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Reporter Gene Insertions Reveal a Strictly B Lymphoid-Specific Expression Pattern of Pax5 in Support of Its B Cell Identity Function

Martin Fuxa and Meinrad Busslinger

The transcription factor Pax5 is essential for B cell commitment and development. Although the detailed Pax5 expression pattern within the hemopoietic system is still largely unknown, we previously reported that Pax5 is monoallelically transcribed in pro-B and mature B cells. In this study, we have investigated the expression of Pax5 at single-cell resolution by inserting a GFP or human Cd2 indicator gene under the translational control of an internal ribosomal entry sequence into the 3' untranslated region of Pax5. These insertions were noninvasive, as B cell development was normal in Pax5iGFP/H11002 and Pax5ihCd2/H11002 mice. Transheterozygous Pax5iGFP/H11002 mice coexpressed GFP and human CD2 at similar levels from pro-B to mature B cells, thus demonstrating biallelic expression of Pax5 at all stages of B cell development. No reporter gene expression could be detected in plasma cells and non-B cells of the hemopoietic system. Moreover, the vast majority of common lymphoid progenitors and pre-pro-B cells in the bone marrow of Pax5iGFP/H11002 mice did not yet express GFP, indicating that Pax5 expression is fully switched on only during the transition from uncommitted pre-pro-B cells to committed pro-B cells. Hence, the transcriptional initiation and B cell-specific expression of Pax5 is entirely consistent with its B cell lineage commitment function. The Journal of Immunology, 2007, 178: 3031–3037.
Pax5 alleles can be individually monitored in B lymphocytes of heterozygous Pax5^{	ext{lacZ/-}} mice carrying a lacZ reporter gene insertion in the mutant Pax5 allele (7, 23). Flow cytometric analyses revealed that Pax5 is predominantly expressed from only one allele in pro-B and mature B cells, whereas it switches to a biallelic transcription mode in pre-B and immature B cells (23, 24). As we could confirm these results in genetically unmanipulated B lymphocytes by single-cell RT-PCR and RNA-FISH analyses, we proposed that Pax5 is initiated in a stochastic manner from only one allele at the onset of B cell development (23, 24). Our hypothesis of stage-specific monoallelic expression of Pax5 was subsequently challenged by Rhoades et al. (25), who provided evidence for biallelic Pax5 expression in unfractionated (immature and mature) B cells of the spleen by single-cell RT-PCR analysis.

To study the regulation of Pax5 at single-cell resolution in vivo, we inserted a GFP or human Cd2 indicator gene under the translational control of an internal ribosomal entry site (IRES) element into the 3′ untranslated region of the endogenous Pax5 gene. B cell development was normal in Pax5^{ihCd2/iGFP} and Pax5^{iGFP/iGFP} mice, indicating that the two noninvasive reporter gene insertions can be used to monitor the expression of individual Pax5 alleles. Transheterozygous Pax5^{ihCd2/iGFP} mice coexpressed GFP and human CD2 at similar levels from committed pro-B cells in the bone marrow to mature B cells in the spleen, thus demonstrating biallelic expression of Pax5 at all stages of B cell development. No reporter gene expression could, however, be detected in plasma cells and non-B cells of the hemopoietic system. Pax5 is therefore exclusively expressed within the B lymphoid lineage and is repressed during terminal plasma cell differentiation. Importantly, the vast majority of CLP and pro-B cells in Pax5^{iGFP/iGFP} mice did not express GFP, whereas a minor fraction of these uncommitted progenitors started to up-regulate GFP expression. These data demonstrate that the onset and B cell specificity of Pax5 expression are entirely consistent with the B cell lineage commitment function of Pax5.

**FIGURE 1.** Generation of two Pax5 alleles with different reporter gene insertions. **A**, Structure of the targeted Pax5 alleles. The indicated expression cassette was inserted 149 bp downstream of the translation stop codon in exon 10 of Pax5. The cassette contained a puromycin (puro) resistance gene, flanked by two IoxP sites (arrowheads), upstream of an IRES and an intracellularly truncated (t) human (h) Cd2 gene or GFP gene. Correct targeting was verified by Southern blot analysis of BamHI-digested DNA using the indicated 1-kb PCR probe. The BamHI (B) fragment indicative of each allele is shown together with its length (in kilobase). The two promoters of Pax5 are shown. DT, diphtheria toxoid A; HSV-tk, herpes simplex virus thymidine kinase; pA, polyadenylation sequence. **B**, Southern blot analysis of BamHI-digested DNA isolated from the indicated mice. **C**, Normal B cell numbers in homozygous knock-in mice. Absolute numbers of CD19^+ B220^+ B cells are shown for the bone marrow and spleen of 6-wk-old mice of the indicated genotypes (n = 3 each).
Materials and Methods

Generation of Pax5 knock-in mice

The Pax5<sup>ho-C2</sup> and Pax5<sup>GFP</sup> targeting vectors were assembled in a pSP64 plasmid containing with a polynucleotide with appropriate loxP and restriction sites. A 1.6-kb XhoI-Sall PCR fragment and a 4.5-kb BamHI fragment of the Pax5 gene were inserted as homology arms together with a floxed puromycin resistance gene (positive selection) and an IRES linked to either an intracellularly truncated human Cd2 gene (hCd2) or to a codon-improved (enhanced) GFP gene. The HSV-k and DTA genes (negative selection) were inserted upstream of the short homology arm. EcoRI-linearized DNA (15 μg) was electroporated into HM-1 ES cells (15) to generate Pax5<sup>ho-C2</sup> and Pax5<sup>GFP</sup> mice, respectively. The floxed puromycin gene was deleted in the germline by crossing Pax5<sup>ho-C2</sup> and Pax5<sup>GFP</sup> mice with the Mmr2<sup>cre</sup> line (26) to generate Pax5<sup>ho-C2</sup> and Pax5<sup>GFP</sup> mice, respectively. The Pax5<sup>ho-C2</sup> and Pax5<sup>GFP</sup> alleles were backcrossed for seven generations or more into the C57BL/6 genetic background. The Pax5<sup>ho-C2</sup> and Pax5<sup>GFP</sup> alleles were genotyped by PCR amplification of a 327- and 448-bp DNA fragment, respectively. These fragments were amplified from the human Cd2 and GFP gene with the forward primers 5′-GAGACAAGAGCCCA CAGAGTA-3′ and 5′-CCGACCCTACCCAGAACA-3′, respectively, and the common reverse primer 5′-ACAGGGACGACAGGGT ATT-3′ in Pax5 exon 10.

Abs and flow cytometry

The following biotin-, PE-, CyChrome-, alkalinecyanine-, PE Cy7-, or alkalinecyanin Cy7-coupled Abs were used for flow cytometry: anti-B220 (RA3-6B2), CD4 (GK1.5), CD5 (53-7.3), CD8α (53-6.7), CD11b/Mac1 (M1/70), CD11c (HL3), CD19 (1D3), CD21 (7G6), CD23 (B3B4), CD44 (1M7), CD117c/Ki (ACK4), F4/80, Gr1 (RB6-8C5), IgD (1.19), IgM (M41.42), Ly6C (6C3), Pan-NK (DX5), Sca1/Ly6A (D7), TCR-β (H57-597), and Ter119 Abs from BD Pharmingen and anti-CD25/IL-2Rα (PC61), CD127/IL-7Rα (H57-597), and Ter119 Abs from BD Pharmingen and anti-CD25/IL-2Rα (PC61), CD127/IL-7Rα (H57-597), and Ter119 Abs from BD Pharmingen and anti-CD25/IL-2Rα (PC61), CD127/IL-7Rα (H57-597), and Ter119 Abs from BD Pharmingen and anti-CD25/IL-2Rα (PC61), CD127/IL-7Rα (H57-597), and Ter119 Abs from BD Pharmingen and anti-CD25/IL-2Rα (PC61).

CD16/CD32 Fc block solution (BD Pharmingen). Single-cell suspensions were stained with the respective Abs and analyzed on a Canto flow cytometer (BD Biosciences). A wide forward and side light scatter gate was used for analysis of the different hematopoietic tissues. Bone marrow cells and thymocytes were stained with the following PE-coupled lineage marker Abs for identifying MPPs (LSK), CLPs, and double-negative (DN)1/2 cells (anti-B220, CD4, CD8α, CD11b/Mac1, CD11c, CD19, Gr1, Ly6C, Pan-NK, TCR-β, and Ter119) and pre-pro-B cells (same Ab mixture but lacking anti-B220 and CD4). Subsequently, the cells were washed once, incubated with anti-PE beads (Miltenyi Biotec), and lineage-depleted by MACS sorting (Miltenyi Biotec). The progenitor-enriched Lin+ cells were then stained with the corresponding Abs for identifying the MPPs (LSK) as Lin<sup>−</sup> Sca1<sup>−</sup> c-Ki<sup>−</sup> IL-7Rα<sup>−</sup> cells, the CLP as Lin<sup>−</sup> Sca1<sup>−</sup> c-Ki<sup>−</sup> IL-7Rα<sup>−</sup> cells, and the pre-pro-B cells as Lin<sup>−</sup> c-Ki<sup>−</sup> B220<sup>−</sup> cells. DN1 and DN2 thymocytes were identified as Lin<sup>−</sup> c-Ki<sup>−</sup> CD4<sup>−</sup> CD8<sup>−</sup> B220<sup>−</sup> cells, respectively. Plasma cells were defined as CD138<sup>−</sup> CD28<sup>−</sup> Lin<sup>−</sup> CD18<sup>−</sup> F4/80<sup>−</sup> CD21<sup>−</sup> cells in the bone marrow and spleen as described (15). For isolating B-1a cells, 6-wk-old mice were i.p. injected with 10 ml of prewarmed RPMI 1640 medium. After massaging the filled peritoneum, the B-1a cell suspension was aspirated, washed, and stained with the corresponding Abs.

Immunization with sheep RBC

Sheep RBC were washed in PBS and resuspended at 10<sup>8</sup> cells/ml followed by i.p. injection of 150 μl into an adult mouse.

Results

Generation of two Pax5 alleles with distinct reporter gene insertions

The expression of Pax5 was so far determined in cultured B cell lines and sorted lymphocyte fractions with methods that allowed quantification of Pax5 expression only at the cell population level (5, 18–20). To be able to measure allele-specific regulation of Pax5 at single-cell resolution, we generated two Pax5 knock-in alleles expressing each a distinct reporter gene from the Pax5 promoters. To this end, we used

FIGURE 2. Similar expression of both Pax5 alleles during B cell development. A, B cell-specific expression of Pax5. Bone marrow cells of 6-wk-old Pax5<sup>ho-C2</sup>GFP mice were analyzed by flow cytometry for expression of the pan-B cell marker CD19, and the expression of human CD2 and GFP is displayed for CD19<sup>−</sup> non-B cells and CD19<sup>+</sup> B cells. B, Pax5 expression at different B cell developmental stages in the bone marrow. Bone marrow cells of 3- and 6-wk-old mice were stained and gated as indicated. GFP and human CD2 expression is shown for pro-B (CD19<sup>−</sup>c-Kit<sup>−</sup>), pre-B (CD19<sup>−</sup>CD25<sup>−</sup> IgM<sup>−</sup>), immature B (B220<sup>−</sup>IgM<sup>−</sup>), and recirculating B (B220<sup>−</sup>IgM<sup>−</sup>) cells. C, Comparison of the GFP expression levels detected in the gated cell populations of the indicated B cell developmental stages.
homologous recombination in ES cells to insert either a GFP or intracellularly truncated human Cd2 indicator gene under the translational control of an IRES element into the 3’ untranslated region (exon 10) of Pax5 (Fig. 1A). Blastocyst injection of correctly targeted ES cells resulted in Pax5\textsuperscript{+/puro-}\Cd2\textsuperscript{-} and Pax5\textsuperscript{+/puro-}\GFP mice, which were crossed with the Mox2-cre line (26) to achieve germline deletion of the puromycin resistance gene (Fig. 1B). Heterozygous Pax5\textsuperscript{+/-}\Cd2 and Pax5\textsuperscript{+/-}\GFP mice were intercrossed to obtain homozygous Pax5\textsuperscript{+/}\Cd2\textsuperscript{-} and Pax5\textsuperscript{+/}\GFP mice (Fig. 1B), which were born at Mendelian frequency and showed no apparent abnormalities. Importantly, flow cytometric analysis revealed similar numbers of B220\textsuperscript{+} CD19\textsuperscript{+} B cells in the bone marrow and spleen of Pax5\textsuperscript{+/}\Cd2\textsuperscript{-} and Pax5\textsuperscript{+/}\GFP mice compared with wild-type control mice (Fig. 1C). We conclude therefore that both reporter gene insertions are noninvasive, as they do not interfere with normal expression and function of Pax5.

Similar expression of both Pax5 alleles throughout B cell development

To monitor the expression of each Pax5 allele, we analyzed the bone marrow of transheterozygous Pax5\textsuperscript{+/}\Cd2\textsuperscript{-}\GFP mice, which express human CD2 from one and GFP from the other Pax5 allele. We used the pan-B cell marker CD19 to separate bone marrow cells into non-B cells (CD19\textsuperscript{-}) and B lymphocytes (CD19\textsuperscript{+}) by flow cytometric analysis and then displayed human CD2 and GFP expression for both cell populations. As shown in Fig. 2A, the GFP and human CD2 proteins were coexpressed in B lymphocytes, but not in non-B cells, which demonstrates exclusive expression of Pax5 within the B cell lineage of the hemopoietic system. We next investigated Pax5 expression during B lymphopoiesis by monitoring GFP and human CD2 expression at different B cell developmental stages (Fig. 2B). Both Pax5 alleles were coexpressed in pre-B (CD19\textsuperscript{-} c-Kit\textsuperscript{+}), pre-B (CD19\textsuperscript{-} CD25\textsuperscript{+} IgM\textsuperscript{-}), immature B (B220\textsuperscript{low} IgM\textsuperscript{-}), and recirculating B (B220\textsuperscript{-} IgM\textsuperscript{high} cells) of the bone marrow. Flow cytometric analysis of Pax5\textsuperscript{+/}\Cd2\textsuperscript{-}\GFP splenocytes also revealed coexpression of GFP and human CD2 in immature (IgM\textsuperscript{high} IgD\textsuperscript{high} and mature (IgM\textsuperscript{low} IgD\textsuperscript{low}) B cells as well as in transitional 1 (B220\textsuperscript{-} CD21\textsuperscript{-} CD23\textsuperscript{high}) marginal zone (MZ; B220\textsuperscript{-} CD21\textsuperscript{-} CD23\textsuperscript{low}) and follicular (FO; B220\textsuperscript{+} CD21\textsuperscript{-}CD23\textsuperscript{low}) B cells. B, Pax5 expression in peritoneal IgM\textsuperscript{+} B-1a cells of Pax5\textsuperscript{+/}\Cd2\textsuperscript{-}\GFP mice. C, Comparison of the GFP expression levels between the indicated B cell subtypes.

Loss of Pax5 expression during terminal plasma cell differentiation

We next studied Pax5 expression in late B lymphopoiesis by analyzing germinal center B cells, which undergo Ig class switch

**FIGURE 3.** Pax5 expression in B cells of peripheral lymphoid organs. A, Pax5 expression in splenic B cells. Splenocytes of 6-wk-old Pax5\textsuperscript{+/}\Cd2\textsuperscript{-}\GFP mice were stained and gated as indicated to reveal human CD2 and GFP expression in immature (IgM\textsuperscript{high} IgD\textsuperscript{low}) and mature (IgM\textsuperscript{low} IgD\textsuperscript{high}) B cells as well as in transitional 1 (T1; B220\textsuperscript{-} CD21\textsuperscript{-} CD23\textsuperscript{+}), transitional 2 (T2; B220\textsuperscript{-} CD21\textsuperscript{hi} CD23\textsuperscript{hi}), marginal zone (MZ; B220\textsuperscript{-} CD21\textsuperscript{-} CD23\textsuperscript{low}) and follicular (FO; B220\textsuperscript{+} CD21\textsuperscript{-}CD23\textsuperscript{low}) B cells. B, Pax5 expression in peritoneal IgM\textsuperscript{+} B-1a cells of Pax5\textsuperscript{+/}\Cd2\textsuperscript{-}\GFP mice. C, Comparison of the GFP expression levels between the indicated B cell subtypes.
Initiation of Pax5 expression in lymphoid progenitors

Turning our attention to early lymphopoiesis, we were particularly interested to see whether the initiation of Pax5 expression would be consistent with the previously described B cell lineage commitment function of Pax5 (9, 10). To determine Pax5 expression in early hemopoietic progenitors, we first eliminated lineage-positive cells from the bone marrow of homozygous Pax5iGFP and wild-type control mice followed by staining with appropriate Abs to identify the different progenitor cell populations (Fig. 5A). No GFP expression could be detected in early MPPs (LSK; Lin−/c-KithighSca1highIL-7Rα−) (or LSK), CLPs (Lin−c-KithighSca1highIL-7Rα−), pre-pro-B cells (Lin−c-KithighB220−CD19−) and pro-B cells (c-KithighCD19+) of Pax5iGFP mice (green and wild-type (dashed) line) mice. The percentages of GFP+ and GFP− cells within each progenitor cell population are shown. B. Absence of Pax5 expression in early thymic progenitors. The earliest CD4−CD8− DN thymocytes were identified by flow cytometry as Lin−c-KithighCD44high cells, which were further subdivided into CD25+ DN1 and CD25− DN2 pro-T cells. GFP expression is shown (right) was absent in DN1 and DN2 cells of Pax5iGFP mice (green) like in wild-type control mice (dashed line).
CLPs (Lin<sup>−</sup>IL-7R<sup>α</sup>−c-Kit<sup>low</sup>Sca1<sup>low</sup>B220<sup>−</sup>CD19<sup>−</sup>) and most (83.7%) of the pre-pro-B cells (Lin<sup>−</sup> c-Kit<sup>+</sup> B220<sup>−</sup>CD19<sup>−</sup>) did not yet express GFP. A small percentage (3.5%) of CLPs and a slightly larger fraction (7.8%) of pre-pro-B cells expressed GFP at a low level, indicating that these cells started to up-regulate Pax5 expression (Fig. 5A). Similar GFP expression levels as found in committed pro-B cells were observed only in 3% and 8.5% of CLPs and pre-pro-B cells, respectively (Fig. 5A). In marked contrast, all pro-B cells (c-Kit<sup>+</sup> B220<sup>−</sup>CD19<sup>−</sup>) homogeneously expressed GFP both in Pax5<sup>IRES-GFP</sup>/GFP (Fig. 5A) and transheterozygous Pax5<sup>IRES-GFP/GFP</sup> mice (Fig. 2B). CLPs and pre-pro-B cells (also referred to as CLP2 or EPLM) are known to have a broad lymphoid and latent myeloid developmental potential in contrast to the B cell lineage-committed CD19<sup>+</sup> pro-B cells (2, 4, 5, 20). As GFP expression of the tagged Pax5 allele is fully switched on only in committed pro-B cells, we conclude that the initiation of Pax5 expression is entirely consistent with the B cell lineage commitment function of Pax5.

In addition to the lymphoid progenitors in bone marrow, the earliest CD4<sup>−</sup>CD8<sup>−</sup> DN progenitors (DN1) in the thymus are also uncommitted and retain some B cell developmental potential (29, 30). No GFP expression could, however, be observed in DN1 (Lin<sup>−</sup> c-Kit<sup>−</sup>CD4<sup>−</sup>CD8<sup>−</sup>CD25<sup>−</sup>) or DN2 (Lin<sup>−</sup> c-Kit<sup>−</sup>CD4<sup>−</sup>CD8<sup>−</sup>CD25<sup>+</sup>) prothymocytes of Pax5<sup>IRES-GFP/GFP</sup> mice (Fig. 5B). Moreover, a subset of plasmacytoid dendritic cells was shown to exhibit low-level expression of lymphoid genes including Pax5 (31). However, plasmacytoid dendritic cells (CD19<sup>+</sup> B220<sup>−</sup>Ly6C<sup>−</sup>CD11c<sup>+</sup>) in the bone marrow of Pax5<sup>IRES-GFP/GFP</sup> mice failed to express detectable levels of GFP, indicating that these cells do not transcribe significant amounts of Pax5 mRNA (data not shown). Hence, the absence of Pax5 expression in uncommitted progenitors and hemopoietic cell types other than B lymphocytes is fully compatible with the known role of Pax5 in controlling the B cell identity.

Discussion

The transcription factor Pax5 is essential for B cell commitment and subsequent development to the mature B cell stage (9, 10, 14). In this study we have analyzed the hemopoietic expression of Pax5 by monitoring the transcription of individual Pax5 alleles, which was achieved by noninvasive insertion of different reporter genes into the two Pax5 alleles. The Pax5 gene was shown to be exclusively expressed within the B lymphoid lineage of the hemopoietic system from the committed pro-B to the mature B cell stage. Both Pax5 alleles were similarly expressed at all stages of B cell development, demonstrating that Pax5 is biallelically transcribed in contrast to our previous report suggesting stage-specific monoaclonic expression of Pax5 (23).

Genes coding for receptors, which convey clonal specificity to a cell, are known to be subject to allele-specific regulation in addition to imprinted and X-chromosomal genes. This class of monoaclonically expressed gene codes for Ag receptors (32), NK cell receptors (33), odorant receptors (34), and cadherin-related neuronal receptors (35). Allele-specific regulation has also been described for certain cytokine genes. For instance, the closely linked Il4 and Il13 genes are monoaclonically transcribed in CD4<sup>+</sup> Th2 cells, which was demonstrated by three independent methods. First, single nucleotide polymorphisms were used to discriminate, by RT-PCR analysis, the allelic origin of Il4 and Il13 mRNA in CD4<sup>+</sup> T cell clones generated from BALB/c x CAST/Ei F<sub>1</sub> hybrid mice (36, 37). Second, nascent transcripts at one or both alleles of the same cytokine genes were visualized by RNA-FISH in nuclei of Th2 cells (38). Finally, FACS analysis was used to monitor the expression of the two Il4 alleles in Th2 cells of mice that carried an inactivating insertion of either a human Cd2 or GFP reporter gene in one of the two Il4 alleles (39, 40). All three methods produced concordant results for the Il4 gene, thus unequivocally demonstrating that this gene is subject to stochastic allele-specific regulation. In contrast, FISH and single-cell RT-PCR analyses revealed tight monoallelic regulation of the Il2 gene in mature CD4<sup>+</sup> T cells (41), whereas the majority of CD4<sup>+</sup> T cells expressed both Il2 alleles in mice containing an Il2<sup>Il2</sup>GFP knock-in allele (42). These discrepancies question whether negative results obtained by FISH and single-cell RT-PCR analyses always reflect the failure of an allele to be expressed or rather result from the limitation of these methods in reliably detecting the expression of both alleles, as discussed by Rhoades et al. (25).

Based on single-cell RT-PCR and RNA-FISH analyses of genetically unmodified B cells as well as FACS analysis of Pax5<sup>Il4<sup>Il2</sup>−/−</sup> B lymphocytes, we previously concluded that the Pax5 gene is monoallelically expressed in pro-B and mature B cells during early and late B cell development (23, 24). In an attempt to verify this hypothesis with a more reliable method for detecting allele-specific gene transcription, we tagged the two Pax5 alleles with IRES-cd2 or IRES-GFP insertions. Analysis of transheterozygous Pax5<sup>Il4<sup>Il2</sup>−/−</sup> mice revealed that Pax5 is biallelically expressed throughout B cell development. Our new data thus confirm and significantly extend the single-cell RT-PCR results of Rhoades et al. (25), who demonstrated biallelic Pax5 expression only in unfractioned splenic B cells. Hence, the negative data of our published single-cell RT-PCR and RNA-FISH analyses have misled us despite careful control experiments (23, 24). We analyzed Pax5 expression by RT-PCR only in those B cells displaying biallelic expression of the equally rare Cd19 mRNA, and the transcription factor gene Ikaro revealed biallelic expression under the same RNA-FISH conditions used for the Pax5 gene (23, 24). Our recent identification of a potent B cell-specific enhancer in intron 5 of Pax5 provides a likely explanation why the lacZ gene is infrequently transcribed from the endogenous Pax5 promoters in pro-B and mature B cells of heterozygous Pax5<sup>Il4<sup>Il2</sup>−/−</sup> mice (23). This Pax5 enhancer may efficiently interact and thus specifically service the promoter of the neomycin resistance gene in intron 2 of the targeted Pax5 allele (7), as this promoter is located more closely to the enhancer than the endogenous Pax5 promoters, thus resulting in a transgenic artifact. Interestingly, five of the nine mammalian Pax genes are associated with mouse developmental mutant and human disease syndromes, as heterozygous loss-of-function mutations in these genes lead to haploinsufficient phenotypes (43). The Pax5 gene, which is associated with aniridia in humans and Small eye (Sev) in mice, was shown to biallelically expressed during lens placode development, thus ruling out the possibility that monoallelic Pax5 expression in this tissue causes the haploinsufficient eye phenotype of Sev mice (44).

The B cell-specific expression of Pax5 from the pro-B to the mature B cell stage is entirely consistent with the B cell identity function of Pax5, as conditional Pax5 inactivation in pro-B or mature B cells results in loss of the B cell phenotype and retrodifferentiation to uncommitted lymphoid progenitors (13, 14) (C. Cobaleda and M. Busslinger, unpublished data). Moreover, the loss of Pax5 expression during plasma cell differentiation is in agreement with the fact that Pax5-repressed, B cell lineage-inappropriate genes are reactivated and Pax5-activated, B cell-specific genes are down-regulated in plasma cells (15) (A. Schebesta and M. Busslinger, unpublished data). Finally, the initiation of Pax5 expression at the onset of B cell development fully supports a critical role for Pax5 in B cell lineage commitment. The Pax5 gene is initiated only in a small subset of the uncommitted CLP and pre-pro-B cell progenitors, whereas it is highly expressed in all committed pro-B cells at
the next B cell developmental stage. Interestingly, the Pax5 mutation arrests B cell development in the bone marrow at an early pro-B cell stage that resembles the pre-pro-B cells (also known as CLP2 or EPLM) of wild-type mice (4, 5, 20) in several aspects including their strong lymphoid and latent myeloid potential (9, 10, 12). Hence, the Pax5 mutation blocks adult B lymphopoiesis precisely at the developmental transition, when expression of the Pax5 gene is fully switched on to give rise to committed pro-B cells.

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Disclosures
The authors have no financial conflict of interest.

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4. Horcher, M., A. Souabni, and M. Busslinger. 2001. Pax5/BSAP maintains the initial aspects including their strong lymphoid and latent myeloid potential (9, 10, 12). Hence, the Pax5 mutation blocks adult B lymphopoiesis precisely at the developmental transition, when expression of the Pax5 gene is fully switched on to give rise to committed pro-B cells.

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