Reporter gene insertions reveal a strictly B lymphoid-specific expression pattern of Pax5 in support of its B cell identity function.

M. Fuxa and M. Busslinger

J Immunol 2007; 178:8221; doi: 10.4049/jimmunol.178.12.8221-a
http://www.jimmunol.org/content/178/12/8221.2
CORRECTIONS


Reference 7 was incorrectly cited. The corrected reference is shown below.


The authors’ proof corrections were not included in the original publication of the article. The editors and staff of *The Journal of Immunology* apologize to the authors and readers for this error.

The entire article is reproduced correctly on the following pages in print only. The errors have been corrected in the online version, which now differs from the print version as originally published.
CORRECTIONS

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 and Pax5iGFP mice. Transheterozygous Pax5iGFP 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 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.

Received for publication October 10, 2006. Accepted for publication December 1, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Boehringer Ingelheim and the Austrian Industrial Research Promotion Fund.

2 Current address: Department of Immunology and Molecular Pathology, University College London, 46 Cleveland Street, London W1T 4JF, U.K.

3 Address correspondence and reprint requests to Dr. Meinrad Busslinger, Research Institute of Molecular Pathology, Vienna Biocenter, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria. E-mail address: busslinger@imp.ac.at

4 Abbreviations used in this paper: MPP, multipotent progenitor; CLP, common lymphoid progenitor; IRES, internal ribosomal entry site; DN, double negative; LSK, Lin−Scal−Flk−Kiry progenitor.

Copyright © 2007 by The American Association of Immunologists, Inc. 0022-1767/07/$2.00
Pax5 alleles can be individually monitored in B lymphocytes of heterozygous Pax5<sup>lacZ/−</sup> mice carrying a <i>lacZ</i> 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 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<sup>hCd2/ihCd2</sup> and Pax5<sup>iGFP/igFp</sup> mice, indicating that the two noninvasive reporter gene insertions can be used to monitor the expression of individual Pax5 alleles. Transheterozygous Pax5<sup>hCd2/ihCd2</sup> 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 pre-pro-B cells in Pax5<sup>iGFP/igFp</sup> 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.
Materials and Methods

Generation of Pax5 knock-in mice

The Pax5<sup>fl<sup>Gl2</sup>C2</sup> and Pax5<sup>GFP</sup> targeting vectors were assembled in a pSp64 plasmid containing a polynucleotide with appropriateloxP and restriction sites. A 1.6-kb HpaI-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 intracellular reporter gene (<sup>GFP</sup>) or to a codon-improved (enhanced) DT-A gene. The HSVtk and DT-A genes (negative selection) were inserted upstream of the short homology arm. Egr1-linearized DNA (15 μg) was electroporated into HM-1 ES cells (1 x 10<sup>6</sup>) followed by selection with 2.5 μg/ml puromycin and 2 μM gancyclovir. PCR-positive clones were verified by Southern blot analysis of BamHI-digested DNA before injection into C57BL/6 blastocysts and the generation of Pax5<sup>fl<sup>Gl2</sup>C2</sup> and Pax5<sup>GFP</sup> mice, respectively. The floxed puromycin gene was deleted in the germ line by massaging the filled peritoneum, the B-1a cell suspension was aspirated, washed, and stained with the corresponding Abs.

Abs and flow cytometry

The following biotin-, PE-, CyChrome-, allophycocyanin-, PE Cy7-, or allophycocyanin Cy7-coupled Abs were used for flow cytometry: anti-CD20 (RA3-6B2), CD4 (GK1.5), CD5 (53-7.3), CD8a (53-6.7), CD11b/Mac1 (M170), CD11c (HL3), CD19 (I3D), CD21 (T6G), CD23 (B23), CD44 (1H7), CD117c/Kit (AIC4), 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 (A7R34), and human CD2 (RPA-2.10) Abs from eBioscience. Unspecific Ab binding was suppressed by preincubation with

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

CORRECTIONS

Sheep RBC

Sheep RBC were washed in PBS and resuspended at 10<sup>9</sup> cells/ml followed by i.p. injection of 200 μl into an adult mouse.

Immunization with sheep RBC

Sheep RBC were washed in PBS and resuspended at 10<sup>9</sup> cells/ml followed by i.p. injection of 200 μl into an adult mouse.
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\(^{+/\text{puro-ihCd2}}\) and Pax5\(^{+/\text{iGFP}}\) mice, which were crossed with the Mox2-cre line (26) to achieve germline deletion of the puromycin resistance gene (Fig. 1B). Heterozygous Pax5\(^{+/\text{ihCd2}}\) and Pax5\(^{+/\text{iGFP}}\) mice were intercrossed to obtain homozygous Pax5\(^{\text{ihCd2/iCd2}}\) and Pax5\(^{\text{iGFP/iGFP}}\) mice (Fig. 1B), which were born at Mendelian frequency and showed no apparent abnormalities. Importantly, flow cytometric analysis revealed similar numbers of B220$^+$ CD19$^+$ B cells in the bone marrow and spleen of Pax5\(^{\text{ihCd2/iCd2}}\) and Pax5\(^{\text{iGFP/iGFP}}\) 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\(^{\text{ihCd2/iCd2}}\)/Pax5\(^{\text{iGFP/iGFP}}\) 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$^+$) and B lymphocytes (CD19$^+$) 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 pro-B (CD19$^+$ c-Kit$^+$), pre-B (CD19$^+$ CD25$^+$ IgM$^+$), immature B (B220$^{\text{low}}$ IgM$^+$), and recirculating B (B220$^+$ IgD$^{\text{high}}$) cells of the bone marrow. Flow cytometric analysis of Pax5\(^{\text{ihCd2/iCd2}}\)/Pax5\(^{\text{iGFP/iGFP}}\) splenocytes also revealed coexpression of GFP and human CD2 in immature (IgM$^{\text{high}}$ IgD$^{\text{low}}$) and mature (IgM$^{\text{low}}$ IgD$^{\text{high}}$) B cells as well as in transitional (T1; B220$^+$ CD21$^{-}$CD23$^+$), transitional 2 (T2; B220$^+$ CD21$^{-}$CD23$^{\text{mid}}$), marginal zone (MZ; B220$^+$ CD21$^{-}$CD23$^{\text{low}}$) and follicular (FO; B220$^+$ CD21$^{-}$CD23$^{\text{high}}$) B cells. B, Pax5 expression in peripheral lymphoid organs.

**FIGURE 3.** Pax5 expression in B cells of peripheral lymphoid organs. A, Pax5 expression in splenic B cells. Splenocytes of 6-wk-old Pax5\(^{\text{ghCd2/iCd2}}\)/iGFP$^+$ mice were stained and gated as indicated to reveal 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 pro-B (CD19$^+$ c-Kit$^+$), pre-B (CD19$^+$ CD25$^+$ IgM$^+$), immature B (B220$^{\text{low}}$ IgM$^+$), and recirculating B (B220$^+$ IgD$^{\text{high}}$) cells of the bone marrow. Flow cytometric analysis of Pax5\(^{\text{ihCd2/iCd2}}\)/Pax5\(^{\text{iGFP/iGFP}}\) splenocytes also revealed coexpression of GFP and human CD2 in immature (IgM$^{\text{high}}$ IgD$^{\text{low}}$) and mature (IgM$^{\text{low}}$ IgD$^{\text{high}}$) B cells as well as in transitional (T1; B220$^+$ CD21$^{-}$CD23$^+$), transitional 2 (T2; B220$^+$ CD21$^{-}$CD23$^{\text{mid}}$), marginal zone (B220$^+$ CD21$^{-}$CD23$^{\text{low}}$), and follicular (B220$^+$ CD21$^{-}$CD23$^{\text{high}}$) B cells (Fig. 3A). Likewise, all B-1a cells (IgM$^{\text{low}}$ CD5$^+$ Mac1$^+$) in the peritoneum of Pax5\(^{\text{ihCd2/iCd2}}\)/GFP mice coexpressed the human CD2 and GFP indicator proteins (Fig. 3B). Direct comparison of GFP expression revealed similar levels of Pax5 expression at all stages of B lymphopoiesis except for marginal zone B cells, which expressed Pax5 at a ~2-fold higher level (Figs. 2C and 3C). Together these data unequivocally demonstrate that Pax5 is expressed within the B lymphoid lineage at similar levels and in a biallelic manner from the pro-B to the mature B cell stage.

**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
recombination and somatic hypermutation before differentiation into memory B or plasma cells (27). Eleven days after immunization with sheep RBC, the germinal center B cells (B220^+ CD19^-PNA^high) of homozygous Pax5^{GFP/GFP} mice expressed similar GFP levels (Fig. 4A) as the mature B cells in the spleen (Fig. 3A). The terminal differentiation of germinal center B cells generates short-lived plasma cells in the spleen as well as long-lived plasma cells that home to the bone marrow (27). We have recently described the unambiguous identification of plasma cells as CD138^{hi}CD28^-Lin^- (CD4^- CD8^- F4/80^- CD21^-) cells in the bone marrow and spleen (15). GFP expression is shown for plasma cells of Pax5^{GFP/GFP} (green) and wild-type (dashed) mice 18 days after immunization.

**FIGURE 4.** Loss of Pax5 expression during terminal plasma cell differentiation. A, Pax5 expression in germinal center (GC) B cells. Pax5^{iGFP} (green) and wild-type (dashed line) mice at the age of 3 mo were immunized with sheep RBC and analyzed 11 days later by flow cytometry for GFP expression in germinal center B cells (B220^-PNA^-) of the spleen. B, Loss of Pax5 expression in plasma cells, which were identified as CD138^{hi}CD28^-Lin^- (CD4^- CD8^- F4/80^- CD21^-) cells in the bone marrow and spleen (15). GFP expression is shown for plasma cells of Pax5^{GFP/GFP} (green) and wild-type (dashed) mice 18 days after immunization.

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 Pax5^{GFP/GFP} 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 (Lin^- c-Kit^{hi}Sca1^{hi}IL-7Ralpha^-) or (LSK), CLPs (Lin^- c-Kit^-Sca1^-IL-7Ralpha^-), pre-pro-B cells (Lin^- c-Kit^-B220^-CD19^-) and pro-B cells (c-Kit^- CD19^+) of Pax5^{GFP/GFP} (green) and wild-type (dashed line) mice. The percentages of GFP^- and GFP^hi 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-Kit^- CD44^hi 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 Pax5^{GFP/GFP} mice (green) like in wild-type control mice (dashed line).

**FIGURE 5.** Initiation of Pax5 expression in early hemopoietic progenitors. A, Pax5 expression pattern in bone marrow progenitors. Bone marrow cells of 4- to 5-wk-old Pax5^{GFP/GFP} and wild-type control mice were depleted of lineage (Lin^-) positive cells before flow cytometric analysis using the indicated Abs (for details, see Materials and Methods). GFP expression is displayed for the MPPs (Lin^- c-Kit^-Sca1^-IL-7Ralpha^-) (or LSK), CLPs (Lin^- c-Kit^-Sca1^-IL-7Ralpha^-), pre-pro-B cells (Lin^- c-Kit^-B220^-CD19^-) and pro-B cells (c-Kit^- CD19^+) of Pax5^{GFP/GFP} (green) and wild-type (dashed line) mice. The percentages of GFP^- and GFP^hi 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-Kit^- CD44^hi 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 Pax5^{GFP/GFP} mice (green) like in wild-type control mice (dashed line).
CLPs (Lin− /Ly5−/Sca1−/B220−/CD19−) and most (83.7%) of the pre-pro-B cells (Lin− /c-KItlow/B220+/CD19−) 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−/B220+/CD19−) homogeneously expressed GFP both in Pax5GFP/GFP (Fig. 5A) and transheterozygous Pax5GFP/Cd2GFP 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+ 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−/CD8−/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− /c-KIt−/CD44−/CD25−) or DN2 (Lin− /c-KIt−/CD44−/CD25+) prothymocytes of Pax5GFP/GFP 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−/B220+/Ly6C−/CD11c+ or Ly6Clow/CD11clow) in the bone marrow of Pax5GFP/GFP 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 hematopoietic 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 hematopoietic 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 hematopoietic 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 monoallelic 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 monoallelically 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 monoallelically transcribed in CD4+ 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+ T cell clones generated from BALB/c × CAST/Ei F1 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 inactivated inserting 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+ T cells (41), whereas the majority of CD4+ T cells expressed both Il2 alleles in mice containing an Il2GFP 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 Pax5GFP+/H11002+ 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-hCd2 or IRES-GFP insertions. Analysis of transheterozygous Pax5GFP/Cd2GFP 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 unfractoned 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 Ikaros 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 Pax5GFP+/H11001+ 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 be 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.

Acknowledgment
We thank C. Theussl for blastocyst injection.

Disclosures
The authors have no financial conflict of interest.

References