) Flippin' lipids. Nature Immunology 12 (5):373-375. doi:10.1038/ni.2024

Clark MR, Mandal M, Ochiai K, Singh H (2014) Orchestrating B cell lymphopoiesis through interplay of IL-7 receptor and pre-B cell receptor signalling. Nat Rev Immunol 14 (2):69-80. doi:10.1038/nri3570

Clayton E, Bardi G, Bell SE, Chantry D, Downes CP, Gray A, Humphries LA, Rawlings D, Reynolds H, Vigorito E, Turner M (2002) A crucial role for the p110delta subunit of phosphatidylinositol 3-kinase in B cell development and activation. The Journal of experimental medicine 196 (6):753-763

Cobaleda C, Schebesta A, Delogu A, Busslinger M (2007) Pax5: the guardian of B cell identity and function. Nat Immunol 8 (5):463-470. doi:10.1038/ni1454

Cooper AB, Sawai CM, Sicinska E, Powers SE, Sicinski P, Clark MR, Aifantis I (2006) A unique function for cyclin D3 in early B cell development. Nature Immunology 7 (5):489-497. doi:10.1038/ni1324

Dakic A, Metcalf D, Di Rago L, Mifsud S, Wu L, Nutt SL (2005) PU.1 regulates the commitment of adult hematopoietic progenitors and restricts granulopoiesis. The Journal of experimental medicine 201 (9):1487-1502. doi:10.1084/jem.20050075

Dakic A, Wu L, Nutt SL (2007) Is PU.1 a dosage-sensitive regulator of haemopoietic lineage commitment and leukaemogenesis? Trends Immunol 28 (3):108-114. doi:10.1016/j.it.2007.01.006

DeKoter RP, Lee HJ, Singh H (2002) PU.1 regulates expression of the interleukin-7 receptor in lymphoid progenitors. Immunity 16 (2):297-309

DeKoter RP, Singh H (2000) Regulation of B lymphocyte and macrophage development by graded expression of PU.1. Science 288 (5470):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 (3):269-281. doi:10.1016/j.immuni.2006.01.012

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 (12):1388-1398. doi:10.1038/ni.1667

Dias S, Månsson R, Gurbuxani S, Sigvardsson M, Kee BL (2008) E2A proteins promote development of lymphoid-primed multipotent progenitors. Immunity 29 (2):217-227. doi:10.1016/j.immuni.2008.05.015

Dias S, Silva H, Cumano A, Vieira P (2005) Interleukin-7 is necessary to maintain the B cell potential in common lymphoid progenitors. The Journal of experimental medicine 201 (6):971-979. doi:10.1084/jem.20042393

Duy C, Yu JJ, Nahar R, Swaminathan S, Kweon SM, Polo JM, Valls E, Klemm L, Shojaee S, Cerchietti L, Schuh W, Jack HM, Hurtz C, Ramezani-Rad P, Herzog S, Jumaa H, Koeffler HP, de Alboran IM, Melnick AM, Ye BH, Muschen M (2010) BCL6 is critical for the development of a diverse primary B cell repertoire. The Journal of experimental medicine 207 (6):1209-1221. doi:10.1084/jem.20091299

Eisenbeis CF, Singh H, Storb U (1995) Pip, a novel IRF family member, is a lymphoid-specific, PU.1-dependent transcriptional activator. Genes Dev 9 (11):1377-1387

Emambokus N, Vegiopoulos A, Harman B, Jenkinson E, Anderson G, Frampton J (2003) Progression through key stages of haemopoiesis is dependent on distinct threshold levels of c-Myb. EMBO J 22 (17):4478-4488. doi:10.1093/emboj/cdg434

Escalante CR, Brass AL, Pongubala JM, Shatova E, Shen L, Singh H, Aggarwal AK (2002) Crystal structure of PU.1/IRF-4/DNA ternary complex. Mol Cell 10 (5):1097-1105

Fahl SP, Crittenden RB, Allman D, Bender TP (2009) c-Myb is required for pro-B cell differentiation. Journal of immunology 183 (9):5582-5592. doi:10.4049/jimmunol.0901187

Ferreiros-Vidal I, Carroll T, Taylor B, Terry A, Liang Z, Bruno L, Dharmalingam G, Khadayate S, Cobb BS, Smale ST, Spivakov M, Srivastava P, Petretto E, Fisher AG, Merkenschlager M (2013) Genome-wide identification of Ikaros targets elucidates its contribution to mouse B-cell lineage specification and pre-B-cell differentiation. Blood 121 (10):1769-1782. doi:10.1182/blood-2012-08-450114

Fruman DA, Snapper SB, Yballe CM, Davidson L, Yu JY, Alt FW, Cantley LC (1999) Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85alpha. Science (New York, NY) 283 (5400):393-397

Fuxa M, Busslinger M (2007) Reporter gene insertions reveal a strictly B lymphoid-specific expression pattern of Pax5 in support of its B cell identity function. Journal of immunology 178 (12):8222-8228

Goetz CA, Harmon IR, O'Neil JJ, Burchill MA, Farrar MA (2004) STAT5 activation underlies IL7 receptor-dependent B cell development. Journal of immunology (Baltimore, Md : 1950) 172 (8):4770-4778

Gounari F, Aifantis I, Martin C, Fehling H-J, Hoeflinger S, Leder P, von Boehmer H, Reizis B (2002) Tracing lymphopoiesis with the aid of a pTalpha-controlled reporter gene. Nat Immunol 3 (5):489-496. doi:10.1038/ni778

Grawunder U, Leu TM, Schatz DG, Werner A, Rolink AG, Melchers F, Winkler TH (1995) Down-regulation of RAG1 and RAG2 gene expression in preB cells after functional immunoglobulin heavy chain rearrangement. Immunity 3 (5):601-608

Greig KT, Carotta S, Nutt SL (2008) Critical roles for c-Myb in hematopoietic progenitor cells. Seminars in immunology 20 (4):247-256. doi:10.1016/j.smim.2008.05.003

Greig KT, de Graaf CA, Murphy JM, Carpinelli MR, Pang SH, Frampton J, Kile BT, Hilton DJ, Nutt SL (2010) Critical roles for c-Myb in lymphoid priming and early B-cell development. Blood 115 (14):2796-2805. doi:10.1182/blood-2009-08-239210

Growney JD, Shigematsu H, Li Z, Lee BH, Adelsperger J, Rowan R, Curley DP, Kutok JL, Akashi K, Williams IR, Speck NA, Gilliland DG (2005) Loss of Runx1 perturbs adult hematopoiesis and is associated with a myeloproliferative phenotype. Blood 106 (2):494-504. doi:10.1182/blood-2004-08-3280

Gyory I, Boller S, Nechanitzky R, Mandel E, Pott S, Liu E, Grosschedl R (2012) Transcription factor Ebf1 regulates differentiation stage-specific signaling, proliferation, and survival of B cells. Genes Dev 26 (7):668-682. doi:10.1101/gad.187328.112

Heizmann B, Kastner P, Chan S (2013) Ikaros is absolutely required for pre-B cell differentiation by attenuating IL-7 signals. The Journal of experimental medicine 210 (13):2823-2832. doi:10.1084/jem.20131735

Heltemes-Harris LM, Willette MJ, Ramsey LB, Qiu YH, Neeley ES, Zhang N, Thomas DA, Koeuth T, Baechler EC, Kornblau SM, Farrar MA (2011) Ebf1 or Pax5

haploinsufficiency synergizes with STAT5 activation to initiate acute lymphoblastic leukemia. The Journal of experimental medicine 208 (6):1135-1149. doi:10.1084/jem.20101947

Herzog S, Reth M, Jumaa H (2009) Regulation of B-cell proliferation and differentiation by pre-B-cell receptor signalling. Nat Rev Immunol 9 (3):195-205. doi:10.1038/nri2491

Holmes ML, Carotta S, Corcoran LM, Nutt SL (2006) Repression of Flt3 by Pax5 is crucial for B-cell lineage commitment. Genes & Development 20 (8):933-938. doi:10.1101/gad.1396206

Holmes ML, Pridans C, Nutt SL (2008) The regulation of the B-cell gene expression programme by Pax5. Immunol Cell Biol 86 (1):47-53. doi:10.1038/sj.icb.7100134

Hu H, Wang B, Borde M, Nardone J, Maika S, Allred L, Tucker PW, Rao A (2006) Foxp1 is an essential transcriptional regulator of B cell development. Nat Immunol 7 (8):819-826. doi:10.1038/ni1358

Igarashi H, Gregory SC, Yokota T, Sakaguchi N, Kincade PW (2002) Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. Immunity 17 (2):117-130

Ikawa T, Kawamoto H, Wright LYT, Murre C (2004) Long-term cultured E2A-deficient hematopoietic progenitor cells are pluripotent. Immunity 20 (3):349-360

Inlay MA, Bhattacharya D, Sahoo D, Serwold T, Seita J, Karsunky H, Plevritis SK, Dill DL, Weissman IL (2009) Ly6d marks the earliest stage of B-cell specification and identifies the branchpoint between B-cell and T-cell development. Genes Dev 23 (20):2376-2381. doi:10.1101/gad.1836009

Iwasaki H, Somoza C, Shigematsu H, Duprez EA, Iwasaki-Arai J, Mizuno S, Arinobu Y, Geary K, Zhang P, Dayaram T, Fenyus ML, Elf S, Chan S, Kastner P, Huettner CS, Murray R, Tenen DG, Akashi K (2005) Distinctive and indispensable roles of PU.1 in maintenance of hematopoietic stem cells and their differentiation. Blood 106 (5):1590-1600. doi:10.1182/blood-2005-03-0860

Jo SH, Schatz JH, Acquaviva J, Singh H, Ren R (2010) Cooperation between deficiencies of IRF-4 and IRF-8 promotes both myeloid and lymphoid tumorigenesis. Blood 116 (15):2759-2767. doi:10.1182/blood-2009-07-234559

John LB, Ward AC (2011) The Ikaros gene family: transcriptional regulators of hematopoiesis and immunity. Mol Immunol 48 (9-10):1272-1278. doi:10.1016/j.molimm.2011.03.006

Johnson K, Hashimshony T, Sawai CM, Pongubala JM, Skok JA, Aifantis I, Singh H (2008) Regulation of immunoglobulin light-chain recombination by the transcription factor IRF-4 and the attenuation of interleukin-7 signaling. Immunity 28 (3):335-345. doi:10.1016/j.immuni.2007.12.019

Joshi I, Yoshida T, Jena N, Qi X, Zhang J, Van Etten RA, Georgopoulos K (2014) Loss of Ikaros DNA-binding function confers integrin-dependent survival on pre-B cells and progression to acute lymphoblastic leukemia. Nature immunology. doi:10.1038/ni.2821

Jou S-T, Carpino N, Takahashi Y, Piekorz R, Chao J-R, Carpino N, Wang D, Ihle JN (2002) Essential, nonredundant role for the phosphoinositide 3-kinase p110delta in signaling by the B-cell receptor complex. Molecular and cellular biology 22 (24):8580-8591

Jumaa H, Bossaller L, Portugal K, Storch B, Lotz M, Flemming A, Schrappe M, Postila V, Riikonen P, Pelkonen J, Niemeyer CM, Reth M (2003) Deficiency of the adaptor SLP-65 in pre-B-cell acute lymphoblastic leukaemia. Nature 423 (6938):452-456. doi:10.1038/nature01608

Karasuyama H, Kudo A, Melchers F (1990) The proteins encoded by the VpreB and lambda 5 pre-B cell-specific genes can associate with each other and with mu heavy chain. The Journal of experimental medicine 172 (3):969-972

Karasuyama H, Rolink A, Shinkai Y, Young F, Alt FW, Melchers F (1994) The expression of Vpre-B/lambda 5 surrogate light chain in early bone marrow precursor B cells of normal and B cell-deficient mutant mice. Cell 77 (1):133-143

Karnowski A, Cao C, Matthias G, Carotta S, Corcoran LM, Martensson IL, Skok JA, Matthias P (2008) Silencing and nuclear repositioning of the lambda5 gene locus at the pre-B cell stage requires Aiolos and OBF-1. PLoS One 3 (10):e3568. doi:10.1371/journal.pone.0003568

Karsunky H, Inlay MA, Serwold T, Bhattacharya D, Weissman IL (2008) Flk2+ common lymphoid progenitors possess equivalent differentiation potential for the B and T lineages. Blood 111 (12):5562-5570. doi:10.1182/blood-2007-11-126219

Kirstetter P, Thomas M, Dierich A, Kastner P, Chan S (2002) Ikaros is critical for B cell differentiation and function. European journal of immunology 32 (3):720-730. doi:10.1002/1521-4141(200203)32:3<720::AID-IMMU720>3.0.CO;2-P

Kitamura D, Kudo A, Schaal S, Müller W, Melchers F, Rajewsky K (1992) A critical role of lambda 5 protein in B cell development. Cell 69 (5):823-831

Kitamura D, Roes J, Kühn R, Rajewsky K (1991) A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene. Nature 350 (6317):423-426. doi:10.1038/350423a0

Kondo M, Weissman IL, Akashi K (1997) Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell 91 (5):661-672

Kosan C, Saba I, Godmann M, Herold S, Herkert B, Eilers M, Moroy T (2010) Transcription factor miz-1 is required to regulate interleukin-7 receptor signaling at early commitment stages of B cell differentiation. Immunity 33 (6):917-928. doi:10.1016/j.immuni.2010.11.028

Kueh HY, Champhekar A, Nutt SL, Elowitz MB, Rothenberg EV (2013) Positive feedback between PU.1 and the cell cycle controls myeloid differentiation. Science 341 (6146):670-673. doi:10.1126/science.1240831

Kuiper RP, Schoenmakers EF, van Reijmersdal SV, Hehir-Kwa JY, van Kessel AG, van Leeuwen FN, Hoogerbrugge PM (2007) High-resolution genomic profiling of childhood ALL reveals novel recurrent genetic lesions affecting pathways involved in lymphocyte differentiation and cell cycle progression. Leukemia 21 (6):1258-1266. doi:10.1038/sj.leu.2404691

Kuiper RP, Waanders E, van der Velden VH, van Reijmersdal SV, Venkatachalam R, Scheijen B, Sonneveld E, van Dongen JJ, Veerman AJ, van Leeuwen FN, van Kessel AG, Hoogerbrugge PM (2010) IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. Leukemia 24 (7):1258-1264. doi:10.1038/leu.2010.87

Kwon K, Hutter C, Sun Q, Bilic I, Cobaleda C, Malin S, Busslinger M (2008) Instructive role of the transcription factor E2A in early B lymphopoiesis and germinal center B cell development. Immunity 28 (6):751-762. doi:10.1016/j.immuni.2008.04.014

Lazorchak AS, Schlissel MS, Zhuang Y (2006) E2A and IRF-4/Pip promote chromatin modification and transcription of the immunoglobulin kappa locus in pre-B cells. Mol Cell Biol 26 (3):810-821. doi:10.1128/MCB.26.3.810-821.2006

Li YS, Hayakawa K, Hardy RR (1993) The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver. The Journal of experimental medicine 178 (3):951-960

Li YS, Wasserman R, Hayakawa K, Hardy RR (1996) Identification of the earliest B lineage stage in mouse bone marrow. Immunity 5 (6):527-535

Lin H, Grosschedl R (1995) Failure of B-cell differentiation in mice lacking the transcription factor EBF. Nature 376 (6537):263-267. doi:10.1038/376263a0

Lin YC, Jhunjhunwala S, Benner C, Heinz S, Welinder E, Mansson 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 (7):635-643. doi:10.1038/ni.1891

Loder F, Mutschler B, Ray RJ, Paige CJ, Sideras P, Torres R, Lamers MC, Carsetti R (1999) B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals. The Journal of experimental medicine 190 (1):75-89

Lorsbach RB, Moore J, Ang SO, Sun W, Lenny N, Downing JR (2004) Role of RUNX1 in adult hematopoiesis: analysis of RUNX1-IRES-GFP knock-in mice reveals differential lineage expression. Blood 103 (7):2522-2529. doi:10.1182/blood-2003-07-2439

Lu R, Medina KL, Lancki DW, Singh H (2003) IRF-4,8 orchestrate the pre-B-to-B transition in lymphocyte development. Genes Dev 17 (14):1703-1708. doi:10.1101/gad.1104803

Ma S, Pathak S, Trinh L, Lu R (2008) Interferon regulatory factors 4 and 8 induce the expression of Ikaros and Aiolos to down-regulate pre-B-cell receptor and promote cell-cycle withdrawal in pre-B-cell development. Blood 111 (3):1396-1403. doi:10.1182/blood-2007-08-110106

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 (10):1069-1077. doi:10.1038/ni1119

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 (2):171-179. doi:10.1038/ni.1827

Mandel EM, Grosschedl R (2010) Transcription control of early B cell differentiation. Curr Opin Immunol 22 (2):161-167. doi:10.1016/j.coi.2010.01.010

Mansson R, Zandi S, Anderson K, Martensson I-L, Jacobsen SEW, Bryder D, Sigvardsson M (2008a) B-lineage commitment prior to surface expression of B220 and CD19 on hematopoietic progenitor cells. Blood 112 (4):1048-1055. doi:10.1182/blood-2007-11-125385

Mansson R, Zandi S, Anderson K, Martensson IL, Jacobsen SE, Bryder D, Sigvardsson M (2008b) B-lineage commitment prior to surface expression of B220 and CD19 on hematopoietic progenitor cells. Blood 112 (4):1048-1055. doi:10.1182/blood-2007-11-125385

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 (13):2601-2609. doi:10.1182/blood-2009-08-236398

McManus S, Ebert A, Salvagiotto G, Medvedovic J, Sun Q, Tamir I, Jaritz M, Tagoh H, Busslinger M (2011) The transcription factor Pax5 regulates its target genes by recruiting chromatin-modifying proteins in committed B cells. EMBO J 30 (12):2388-2404. doi:10.1038/emboj.2011.140

Medina KL, Pongubala JM, Reddy KL, Lancki DW, Dekoter R, Kieslinger M, Grosschedl R, Singh H (2004a) Assembling a gene regulatory network for specification of the B cell fate. Dev Cell 7 (4):607-617. doi:10.1016/j.devcel.2004.08.006

Medina KL, Pongubala JMR, Reddy KL, Lancki DW, Dekoter R, Kieslinger M, Grosschedl R, Singh H (2004b) Assembling a gene regulatory network for specification of the B cell fate. Dev Cell 7 (4):607-617. doi:10.1016/j.devcel.2004.08.006

Mercer EM, Lin YC, Benner C, Jhunjhunwala S, Dutkowski J, Flores M, Sigvardsson M, Ideker T, Glass CK, Murre C (2011) Multilineage priming of enhancer repertoires precedes commitment to the B and myeloid cell lineages in hematopoietic progenitors. Immunity 35 (3):413-425. doi:10.1016/j.immuni.2011.06.013

Merup M, Moreno TC, Heyman M, Ronnberg K, Grander D, Detlofsson R, Rasool O, Liu Y, Soderhall S, Juliusson G, Gahrton G, Einhorn S (1998) 6q deletions in acute lymphoblastic leukemia and non-Hodgkin's lymphomas. Blood 91 (9):3397-3400

Mikkola I, Heavey B, Horcher M, Busslinger M (2002) Reversion of B cell commitment upon loss of Pax5 expression. Science 297 (5578):110-113. doi:10.1126/science.1067518

Mittrücker HW, Matsuyama T, Grossman A, Kündig TM, Potter J, Shahinian A, Wakeham A, Patterson B, Ohashi PS, Mak TW (1997) Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. Science (New York, NY) 275 (5299):540-543

Moroy T, Khandanpour C (2011) Growth factor independence 1 (Gfi1) as a regulator of lymphocyte development and activation. Seminars in immunology 23 (5):368-378. doi:10.1016/j.smim.2011.08.006

Moroy T, Saba I, Kosan C (2011) The role of the transcription factor Miz-1 in lymphocyte development and lymphomagenesis-Binding Myc makes the difference. Seminars in immunology 23 (5):379-387. doi:10.1016/j.smim.2011.09.001

Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, Girtman K, Mathew S, Ma J, Pounds SB, Su X, Pui CH, Relling MV, Evans WE, Shurtleff SA, Downing JR (2007) Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 446 (7137):758-764. doi:10.1038/nature05690

Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, Ma J, Liu W, Cheng C, Schulman BA, Harvey RC, Chen IM, Clifford RJ, Carroll WL, Reaman G, Bowman WP, Devidas M, Gerhard DS, Yang W, Relling MV, Shurtleff SA, Campana D, Borowitz MJ, Pui CH, Smith M, Hunger SP, Willman CL, Downing JR, Children's Oncology G (2009) Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med 360 (5):470-480. doi:10.1056/NEJMoa0808253

Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, Heatley SL, Holmfeldt L, Collins-Underwood JR, Ma J, Buetow KH, Pui CH, Baker SD, Brindle PK, Downing JR (2011) CREBBP mutations in relapsed acute lymphoblastic leukaemia. Nature 471 (7337):235-239. doi:10.1038/nature09727

Murre C (2007) Regulation and function of the E2A proteins in B cell development. Adv Exp Med Biol 596:1-7. doi:10.1007/0-387-46530-8_1

Muto A, Tashiro S, Nakajima O, Hoshino H, Takahashi S, Sakoda E, Ikebe D, Yamamoto M, Igarashi K (2004) The transcriptional programme of antibody class switching involves the repressor Bach2. Nature 429 (6991):566-571. doi:10.1038/nature02596

Nahar R, Ramezani-Rad P, Mossner M, Duy C, Cerchietti L, Geng H, Dovat S, Jumaa H, Ye BH, Melnick A, Muschen M (2011) Pre-B cell receptor-mediated activation of BCL6 induces pre-B cell quiescence through transcriptional repression of MYC. Blood 118 (15):4174-4178. doi:10.1182/blood-2011-01-331181

Nechanitzky R, Akbas D, Scherer S, Gyory I, Hoyler T, Ramamoorthy S, Diefenbach A, Grosschedl R (2013) Transcription factor EBF1 is essential for the maintenance of B cell identity and prevention of alternative fates in committed cells. Nat Immunol 14 (8):867-875. doi:10.1038/ni.2641

Niebuhr B, Kriebitzsch N, Fischer M, Behrens K, Gunther T, Alawi M, Bergholz U, Muller U, Roscher S, Ziegler M, Buchholz F, Grundhoff A, Stocking C (2013a) Runx1 is essential at two stages of early murine B-cell development. Blood 122 (3):413-423. doi:10.1182/blood-2013-01-480244

Niebuhr DW, Gubata ME, Oetting AA, Weber NS, Feng X, Cowan DN (2013b) Personality Assessment Questionnaire as a pre-accession screen for risk of mental disorders and early attrition in U. S. Army recruits. Psychol Serv 10 (4):378-385. doi:10.1037/a0032783

Nutt SL, Heavey B, Rolink AG, Busslinger M (1999) Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. Nature 401 (6753):556-562. doi:10.1038/44076

Nutt SL, Kee BL (2007) The transcriptional regulation of B cell lineage commitment. Immunity 26 (6):715-725. doi:10.1016/j.immuni.2007.05.010

Nutt SL, Metcalf D, D'Amico A, Polli M, Wu L (2005) Dynamic regulation of PU.1 expression in multipotent hematopoietic progenitors. The Journal of experimental medicine 201 (2):221-231. doi:10.1084/jem.20041535

Nutt SL, Urbánek P, Rolink A, Busslinger M (1997) Essential functions of Pax5 (BSAP) in pro-B cell development: difference between fetal and adult B lymphopoiesis and reduced V-to-DJ recombination at the IgH locus. Genes & Development 11 (4):476-491

Ochiai K, Maienschein-Cline M, Mandal M, Triggs JR, Bertolino E, Sciammas R, Dinner AR, Clark MR, Singh H (2012) A self-reinforcing regulatory network triggered by limiting IL-7 activates pre-BCR signaling and differentiation. Nat Immunol 13 (3):300-307. doi:10.1038/ni.2210

Ochiai K, Maienschein-Cline M, Simonetti G, Chen J, Rosenthal R, Brink R, Chong AS, Klein U, Dinner AR, Singh H, Sciammas R (2013) Transcriptional regulation of germinal center B and plasma cell fates by dynamical control of IRF4. Immunity 38 (5):918-929. doi:10.1016/j.immuni.2013.04.009

Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek SE, Salpekar A, Waterfield MD, Smith AJH, Vanhaesebroeck B (2002) Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. Science (New York, NY) 297 (5583):1031-1034. doi:10.1126/science.1073560

Pang SHM, Nutt SL (2011) B cells need their flip-flops. Immunol Cell Biol 89 (7):751-752. doi:10.1038/icb.2011.48

Parker H, An Q, Barber K, Case M, Davies T, Konn Z, Stewart A, Wright S, Griffiths M, Ross FM, Moorman AV, Hall AG, Irving JA, Harrison CJ, Strefford JC (2008) The complex genomic profile of ETV6-RUNX1 positive acute lymphoblastic leukemia highlights a recurrent deletion of TBL1XR1. Genes Chromosomes Cancer 47 (12):1118-1125. doi:10.1002/gcc.20613

Pathak S, Ma S, Trinh L, Lu R (2008) A role for interferon regulatory factor 4 in receptor editing. Mol Cell Biol 28 (8):2815-2824. doi:10.1128/MCB.01946-07

Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, Gliniak BC, Park LS, Ziegler SF, Williams DE, Ware CB, Meyer JD, Davison BL (1994) Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. The Journal of experimental medicine 180 (5):1955-1960

Polli M, Dakic A, Light A, Wu L, Tarlinton DM, Nutt SL (2005) The development of functional B lymphocytes in conditional PU.1 knock-out mice. Blood 106 (6):2083-2090. doi:10.1182/blood-2005-01-0283

Pongubala JM, Nagulapalli S, Klemsz MJ, McKercher SR, Maki RA, Atchison ML (1992) PU.1 recruits a second nuclear factor to a site important for immunoglobulin kappa 3' enhancer activity. Mol Cell Biol 12 (1):368-378

Pongubala JMR, 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 (2):203-215. doi:10.1038/ni1555

Pridans C, Holmes ML, Polli M, Wettenhall JM, Dakic A, Corcoran LM, Smyth GK, Nutt SL (2008) Identification of Pax5 target genes in early B cell differentiation. Journal of immunology 180 (3):1719-1728

Ramadani F, Bolland DJ, Garcon F, Emery JL, Vanhaesebroeck B, Corcoran AE, Okkenhaug K (2010) The PI3K isoforms p110alpha and p110delta are essential for pre-B cell receptor signaling and B cell development. Science signaling 3 (134):ra60. doi:10.1126/scisignal.2001104

Revilla IDR, Bilic I, Vilagos B, Tagoh H, Ebert A, Tamir IM, Smeenk L, Trupke J, Sommer A, Jaritz M, Busslinger M (2012) The B-cell identity factor Pax5 regulates distinct transcriptional programmes in early and late B lymphopoiesis. EMBO J 31 (14):3130-3146. doi:10.1038/emboj.2012.155

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 (8):927-936. doi:10.1038/ni.1626

Roessler S, Gyory 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 (2):579-594. doi:10.1128/MCB.01192-06

Rolink A, Grawunder U, Winkler TH, Karasuyama H, Melchers F (1994) IL-2 receptor alpha chain (CD25, TAC) expression defines a crucial stage in pre-B cell development. Int Immunol 6 (8):1257-1264

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 (6753):603-606. doi:10.1038/44164

Rumfelt LL, Zhou Y, Rowley BM, Shinton SA, Hardy RR (2006) Lineage specification and plasticity in CD19- early B cell precursors. The Journal of experimental medicine 203 (3):675-687. doi:10.1084/jem.20052444

Sandberg ML, Sutton SE, Pletcher MT, Wiltshire T, Tarantino LM, Hogenesch JB, Cooke MP (2005) c-Myb and p300 regulate hematopoietic stem cell proliferation and differentiation. Dev Cell 8 (2):153-166. doi:10.1016/j.devcel.2004.12.015

Schebesta A, McManus S, Salvagiotto G, Delogu A, Busslinger GA, Busslinger M (2007) Transcription factor Pax5 activates the chromatin of key genes involved in B cell signaling, adhesion, migration, and immune function. Immunity 27 (1):49-63. doi:10.1016/j.immuni.2007.05.019

Schulz D, Vassen L, Chow KT, McWhirter SM, Amin RH, Moroy T, Schlissel MS (2012) Gfi1b negatively regulates Rag expression directly and via the repression of FoxO1. The Journal of experimental medicine 209 (1):187-199. doi:10.1084/jem.20110645

Schwickert TA, Tagoh H, Gultekin S, Dakic A, Axelsson E, Minnich M, Ebert A, Werner B, Roth M, Cimmino L, Dickins RA, Zuber J, Jaritz M, Busslinger M (2014)

Stage-specific control of early B cell development by the transcription factor Ikaros. Nat Immunol. doi:10.1038/ni.2828

Sciammas R, Li Y, Warmflash A, Song Y, Dinner AR, Singh H (2011) An incoherent regulatory network architecture that orchestrates B cell diversification in response to antigen signaling. Mol Syst Biol 7:495. doi:10.1038/msb.2011.25

Sciammas R, Shaffer AL, Schatz JH, Zhao H, Staudt LM, Singh H (2006) Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation. Immunity 25 (2):225-236. doi:10.1016/j.immuni.2006.07.009

Scott EW, Simon MC, Anastasi J, Singh H (1994) Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Science 265 (5178):1573-1577

Seet CS, Brumbaugh RL, Kee BL (2004) Early B cell factor promotes B lymphopoiesis with reduced interleukin 7 responsiveness in the absence of E2A. The Journal of experimental medicine 199 (12):1689-1700. doi:10.1084/jem.20032202

Seo W, Ikawa T, Kawamoto H, Taniuchi I (2012) Runx1-Cbfbeta facilitates early B lymphocyte development by regulating expression of Ebf1. The Journal of experimental medicine 209 (7):1255-1262. doi:10.1084/jem.20112745

Shaffer AL, Emre NC, Lamy L, Ngo VN, Wright G, Xiao W, Powell J, Dave S, Yu X, Zhao H, Zeng Y, Chen B, Epstein J, Staudt LM (2008) IRF4 addiction in multiple myeloma. Nature 454 (7201):226-231. doi:10.1038/nature07064

Shah S, Schrader KA, Waanders E, Timms AE, Vijai J, Miething C, Wechsler J, Yang J, Hayes J, Klein RJ, Zhang J, Wei L, Wu G, Rusch M, Nagahawatte P, Ma J, Chen SC, Song G, Cheng J, Meyers P, Bhojwani D, Jhanwar S, Maslak P, Fleisher M, Littman J, Offit L, Rau-Murthy R, Fleischut MH, Corines M, Murali R, Gao X, Manschreck C, Kitzing T, Murty VV, Raimondi SC, Kuiper RP, Simons A, Schiffman JD, Onel K, Plon SE, Wheeler DA, Ritter D, Ziegler DS, Tucker K, Sutton R, Chenevix-Trench G, Li J, Huntsman DG, Hansford S, Senz J, Walsh T, Lee M, Hahn CN, Roberts KG, King MC, Lo SM, Levine RL, Viale A, Socci ND, Nathanson KL, Scott HS, Daly M, Lipkin SM, Lowe SW, Downing JR, Altshuler D, Sandlund JT, Horwitz MS, Mullighan CG, Offit K (2013) A recurrent germline PAX5 mutation confers susceptibility to pre-B cell acute lymphoblastic leukemia. Nat Genet 45 (10):1226-1231. doi:10.1038/ng.2754

Shukla V, Ma S, Hardy RR, Joshi SS, Lu R (2013) A role for IRF4 in the development of CLL. Blood 122 (16):2848-2855. doi:10.1182/blood-2013-03-492769

Siggs OM, Arnold CN, Huber C, Pirie E, Xia Y, Lin P, Nemazee D, Beutler B (2011) The P4-type ATPase ATP11C is essential for B lymphopoiesis in adult bone marrow. Nature Immunology 12 (5):434-440. doi:10.1038/ni.2012

Singh H, Medina KL, Pongubala JM (2005) Contingent gene regulatory networks and B cell fate specification. Proc Natl Acad Sci U S A 102 (14):4949-4953. doi:10.1073/pnas.0500480102

Singh H, Pongubala JM, Medina KL (2007) Gene regulatory networks that orchestrate the development of B lymphocyte precursors. Adv Exp Med Biol 596:57-62. doi:10.1007/0-387-46530-8_5

Sokalski KM, Li SK, Welch I, Cadieux-Pitre HA, Gruca MR, DeKoter RP (2011) Deletion of genes encoding PU.1 and Spi-B in B cells impairs differentiation

and induces pre-B cell acute lymphoblastic leukemia. Blood 118 (10):2801-2808. doi:10.1182/blood-2011-02-335539

Souabni A, Cobaleda C, Schebesta M, Busslinger M (2002) Pax5 promotes B lymphopoiesis and blocks T cell development by repressing Notch1. Immunity 17 (6):781-793

Speck NA, Gilliland DG (2002) Core-binding factors in haematopoiesis and leukaemia. Nat Rev Cancer 2 (7):502-513. doi:10.1038/nrc840

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 (4):576-586. doi:10.1016/j.immuni.2009.07.011

Su GH, Chen HM, Muthusamy N, Garrett-Sinha LA, Baunoch D, Tenen DG, Simon MC (1997) Defective B cell receptor-mediated responses in mice lacking the Ets protein, Spi-B. EMBO J 16 (23):7118-7129. doi:10.1093/emboj/16.23.7118

Suzuki H, Terauchi Y, Fujiwara M, Aizawa S, Yazaki Y, Kadowaki T, Koyasu S (1999) Xid-like immunodeficiency in mice with disruption of the p85alpha subunit of phosphoinositide 3-kinase. Science (New York, NY) 283 (5400):390-392

Swaminathan S, Duy C, Muschen M (2013a) BACH2-BCL6 balance regulates selection at the pre-B cell receptor checkpoint. Trends Immunol. doi:10.1016/j.it.2013.11.002

Swaminathan S, Huang C, Geng H, Chen Z, Harvey R, Kang H, Ng C, Titz B, Hurtz C, Sadiyah MF, Nowak D, Thoennissen GB, Rand V, Graeber TG, Koeffler HP, Carroll WL, Willman CL, Hall AG, Igarashi K, Melnick A, Muschen M (2013b) BACH2 mediates negative selection and p53-dependent tumor suppression at the pre-B cell receptor checkpoint. Nat Med 19 (8):1014-1022. doi:10.1038/nm.3247

Tagoh H, Ingram R, Wilson N, Salvagiotto G, Warren AJ, Clarke D, Busslinger M, Bonifer C (2006) The mechanism of repression of the myeloid-specific c-fms gene by Pax5 during B lineage restriction. Embo J 25 (5):1070-1080. doi:10.1038/sj.emboj.7600997

ten Boekel E, Melchers F, Rolink A (1995) The status of Ig loci rearrangements in single cells from different stages of B cell development. Int Immunol 7 (6):1013-1019

Thompson EC, Cobb BS, Sabbattini P, Meixlsperger S, Parelho V, Liberg D, Taylor B, Dillon N, Georgopoulos K, Jumaa H, Smale ST, Fisher AG, Merkenschlager M (2007) Ikaros DNA-binding proteins as integral components of B cell developmental-stage-specific regulatory circuits. Immunity 26 (3):335-344. doi:10.1016/j.immuni.2007.02.010

Timblin GA, Schlissel MS (2013) Ebf1 and c-Myb repress rag transcription downstream of Stat5 during early B cell development. Journal of immunology 191 (9):4676-4687. doi:10.4049/jimmunol.1301675

Treiber T, Mandel EM, Pott S, Gyory I, Firner S, Liu ET, Grosschedl R (2010) Early B cell factor 1 regulates B cell gene networks by activation, repression, and transcription- independent poising of chromatin. Immunity 32 (5):714-725. doi:10.1016/j.immuni.2010.04.013

Trinh DL, Scott DW, Morin RD, Mendez-Lago M, An J, Jones SJ, Mungall AJ, Zhao Y, Schein J, Steidl C, Connors JM, Gascoyne RD, Marra MA (2013) Analysis of FOXO1 mutations in diffuse large B-cell lymphoma. Blood 121 (18):3666-3674. doi:10.1182/blood-2013-01-479865

Vassen L, Okayama T, Moroy T (2007) Gfi1b:green fluorescent protein knock-in mice reveal a dynamic expression pattern of Gfi1b during hematopoiesis that is largely complementary to Gfi1. Blood 109 (6):2356-2364. doi:10.1182/blood-2006-06-030031

Vilagos B, Hoffmann M, Souabni A, Sun Q, Werner B, Medvedovic J, Bilic I, Minnich M, Axelsson E, Jaritz M, Busslinger M (2012) Essential role of EBF1 in the generation and function of distinct mature B cell types. The Journal of experimental medicine 209 (4):775-792. doi:10.1084/jem.20112422

Vosshenrich CA, Cumano A, Muller W, Di Santo JP, Vieira P (2003) Thymic stromal-derived lymphopoietin distinguishes fetal from adult B cell development. Nat Immunol 4 (8):773-779. doi:10.1038/ni956

Wang H, Lee CH, Qi C, Tailor P, Feng J, Abbasi S, Atsumi T, Morse HC, 3rd (2008) IRF8 regulates B-cell lineage specification, commitment, and differentiation. Blood 112 (10):4028-4038. doi:10.1182/blood-2008-01-129049

Wang JH, Nichogiannopoulou A, Wu L, Sun L, Sharpe AH, Bigby M, Georgopoulos K (1996) Selective defects in the development of the fetal and adult lymphoid system in mice with an Ikaros null mutation. Immunity 5 (6):537-549

Welinder E, Mansson R, Mercer EM, Bryder D, Sigvardsson M, Murre C (2011) The transcription factors E2A and HEB act in concert to induce the expression of FOXO1 in the common lymphoid progenitor. Proceedings of the National Academy of Sciences of the United States of America 108 (42):17402-17407. doi:10.1073/pnas.1111766108

Willis SN, Good-Jacobson KL, Curtis J, Light A, Tellier J, Shi W, Smyth GK, Tarlinton DM, Belz GT, Corcoran LM, Kallies A, Nutt SL (2014) Transcription factor IRF4 regulates germinal center formation through a B-cell intrinsic mechanism. J Immunol in press 27 Jan

Xiao C, Calado DP, Galler G, Thai TH, Patterson HC, Wang J, Rajewsky N, Bender TP, Rajewsky K (2007) MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. Cell 131 (1):146-159. doi:10.1016/j.cell.2007.07.021

Xu LS, Sokalski KM, Hotke K, Christie DA, Zarnett O, Piskorz J, Thillainadesan G, Torchia J, DeKoter RP (2012) Regulation of B cell linker protein transcription by PU.1 and Spi-B in murine B cell acute lymphoblastic leukemia. Journal of immunology 189 (7):3347-3354. doi:10.4049/jimmunol.1201267

Yabas M, Teh CE, Frankenreiter S, Lal D, Roots CM, Whittle B, Andrews DT, Zhang Y, Teoh NC, Sprent J, Tze LE, Kucharska EM, Kofler J, Farell GC, Bröer S, Goodnow CC, Enders A (2011) ATP11C is critical for the internalization of phosphatidylserine and differentiation of B lymphocytes. Nature Immunology 12 (5):441-449. doi:10.1038/ni.2011

Yao Z, Cui Y, Watford WT, Bream JH, Yamaoka K, Hissong BD, Li D, Durum SK, Jiang Q, Bhandoola A, Hennighausen L, O'Shea JJ (2006) Stat5a/b are essential for normal lymphoid development and differentiation. Proceedings of the

National Academy of Sciences of the United States of America 103 (4):1000-1005. doi:10.1073/pnas.0507350103

Ye M, Ermakova O, Graf T (2005) PU.1 is not strictly required for B cell development and its absence induces a B-2 to B-1 cell switch. The Journal of experimental medicine 202 (10):1411-1422. doi:10.1084/jem.20051089

Yoshida T, Ng SY, Zuniga-Pflucker JC, Georgopoulos K (2006) Early hematopoietic lineage restrictions directed by Ikaros. Nat Immunol 7 (4):382-391. doi:10.1038/ni1314

Zhang J, Mullighan CG, Harvey RC, Wu G, Chen X, Edmonson M, Buetow KH, Carroll WL, Chen IM, Devidas M, Gerhard DS, Loh ML, Reaman GH, Relling MV, Camitta BM, Bowman WP, Smith MA, Willman CL, Downing JR, Hunger SP (2011) Key pathways are frequently mutated in high-risk childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood 118 (11):3080-3087. doi:10.1182/blood-2011-03-341412

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 (18):4759-4769. doi:10.1093/emboj/cdg464

Zhuang Y, Soriano P, Weintraub H (1994) The helix-loop-helix gene E2A is required for B cell formation. Cell 79 (5):875-884

Figure Legends

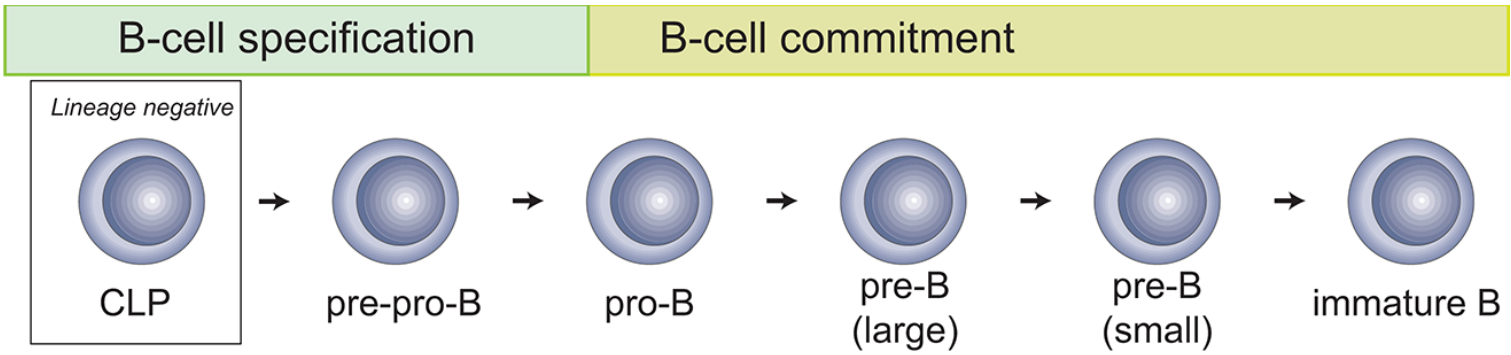
Figure 1. B cell development in the bone marrow of adult mice.

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.

Figure 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 and build on earlier work by (Medina et al. 2004a; Nutt and Kee 2007; Singh et al. 2005).

Index



	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	-	++	+++	++	+++	++

Figure 1. Pang et al

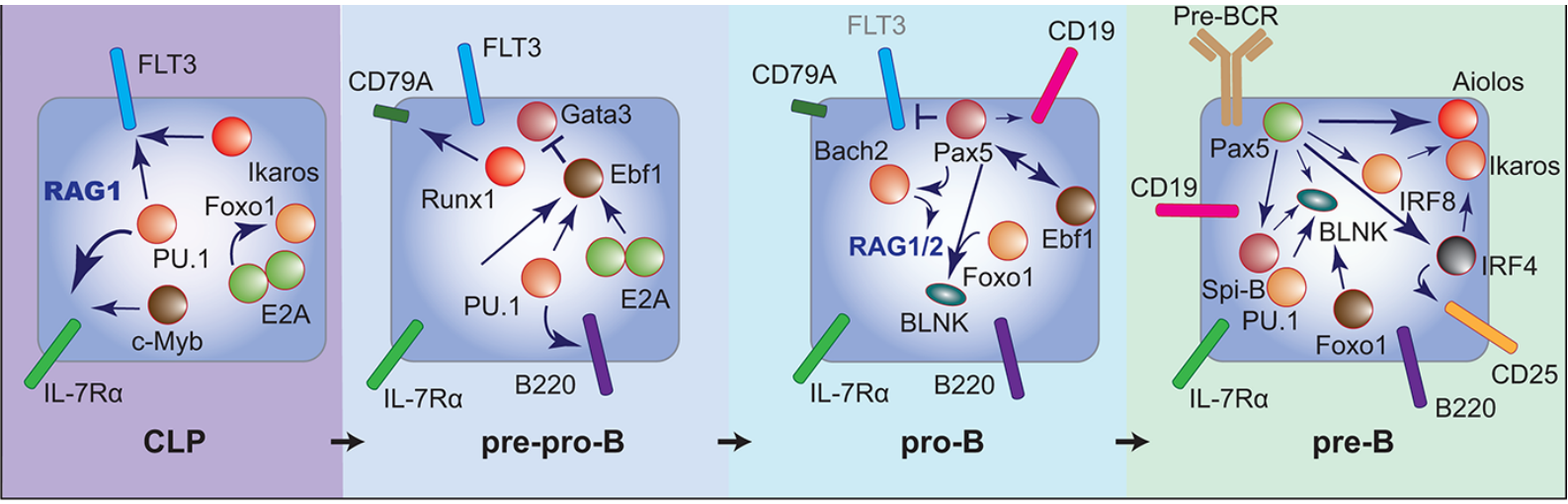


Figure 2 pang et al