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The regulation of the B-cell gene expression programme by Pax5

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Summary

The activity of the transcription factor paired box gene 5 (Pax5) is essential for many aspects of B lymphopoiesis including the initial commitment to the lineage, immunoglobulin rearrangement, pre-B cell receptor signalling and maintaining cell identity in mature B cells. Deregulated or reduced Pax5 activity has also been implicated in B-cell malignancies both in human disease and mouse models. Candidate gene approaches and biochemical analysis have revealed that Pax5 regulates B lymphopoiesis by concurrently activating B cell-specific gene expression as well as repressing the expression of genes, many of which are associated with non-B cell lineages. These studies have been recently complemented with more exhaustive microarray studies, which have identified and validated a large panel of Pax5 target genes. These target genes reveal a gene regulatory network, with Pax5 at its centre that controls the B-cell gene expression programme.

Overview of B-cell development

B cells derive from multipotent haematopoietic stem cells (HSCs), which differentiate through a number of intermediate stages into lymphoid-restricted progenitors.[1,2] B lymphopoiesis occurs in the foetal liver and post-natal bone marrow. The first B cell-specified progenitors, variously termed pre-pro-B cells, fraction A or CLP-2, are defined by expression of the B cell-associated marker B220 and activation of many B cell lineage-associated genes.[1]

The earliest committed B-cell progenitors are large cycling cells with their immunoglobulin-heavy chain (Igh) gene in either germ line or D-J configuration and express CD19, a direct target of paired box gene 5 (Pax5, see below).[1,2] Those pro-B cells that successfully undergo VDJ recombination of the Igh locus proceed to the pre-B cell stage and express the pre-B cell receptor (pre-BCR), which consists of the productively rearranged IgH protein and the surrogate light chains, l5 and VpreB.[3] Signalling through the pre-BCR results in a proliferation phase and initiates immunoglobulin-light chain (Igl) chain recombination. Both the pro-B and pre-B cell stages are dependent on the cytokine interleukin (IL)-7.[4] Productive Igl rearrangement leads to the expression of the BCR and the immature B-cell stage. These immature B cells migrate to the periphery where they pass through several transitional stages before becoming quiescent mature B cells that circulate through blood and lymphoid organs.[5]

Upon encounter with antigen, mature B-cell differentiation can take one of two forms. Direct stimulation with T-independent antigens, often in multimeric form, leads to the rapid formation of antibody-secreting plasmablasts.[6] In contrast, the response to T-dependent antigens results in the formation of a specialized structure called the germinal centre (GC), where B cells are provided with T-cell help and undergo proliferation, somatic hypermutation and immunoglobulin class switching.[7] Positively selected GC B cells are then induced through a poorly understood process to become either terminally differentiated plasma cells, or memory cells that provide the basis for acquired immunity.

As well as being guided by the status of the immunoglobulin receptor recombinations and the specificity of the BCR, B-cell development is controlled by the coordinated activity of a number of transcription factors.[2,8,9] In this review we discuss the role of one such factor, Pax5, focusing on recent data that have highlighted its dual role in both positively and negatively regulating gene expression during B-cell development.

Pax5 is expressed throughout B-cell development

The DNA-binding activity of Pax5 was independently identified in a number of laboratories and the protein was purified and cloned as the B cell-specific activator protein (reviewed by Cobaleda et al.[10]). Pax5 belongs to a family of nine transcription factors that are important for the control of tissue-specific transcription during many types of cellular differentiation. Pax5 is the only family member found within the haematopoietic system, where its expression appears to be confined to B cells. Analysis of mice carrying a reporter gene inserted into the Pax5 locus revealed that Pax5 is absent from multipotent pro-genitors and the vast majority of common lymphoid progenitors (95% are Pax5 negative11). Pax5 expression is initiated in pre-pro-B cells and by the pro-B cell stage all cells are uniformly Pax5 positive (Figure 1). Pax5 expression is then maintained at a remarkably stable level throughout the life of a B cell before its downregulation during plasma cell differentiation.[11,12]

Pax5 is required for B-cell commitment

Early B-cell development and lineage commitment critically depends on the activity of two growth factor receptors, the IL-7R and Flt3, as well as a number of transcription factors, including Ikaros, PU.1, E2A, early B cell factor 1 (EBF1) and Pax5 that function in a transcriptional network.[9] Ikaros and PU.1 act primarily in promoting the formation of lymphoid progenitors, as Ikaros-deficient mice lack expression of Flt3,[13] whereas PU.1 promotes the initiation of IL-7Rα and Ebf1 expression.[14,15] Once the earliest B-lymphoid progenitors are formed, E2A and EBF1 act together to initiate expression of many B cellspecific genes, including Pax5.[16,17]

Much of what we know about the role of Pax5 in early B lymphopoiesis derives from the study of mice lacking Pax5, both throughout development[18] and after the conditional inactivation in B cells.[19] In the absence of Pax5, foetal B-cell development is arrested at a very early stage, prior to the appearance of identifiable B-cell progenitors and B cell-specific gene expression.[20] In contrast B-cell development in the post-natal Pax5-deficient bone marrow proceeds to the early pro-B cell (or pre-BI) stage of differentiation. The reason for the difference between foetal and adult phenotypes in $Pax5^{-/-}$ mice is unknown, but likely to involve cell survival, as the introduction of a Bcl2 transgene onto the Pax5-deficient background rescues some foetal pro-B cell differentiation in vitro (SLN unpublished). Cultured Pax5-deficient pro-B cells derived from adult bone marrow are characterized by the expression of many B cell-specific transcripts and D-J rearrangements at the Igh locus.[20] Although it is beyond the scope of this review, it is important to note that Pax5, through an

unknown mechanism, also regulates the V-DJ recombination of the distal, but not proximal, V genes of the Igh locus[21,22] (Figure 1). Intriguingly, while being unable to differentiate into mature B cells, Pax5$^{-/-}$ pro-B cells can be cultivated indefinitely in vitro in the presence of IL-7 and a stromal cell layer. Again the mechanism underlying this self-renewal capacity is unknown, but serial transplantation studies have found that Pax5$^{-/-}$ pro-B cells have similar in vivo self-renewal abilities to wild-type HSCs.[23] Most surprisingly, however, is that these pro-B cells are not committed to the B-cell lineage and are capable of differentiating into a broad spectrum of haematopoietic cell types.[24–27] The restoration of Pax5 expression in deficient cells suppresses this multi-lineage potential, whereas the conditional inactivation of Pax5 in pro-B cells reverts lineage commitment and again generates multipotent cells.[28] A similar capacity was subsequently reported for E2A-deficient lymphoid progenitors, a finding in keeping with the lack of Pax5 expression in these cells.[29]

The important role for Pax5 in B-cell commitment does not end at the pro-B cell stage as the conditional inactivation of Pax5 in mature B cells or its physiological downregulation during plasma cell differentiation results in the re-expression of many Pax5-repressed genes.[12,19,30] Remarkably this maintenance of B-cell identity also extends to the control of lineage commitment itself as conditional inactivation of Pax5 in mature B cells results in the de-differentiation of a proportion of cells to the pro-B cell stage, which are then capable of reconstituting T-cell development.[31] These studies unequivocally demonstrate that B-cell commitment is not a discrete event but is continually maintained by Pax5 throughout the life of a B cell. Interestingly, the conditional inactivation of Pax5 in committed B cells also results in pre-B cell leukaemia that arises from mature B cells.[31] This observation has important implications for our understanding of human leukaemia, in particular B-cell acute lymphoblastic leukaemia, where Pax5 is mutated in 430% of cases,[32] that may arise from similarly de-differentiated mature B cells. Similarly it will be important to assess whether mixed-lineage leukaemia, which is comprised of multiple malignant cell types, is derived from an analogous de-differentiation event.

The mechanism of gene regulation by Pax5

The developmental plasticity observed in Pax5-deficient pro-B cells highlights a dual role for Pax5 in lymphopoiesis: activating genes associated with the B-cell lineage while at the same time repressing genes involved in other haematopoietic cell lineages. Pax5 binds DNA via its N-terminal DNA-binding domain, termed the paired domain (Figure 2a).[33] Structural and mutagenesis studies have revealed that the paired domain consists of two sub-domains that

each independently bind to a distinct half-site in the recognition sequence.[34,35] This bipartite binding allows the highly degenerate Pax5 consensus-binding sequence, as multiple sequence changes can increase the affinity of one half-site while decreasing affinity at the other half-site. The current consensus Pax5 recognition sequence, derived by the Busslinger laboratory from the direct comparison of 30 high-affinity sites is shown in Figure 2b.[10] The degree of sequence redundancy in the recognition sequence has made the identification of Pax5-binding sites from DNA sequence analysis alone problematic.

While numerous studies have shown that Pax5 has the ability to both activate and repress gene transcription, the mechanisms by which this is achieved concurrently in the same cell are less clear. Transcriptional reporter assays have shown that Pax5 can regulate gene expression via its C-terminal domain, which consists of a transactivation and inhibitory motifs,[36] and that a centrally located conserved octapeptide motif is required for gene repression through the recruitment of Groucho co-repressors (Figure 2a[37]). Pax5 has also been shown to interact with the basal transcriptional machinery, the retinoblastoma protein[38,39] and co-activator complexes[40] (Figure 2a). The relative importance of these biological activities is at present difficult to assess, as they have not been demonstrated to be functionally relevant on endogenous target genes.

Pax5 activity is also influenced by interactions with other sequencespecific transcription factors including Ets proteins[41,42] Runx1,[43] c-Myb44 and Id proteins.[45,46] For example, Pax5 contributes to the regulation of the B cell-specific Cd79a promoter through the cooperative binding with various Ets family members,[41] a process that also requires demethylated DNA.[47] The binding of Pax5 to the Cd79a promoter is inhibited by Id proteins (Id1-3),[46] with Id2 antagonizing Pax5 binding to the Aicda promoter through a potentially similar mechanism.[45] In contrast, interactions between Pax5 and Runx1[43] or c-Myb[44] are proposed to positively influence gene expression through cooperative DNA binding. Pax5 also physically interacts with another Ets family member, PU.1[42,48,49] (Figure 2a). The outcome of this interaction appears to be context specific, as Pax5 and PU.1 mutually antagonize each other's activity on the Igk locus,42 while functioning together to recruit Groucho proteins and repress the Igh locus.[49]

Gene Repression by Pax5

Repression of non-B cell lineage genes by Pax5 is essential for B-cell commitment

One of the most striking features of Pax5-deficient pro-B cells is their expression of genes usually associated with non-B cell lineages.[24] Microarray studies have now identified greater than 100 Pax5 repressed genes, the majority of which are normally expressed in other haematopoietic lineages, including T cells and macrophages.[30,50] This phenomenon is exemplified by the expression of the cell surface receptors macrophage colony-stimulating factor receptor (M-CSFR, coded for by Csf1r[24,51]) and Notch1,[52] which allows Pax5-deficient pro-B cells to respond to external signals and differentiate into macrophages and T cells, respectively (reviewed in Carotta et al.[53]). Re-introduction of Pax5 into $Pax5^{-/-}$ pro-B cells leads to repression of Csf1r and Notch1, abolishing multi-lineage potential, whereas the inactivation of Pax5 in committed pro-B cells results in the reexpression of these genes.[28,52] In the case of the Csf1r promoter it appears that Pax5 directly represses transcription by decreasing the binding of PU.1, an essential regulator of Csf1r expression in macrophages.[51] Pax5 binds to the Csf1r promoter directly adjacent to a PU.1 site, inhibiting PU.1 function but not binding.Whether this represents a general mechanism for Pax5-mediated gene repression remains to be determined.

A role for Pax5 in repressing lineage-inappropriate genes is compatible with other studies that have shown that multipotent cells maintain the concurrent expression of genes associated with different lineages. This phenomenon is termed ‘lineage priming’ and it is proposed that uncommitted progenitors maintain a relatively open chromatin configuration that allows the low-level expression of genes reflective of multiple lineage fates.[54,55] By this model, lineage commitment results in the progressive repression of this lineage-promiscuous gene expression until a stable transcriptional profile is reached. While the repression of such non-B cell genes as Notch1 or Csf1r by Pax5 provides a molecular explanation for the lineage-plasticity of $Pax5^{-/-}$ pro-B cells, studies on another repressed gene, Flt3[30,56] have shown the importance of the process for B-cell commitment.

Flt3 is expressed on early haematopoietic progenitors and is important for multi-lineage potency and the generation of B-cell progenitors.[57] As B cells downregulate Flt3 expression during B-cell commitment, this gene represented an attractive candidate for Pax5 repression (Figure 1). Indeed, we recently reported that Pax5 directly represses Flt3, through binding to two sites in the proximal promoter.[56] More importantly, enforced Flt3 expression throughout haematopoiesis results in a pronounced decrease in bone marrow B lymphopoiesis while thymocyte and myeloid differentiation was relatively unaffected.[56] Similarly, high doses of

exogenous Flt3L exposure result in reduced numbers of B-cell progenitors in vivo, whereasmyeloid and T cells are again unaffected.[58] Flt3L exposure results in the expansion of a population of the wild-type progenitor cells termed the early progenitor of lymphoid and myeloid developmental potential (EPLM).58 EPLMs have a cell surface phenotype and developmental capacity that is similar to $Pax5^{-/-}$ pro-B cells, suggesting that the developmental pathway characterized in the absence of Pax5 also occurs in a transient manner in wild-type cells.[59] One interpretation of these experiments is that the inability of pro-B cells to silence Flt3 expression enables these multipotent cells to be influenced by other signals and thus allows the commitment to alternate lineages at the expense of B cells.

Gene repression by Pax5 in mature B cells

Pax5-mediated repression of non-B cell genes is not only critical for lineage commitment but is also important in maintaining B-cell identity and function of committed pro-B cells as inactivation of Pax5 in pro-B cells induces the rapid expression of a large number of previously repressed genes.[30] Surprisingly, this repression is still essential in mature B cells as the deletion of Pax5 results in the reexpression of many of the formerly silent genes, including Flt3[30] (Figure 1). The re-expression of these Pax5-repressed genes also occurs during the physiological downregulation of Pax5 during the terminal differentiation of activated B cells into antibody-secreting plasma cells.[11,12,30] This re-expression has direct biological relevance as the products of two Pax5-repressed genes, the co-receptor CD28 and the chemokine receptor CCR2, are important for plasma cell function in an immune response.[30]

Interestingly, the conditional deletion of Pax5 in mature B cells also results in premature expression of genes coding for proteins involved in plasma cell differentiation and function, including X-boxbinding protein 1,[60,61] B lymphocyte-induced maturation protein 1 (Blimp-1),[30,61] Immunoglobulin J chain[62] and the Igh 3` enhancer.[48,49] Together these studies suggest that a key function of Pax5 in mature B cells is to repress the plasma cell pathway. As Blimp-1, an essential regulator of plasma cell development,[63] is known to directly repress Pax5 expression, it was proposed that the mutually antagonistic functions of these two pivotal transcription factors control B-cell terminal differentiation.[64] However, recent experiments from our laboratory suggest that this interaction is more complex than anticipated, as the expression of multiple Pax5 target genes, including Flt3, Cd79a and Blnk is deregulated in late stage B cells prior to the onset of Blimp-1 expression.[12] This altered Pax5 activity is not the result of reduced Pax5 protein amounts and, at least in the case of Flt3, appears not to be

mediated by differential binding to regulatory sequences, suggesting that another protein or a modification of Pax5 itself suppresses Pax5 function and promotes terminal differentiation (Figure 1). Therefore, it appears that plasma cell development occurs via a multi-step model whereby differentiation is initiated by the inhibition of Pax5 function followed by the induction of low-level Ig secretion and Blimp-1 transcription. Only subsequently does Blimp-1 drive the terminal-differentiation programme by silencing Pax5 transcription, thereby promoting high-level Ig secretion and plasma cell maturation.[12] These studies highlight the central role played by Pax5, in particular its gene repression function, in controlling many aspects of the B-cell fate.

The activation of gene expression by Pax5

Pax5 regulates multiple components of the pre-BCR

In early studies, several B cell-specific genes were proposed to be activated by Pax5 on the basis of paired domain-binding sites in their regulatory regions and protein–DNA binding and transient transfection assays. Reported Pax5-activated target genes included genes coding for the cell surface protein CD19,[65] the tyrosine kinase Blk,[66] the signalling molecule Igα (CD79a)[41] and the surrogate light chains λ5 and VpreB[67] (Figure 3). The first systematic screens for Pax5-activated genes took advantage of cultured Pax5-deficient and wildtype early pro-B cells to screen numerous candidate B-lymphoid genes for additional Pax5-target genes.[68,69] These screens confirmed the importance of Pax5 for CD19 and Igα expression and found a role for Pax5 in regulating the expression of the transcription factors N-myc and LEF-1 and the adaptor protein Blnk.[68,69]

Pax5 plays an important role in activating the expression of many components of the pre-BCR including V-DJ recombination at the Igh locus[21] and the expression of Blnk, CD19, Igα, λ5 and VpreB (Figure 3). Since mice that lack one or more components of the pre-BCR also display a partial arrest in B-cell development at the pro-B cell stage,[3] the inability to express a functional pre-BCR might have been the cause of the developmental block in Pax5-deficient mice. The re-introduction of functionally rearranged Igh and chimeric Igh–Igβ transgenes into the Pax5-mutant background neither advances B-cell development past the pro-B cell stage, nor elicits a normal signalling response.[70] Moreover, restoration of Blnk expression in $Pax5^{-/-}$ Igh^{+} pro-B cells results in constitutive pre-BCR signalling and proliferation but again fails to induce pre-B cell development.69 Together, these data demonstrate that while Pax5-controlled expression of the pre-BCR is essential for early B-

cell development, the block at the pro-B cell stage in $Pax5^{-/-}$ mice is not simply a consequence of impaired pre-BCR signalling.

Identification of Pax5-activated genes using global expression analyses

More recent studies have also utilized the $Pax5^{-/-}$ early pro-B culture system in combination with various microarray approaches to investigate novel Pax5-activated genes. The study from the Busslinger laboratory made use of two different microarray approaches to compare gene expression between wild-type and Pax5-deficient pro-B cells.[71] These included a mouse 'lymphochip' and an array containing unselected mouse ESTs that were hybridized with short-term cultured or ex vivo sorted pro-B cells. Using a similar, but complementary, approach our laboratory has screened a custom-generated IgM^{+} mature B cell array and a 15000 clone library from the National Institute on Aging derived from a variety of sources including embryonic, neural and malignant samples.[50] Together these approaches identified greater than 200 potential Pax5-activated genes belonging to numerous functional classes such as secreted proteins, cell surface receptors, cell cycle regulators and transcription factors. Approximately 90 of these genes were validated by investigating expression in cultured wild-type and Pax5-deficient cells and a number have been shown to be direct Pax5 targets using an inducible Pax5 culture system and chromatin immunoprecipitation assays.[50,71] The genes identified in these studies are either previously known, or predicted, to be involved in many functions within the B-cell lineage. Two groups of target genes stand out and will be further discussed here; those involved in adhesion and migration and those involved in gene regulation.

Both the studies found that Pax5 is required for the expression of a number of genes involved in adhesion and migration. This includes cell surface receptors such as CD157 (Bst1), Syndecan4, CD97, Troy (tnfrsf17) and CD55, as well as intracellular signalling components such as Nedd9 and calpain2 and Eps8.[50,71] Importantly, Schebesta et al.[71] found that $Pax5^{-/-}$ pro-B cells show increased migration in response to the chemokine, CXCL12, while wild-type pro-B cells display much stronger integrin-mediated cell adherence, demonstrating a functional consequence to the deregulated gene expression observed. Pax5 also has an indirect role in regulating B-cell trafficking by activating the expression of the transcription factor Klf2, a known regulator of Edg1, whose expression was, as expected, also reduced in $Pax5^{-/-}$ B cells.[30] Edg1 codes for the sphingosine-1-phosphate receptor 1 that is required for lymphocyte egress from lymph nodes.[72]

Pax5 also activates the expression of a number of the genes that are well established as regulators of B-cell differentiation, including Aiolos (Ikzf3), Spib, Irfr4, Bach2 and Irf8 (Icsbp)[50,71] as well as the previously identified regulators Lef1[68] and Ebf1.[73] Ikzf3 and Ebf1 expression is also dependent on Pax5 in the chicken DT40 B-cell line.[61] It is interesting to note that several of these transcription factors play crucial roles at the pre-B cell stage, where Irf8 and Irf4 function to regulate Igk gene expression and the pre-B-to-B transition during development[74,75] while Aiolos represses l5 to end the pre-B cell stage[76] (Figure 3). The genes encoding the related proteins Tcf7l2 and Tcf4 (encoding E2-2) are also differentially expressed, as is the inhibitor of E proteins, Id3. E2-2 is required for optimal pro-B cell expansion,[77] whereas Id3 functions to inhibit growth and induce apoptosis in pro-B cells as well as late-stage B cells.[78,79] Finally, Bach2 and SpiB function in activated B cells to control the GC response.[80,81] For most of the transcription factors this regulation is direct, suggesting that an important aspect of Pax5 function is to reinforce the B-cell programme by further activating the downstream transcriptional cascade.

Gene activation by Pax5 in mature B cells

Pax5 also plays a role in activating gene expression during late B-cell differentiation, however this could not be detected by the microarray approaches that concentrated on differentially expressed genes during early B lymphopoiesis. For example, Pax5 regulates the class switch recombination of immunoglobulin genes by regulating Aicda (coding for AID).[45,61] In vitro stimulation of B cells results in the recruitment of Pax5 to the Aicda locus, whereas enforced expression of Pax5 induces endogenous Aicda gene expression in pro-B cell lines.[45] The recruitment of Pax5 to the Aicda promoter is inhibited by Id2, suggesting another level of regulation of Pax5 function during B-cell differentiation.[45] Furthermore, Pax5 positively regulates germ line transcription of the IgE locus (Iε), which is a prerequisite for a switch to IgE synthesis, while repressing IgA switching.82,83 Pax5 has also been shown to bind to the promoter regions of both Cr2 (CD21) and Fcer2a (CD23).[84,85] Both genes play a role in the late stages of B-lymphocyte differentiation as CD23 is the low-affinity IgE receptor and CD21 is critical during the antibody response. These results highlight that Pax5 is not only involved in the earliest stages of B-cell development and commitment, but also plays a role in mature B-cell differentiation.

Concluding remarks

The gene activation and repression functions of Pax5 control many aspects of B-cell biology including lineage commitment, antigen receptor rearrangement and signalling, and the control of terminal differentiation (Figure 1). Deciphering how one factor can achieve all these disparate functions is a difficult challenge, however the recent identification of a large number of Pax5 target genes promises to shed light on these processes. Many questions, however, remain. For example, little is known about the interactions of Pax5 with other proteins that activate and repress gene expression and how participation in these distinct complexes is concurrently regulated in the same cell. Moreover how Pax5 regulates endogenous targets remains obscure. In this regard the recent report of chromatin profiling of a selection of Pax5 target genes provides one approach that will facilitate future studies. That study used a ChIP-on-chip approach to identify areas of active histone marks at the promoters and putative enhancers of Pax5 target genes.[71] These active areas were noticeably absent in Pax5-deficient pro-B cells. Future genome-wide binding studies will undoubtedly extend these findings to determine if Pax5 functions predominantly in promoter, enhancer or silencer elements. The many putative protein interaction partners suggest that Pax5 may often function as part of an enhanceosome, whereby specificity in gene transcription is achieved by the assembly of three-dimensional transcription factor/enhancer complexes.[86] Finally, the recent demonstration that Pax5 is commonly mutated in human acute B-cell leukaemia[32] and is a tumour suppressor in the mouse[31] suggests that mapping the gene regulation by Pax5 in these malignant situations will also be important for our understanding of acute leukaemia.

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Legends

Fig.1 - The expression and function of paired box gene 5 (Pax5) during B-cell development. Simplified model of the stages of B-cell development. Pax5 expression is initiated at the pre-pro-B cell stage and maintained at a stable level throughout B-cell ontogeny before being downregulated during plasma cell development. Major functions of Pax5 are indicated on the upper portion of the figure. The cell surface expression of the protein products of two Pax5- repressed genes, Flt3 and Notch1, and one activated gene, CD19, is indicated. Pax5 function in activated B cells is inhibited on a post-translational level, allowing for the re-expression of Flt3 and Notch. Pax5 is then transcriptionally silenced in plasma cells. Pre-BCR, pre-B cell receptor; BCR, B cell receptor.

Fig.2 - Schematic representation of the structural domains and interacting partners of paired box gene 5 (Pax5). (a) Locations of the DNA-binding paired domain, the conserved octapeptide (oct.), the partial homoeodomain, and the C-terminal transactivation (transact'n) and inhibitory domains are indicated. The Pax5 protein is coded for by two alternative first exons (ex1A/B). Putative Pax5-interacting proteins are indicated. Pax5 interacts with all four members of the Groucho family (Grg 1–4). The Pax5 homoeodomain region interacts with Daxx that is potentially in a complex with the CREB-binding protein (CBP) as well as the retinoblastoma protein (Rb) and the TATAbinding protein (TBP). Similarly, the physical association of PU.1 and P300 with the Pax5 inhibitory domain has not been directly shown. (b) Current consensus high-affinity Pax5-binding sequence, derived by the Busslinger laboratory. N indicates that any nucleotide may be present.

Fig.3 - Paired box gene 5 (Pax5) regulates multiple components of pre-B cell receptor (BCR) signalling. Schematic of pre-BCR structure and the events initiated by pre-BCR signals. Pax5-regulated genes are shown in bold. The pre-BCR consists of a V-DJ recombined IgH chain complexed with the surrogate light chain (VpreB and λ5) and the Igα/b dimer. The CD19 coreceptor acts to positively amplify the pre-BCR signal. Pre-BCR signalling results in pre-B cell proliferation (partially through cyclin D3), Igκ locus activation (through the initiation of germ line transcripts and V-J recombination) and allelic exclusion at the Igh locus. Pax5 influences the Igκ gene both positively by promoting germ line transcription and activating the expression of Irf4 and Irf8, required for Igκ recombination and expression, and negatively by repressing the 3` Igκ enhancer (3` enh). Pax5 also activates Aiolos (Ikzf3) expression, which in turn represses Igll1 (λ5) to terminate pre-BCR signalling and the pre-B cell stage.

Fig.1

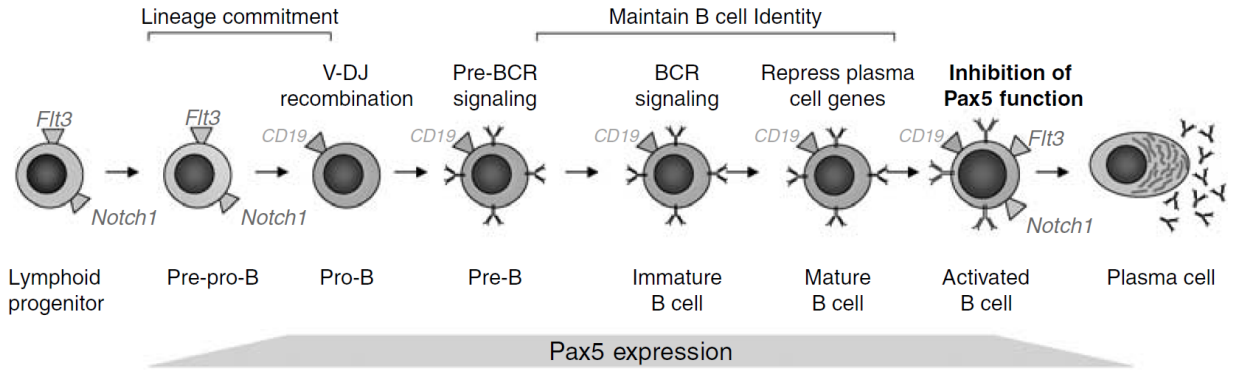
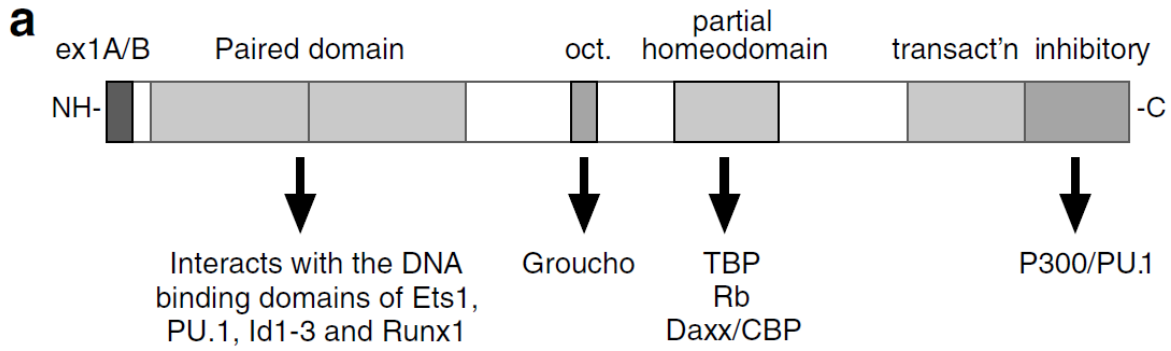

Lineage commitment
Maintain B cell Identity
V-DJ recombination
Pre-BCR signaling
BCR signaling
Repress plasma cell genes
Inhibition of Pax5 function
Flt3
Notch1
CD19
Lymphoid progenitor
Pre-pro-B
Pro-B
Pre-B
Immature B cell
Mature B cell
Activated B cell
Plasma cell
Pax5 expression

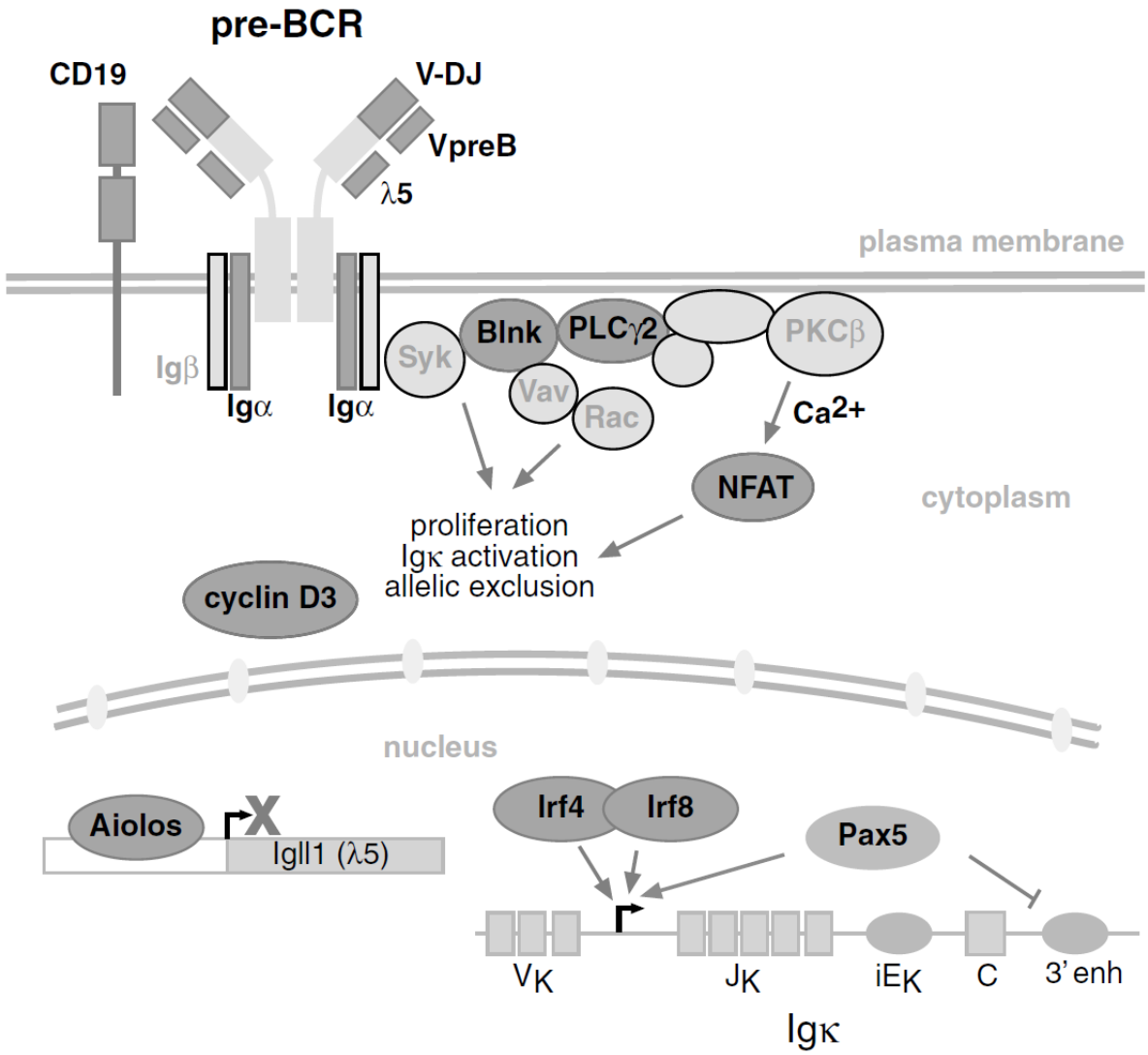
Fig.2



b Pax5 consensus binding site

5'-ANCTANTCATGCGGATA-3'
G G G A TGAC
C

Fig.3


pre-BCR
CD19
V-DJ
VpreB
λ5
plasma membrane
Igβ
Igα
Igα
Syk
Blnk
PLCγ2
PKCβ
Vav
Rac
Ca2+
NFAT
cytoplasm
proliferation
Igκ activation
allelic exclusion
cyclin D3
nucleus
Aiolos
Igll1 (λ5)
Irf4
Irf8
Pax5
VK
JK
iEK
C
3' enh
Igκ