The Ets factor Spi-B is a direct critical target of the coactivator OBF-1

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OBF-1 (Bob.1, OCA-B) is a lymphoid-specific transcriptional coactivator that associates with the transcription factors Oct-1 or Oct-2 on the conserved octamer element present in the promoters of several ubiquitous and lymphoid-specific genes. OBF-1-deficient mice have B cell-intrinsic defects, lack germinal centers, and have severely impaired immune responses to T cell-dependent antigens. Crucial genes that are regulated by OBF-1 and that might explain the observed phenotype of OBF-1 deficiency have remained elusive to date. Here we have generated transgenic mice expressing OBF-1 specifically in T cells and examined these together with mice lacking OBF-1 to discover transcriptional targets of this coactivator. Using microarray analysis, we have identified the Ets transcription factor Spi-B as a direct target gene critically regulated by OBF-1 that can help explain the phenotype of OBF-1-deficient mice. Spi-B has been implicated in signaling pathways downstream of the B cell receptor and is essential for germinal center formation and maintenance. The present findings establish a hierarchy between these two factors and provide a molecular link between OBF-1 and B cell receptor signaling.

B cell development | gene regulation | transcription factors | transgenic mice

The proline-rich, lymphoid-specific protein OBF-1 (Bob.1, OCA-B; see refs 1 and 2) forms a ternary complex with the POU proteins Oct-1 or Oct-2 and DNA on the conserved octamer element (5'-ATGCCAAT-3'). This motif is present in the promoters of a number of eukaryotic genes, including ubiquitously active genes, such as H2B and U snRNA genes, but also genes that are restricted to distinct cell types (reviewed in ref. 3). Notably, the octamer element is present in all Ig heavy and light chain gene variable (V) region promoters as well as in the heavy chain intronic enhancer (4) and is critical for Ig gene transcription (3, 5–9). OBF-1 strongly potentiates transcription from octamer-containing promoters such as the Ig k light chain promoter in transfection assays and in vitro (1, 2, 10). In B cells containing an inducible OBF-1 allele, it was found that the activity of octamer-dependent reporters depended on OBF-1 (11). Yet, in vivo data from OBF-1−/− mice indicated that OBF-1 is dispensable for early antigen-independent B cell development and that the level of unswitched Ig mu gene transcription in mature B cells is unaffected in the absence of this coactivator (10, 12–14). OBF-1 appears to play a role in cell survival at early B cell stages, and, in the absence of OBF-1, a reduction of transitional B cells in the spleen was observed. This finding suggests that OBF-1 is important for production of transitional B cells in the bone marrow (15) or B cell homing from the bone marrow to the spleen (16).

Importantly, histochemical studies have shown that OBF-1 function is essential for the formation of germinal centers (GC) in secondary lymphoid organs. As a consequence, OBF-1-deficient mice have defects in antigen-dependent B cell development and show a dramatically impaired immune response to T cell-dependent (TD) antigens (10, 13). In addition, it was recently found in an in vitro culture system that OBF-1 is critical for the final stages of antibody-secreting cell differentiation; in the absence of OBF-1, the repressor protein Blimp1/PRDM1 fails to be induced, and downstream targets, such as Pax-5 or Bel6, are not down-regulated (17).

OBF-1 expression is largely restricted to B cells (10), but it can also be induced in T lymphocytes by costimulation in vitro (18, 19). The phenotype observed in OBF-1-deficient mice clearly coincides with the expression pattern of the OBF-1 gene in B cells, which peaks at two distinct time points in B cell development: at the immature B cell stage in the bone marrow and with the highest expression levels in GCs and GC-derived B cell lymphomas (20, 21). The increased amount of OBF-1 protein in GC B cells is partly due to posttranscriptional regulation (22, 23).

To date, known direct target genes depending on OBF-1 are the PRDM1 gene, which is regulated by NF-kB, OBF-1, and Oct-2 in B cells (24), the CCR5 gene in T cells (25), and the B cell-specific B29 and mb1 genes (26). Recently, the Kcnd4 promoter, the Lck distal promoter (27), and the Adh1-like promoter (28) have been identified to be directly bound and activated by OBF-1. However, the physiological relevance of these different target genes for GC formation and TD immune responses remains unclear. Here we present evidence that the Ets factor Spi-B is a crucial target of OBF-1.

Spi-B is a member of the Ets family of transcription factors that are characterized by the presence of a conserved DNA-binding domain (the Ets domain), a motif of 85 aa that forms a unique helix-loop-helix structure binding purine-rich DNA sequences (29, 30). Spi-B is closely related to another Ets factor crucial for hematopoiesis, PU.1/Spi-1, through high structural homology and by its ability to transactivate in vitro target genes identical to those transactivated by PU.1 (31, 32). Spi-B also interacts with the coactivator PU.1 interacting protein ( Pip/IRF-4) (33). In vivo data show, however, that the target genes for PU.1 and Spi-B overlap only partially (34). Both PU.1 and Spi-B are required for normal B cell receptor (BCR) signaling (34), but whereas Spi-B can replace PU.1 in myeloid development, it cannot replace PU.1 in lymphoid development (35). Spi-B-deficient mice exhibit severe abnormalities in B cell function and selective TD humoral immune responses accompanied by a defect in GC formation and maintenance (36). Spi-B-deficient mice have a BCR signaling defect and appear to initiate the production of GCs within splenic primary B cell follicles, but these structures decay prematurely due to BCR-mediated apoptosis. The Peyer’s patches in Spi-B-deficient mice, just as in OBF-1-deficient mice, are normal in appearance and cell numbers.

Here we show conclusively that OBF-1-deficient mice have strongly reduced levels of Spi-B and that OBF-1 acts directly on...
the Spi-B promoter to enhance transcription of this Ets factor. These results establish a molecular hierarchy between these two transcription factors, which are both crucial for the TD immune response and GC formation.

**Results**

**Generation and Phenotype of lck-OBF-1 Mice.** OBF-1 expression was previously found to be mostly B cell-restricted but could be induced in T cell lines and primary murine thymocytes after *in vitro* stimulation with phorbol myristic acetate (PMA) and ionomycin (18, 19). However, the physiological role of OBF-1 in T cells has remained unclear and in *OBF-1−/−* mice T cell development and function appear normal (10, 20). To further define the potential function of OBF-1 in T cells, we generated transgenic mice expressing an HA epitope-tagged OBFB-1 cDNA under the control of the murine proximal lck promoter (see Fig. 6A, which is published as supporting information on the PNAS web site). This promoter is highly active in thymocytes throughout their development and then shows low activity in peripheral T cells (37). In agreement with this activity pattern, high levels of transgenic OBF-1 mRNA and protein were found in the thymus, and lower levels were found in the spleen (Fig. 7, which is published as supporting information on the PNAS web site); by contrast, transgenic OBFB-1 mRNA or protein were not detectable in nonlymphoid organs of these transgenic lines (data not shown). Several independent transgenic mouse lines were generated, which all showed the phenotype described below.

We first examined overall T cell development by flow cytometry by using antibodies against the T lineage surface markers CD4 and CD8. Total thymocyte numbers, as well as the numbers of double positive, double negative, or single positive CD4 or CD8 T cells were all found to be normal in these transgenic mice (Fig. 6B). Upon further characterization by FACScan analysis, some specific alterations in distinct T cell populations were observed in the thymus or in the periphery. For example, a subset of CD4+CD8+ thymocytes also coexpressed the IL-2 receptor α-chain (CD25; Fig. 8, which is published as supporting information on the PNAS web site). These CD4+CD8+CD25+ cells were found to express a higher level of OBFB-1 than the CD25− T cells, thus establishing a link between CD25 expression and OBFB-1 levels (Fig. 9, which is published as supporting information on the PNAS web site). These cells, however, do not appear to correspond to CD25−CD4+ regulatory helper T cells based on the absence of Foxp3 expression, and no elevated number of CD25+ cells was observed in the spleen (data not shown). In addition, other specific T cell populations such as γδ and dendritic epidermal T cells were also influenced by OBFB-1 expression (Figs. 10 and 11, which are published as supporting information on the PNAS web site). Together, these results indicate that OBFB-1 overexpression in the T cell compartment leads to very specific effects on a number of minor T cell subsets but does not overtly perturb normal T cell development.

Because overall T cell development and total T cell numbers were largely unchanged by the ectopic expression of OBFB-1, we decided to use thymocytes RNA for microarray experiments to identify potential OBFB-1 target genes. To this end, two independent comparisons were performed: (i) transgenic vs. WT total thymocytes and (ii) transgenic thymocytes, CD4+CD8+CD25+ vs. CD4+CD8−CD25− (Fig. 9). The results of these independent microarray experiments were combined and allowed to identify overlapping subsets of genes that are modulated by OBFB-1 expression.

**The Expression of Spi-B is Proportional to OBFB-1 Levels in Mouse Thymocytes and Reflects the Activity of the Octamer-Containing Promoter 2 (P2).** The lymphoid-specific Ets family transcription factor Spi-B was identified as one interesting potential OBFB-1 target gene in the microarray analysis. Its elevated expression in the transgenic mice was confirmed by Northern blot analysis and was compared with the expression in *OBF-1−/−* thymocytes. Whereas the WT mice express low levels of *Spi-B* mRNA, the expression of this gene is severely reduced in *OBF-1−/−* thymocytes (Fig. 1A). Thus, expression of *Spi-B* and *OBFB-1* mRNA are directly correlated in thymocytes in vivo.

The *Spi-B* gene is transcribed from two distinct promoters (38); the downstream promoter (P2) contains a perfect octamer site and is therefore a good potential target of coactivation by OBFB-1. The use of an RNase protection probe that distinguishes between the
mRNA isoforms transcribed from the two different promoters revealed that promoter 1 is equally active in cells of the different OBF-1 genotypes, whereas P2 activity is strongly modulated in thymocytes lacking or overexpressing OBF-1 (Fig. 1C).

Transcription from Spi-B P2 is Controlled by OBF-1. To verify the importance of the octamer site in P2, we performed transient transfection assays using luciferase reporter genes driven by the Spi-B gene P1 (P1-luc) or P2 or mutated (P2-S, P2-M) octamer site DNA probe derived from the Spi-B promoter and in vitro translated proteins. Either full-length Oct-1 (lanes 4–9) or its isolated POU domain (lanes 10–15) was used for complex formation.

Oct-1 or the Oct-1 POU domain and OBF-1 on the octamer site of P2 (lanes 9 and 15), which is visible as a supershifted complex, compared with the complex obtained with Oct-1 or POU-1 alone (lanes 6 and 12). These results demonstrate that the octamer site in the Spi-B P2 is necessary for allowing OBF-1 binding in a ternary complex with Oct-1 (or Oct-2) and that it mediates coactivation by OBF-1.

OBF-1 Binds in Vivo on Spi-B P2. We next performed ChIP experiments. For these experiments, primary thymocytes from WT or transgenic mice were isolated, chromatin-bound proteins were cross-linked with formaldehyde, and OBF-1 was immunoprecipitated with an anti-HA antibody. As shown in Fig. 3A, the Spi-B P2 could be amplified by PCR in the precipitate from transgenic thymocytes but not from WT samples. The BLR-1 promoter, which has been shown to be regulated by OBF-1 (24), was used as a positive control and was robustly amplified in the immunoprecipitated transgenic sample. To establish a link between expression of OBF-1 and Spi-B in B cells, ChIP experiments were first repeated with primary splenic B cells isolated from WT mice. However, because OBF-1 is expressed only at low levels in these cells (20) and the available anti-OBF-1 antibodies are not sensitive enough, no conclusive results were obtained (data not shown). We next used Abelson virus-transformed pro-B cell lines established from WT or OBF-1−/− mice. Unlike primary mouse splenic B cells, v-Abl transformed pro-B cells are homogeneous and express high levels of OBF-1 (our unpublished data). In this case, ChIP analysis with an anti-OBF-1 antibody showed that the Spi-B P2 could be amplified in immunoprecipitates from WT but not from OBF-1−/− cells (Fig. 3B). In contrast, control primers in the coding region of the Spi-B gene failed to amplify a fragment (data not shown). These results thus demonstrate unambiguously the presence of OBF-1 on the Spi-B P2 in vivo.
OBF-1 Is Essential for Spi-B Transcription in B Cells. It was shown recently that transcription of Spi-B (and of its coactivator IRF-4) is induced upon treatment of v-Abl-transformed pro-B cells with the Abelson kinase inhibitor STI571 (Gleevec); this treatment elicits further differentiation of the cells, as characterized by increased transcription of Rag-1 and Rag-2, Ig κ gene rearrangement, and enhanced expression of CD25 at the cell surface (40). We therefore used v-Abl transformed pro-B cells to test whether OBF-1 is essential for STI571-induced expression of the Spi-B gene. For this, WT or OBF-1−/− Abl pro-B cells were cultured in the presence (open bars) or absence (solid bars) of STI571 (10 nM) for 10 h, and total RNA was extracted. After RT-PCR, expression of the Spi-B gene was measured by real-time PCR, with primers specific for RNA originating from P1 or P2. The histograms show the mean ± SD of three independent experiments. (B) Mature B cells from OBF-1−/− mice have reduced levels of Spi-B mRNA originating from promoter P2. Real-time PCR was performed on sorted CD43−/−depleted mature splenocytes of OBF-1+/− and OBF-1−/− mice. (C) Rescue of Spi-B transcription by expression of OBF-1 in a mature OBF-1−/− B cell line. (Upper) The Northern blot shows the OBF-1-deficient BM4 parental cell line and two clones thereof expressing an inducible OBF-1/estrogen receptor fusion protein (lanes 1 and 2); all samples were treated with hormone before RNA isolation. (Lower) The blot was reprobed with β-actin as a loading control.

Spi-B Expression in Spleens of Immunized WT and OBF-1−/− Mice. It was shown previously that OBF-1−/− mice fail to form GCs upon challenge with a TD antigen such as 2,4-dinitrophenyl-conjugated keyhole limpet hemocyanin (DNP-KLH) (10). Similarly, Spi-B−/− mice have a severe defect in formation of GCs, which are only small and short-lived (36). To investigate whether down-regulation of Spi-B could explain the lack of GCs in OBF-1−/− mice, we examined, by in situ hybridization, the expression of Spi-B in spleens of immunized mice. As can be seen in Fig. 5A, peanut agglutinin (PNA)-positive GCs could be detected at day 10 after immunization in WT but not OBF-1−/− mice. Accordingly, in these immunized samples, expression of Spi-B mRNA was clearly detected in WT but not OBF-1-deficient spleens (Fig. 5A). As transient GC form in Spi-B−/− mice, we tested whether OBF-1-deficient mice might be able to form transient GCs and examined their formation at an early time point; as shown in Fig. 12, which is published as supporting information on the PNAS web site, small GCs could be detected at day 6 in control mice but not in mice lacking OBF-1.

Because the in situ hybridization did not allow us to discriminate between GC and follicular B cells for Spi-B expression, these two cell fractions were isolated by FACs, and RNA was used to determine the levels of Spi-B transcripts from each promoter. Restricted expression of AID (activation-induced cytidine deaminase protein gene) to the GC B cell fraction confirmed the identity of each sorted population (Fig. 5B Lower). Spi-B expression from promoter P2 was observed in both populations, albeit to a slightly reduced level in GC B cells (Fig. 5B Upper). In contrast, expression from promoter P1 was dramatically reduced in the GC fraction, indicating that OBF-1-dependent expression of Spi-B is critical in GC B cells (Fig. 5B Upper). Together, these data suggest that OBF1 is required for the formation and maintenance of GCs, possibly by way of Spi-B.

Discussion

Here we present compelling evidence that the coactivator OBF-1 is necessary for efficient transcription of the Spi-B gene in mouse B cells.

Fig. 4. OBF-1 is required for Spi-B expression in B cells. (A) OBF-1 is necessary for STI571-induced expression of Spi-B in Abelson pro-B cells. WT or OBF-1−/− Abl pro-B cells were cultured in the presence (open bars) or absence (solid bars) of STI571 (10 nM) for 10 h, and total RNA was extracted. After RT-PCR with primer pairs that discriminate mRNA originating from promoter P1 or P2, real-time PCR was performed on sorted CD43−/−depleted mature splenocytes of OBF-1+/− and OBF-1−/− mice. (B) Rescue of Spi-B transcription by expression of OBF-1 in a mature OBF-1−/− B cell line. (Upper) The Northern blot shows the OBF-1-deficient BM4 parental cell line and two clones thereof expressing an inducible OBF-1/estrogen receptor fusion protein (lanes 1 and 2); all samples were treated with hormone before RNA isolation. (Lower) The blot was reprobed with β-actin as a loading control.

Fig. 5. Absence of Spi-B expression in OBF-1−/− follicular B cells. (A) Ten days after immunization with 2,4-dinitrophenyl-conjugated keyhole limpet hemocyanin (DNP-KLH) splenic cryosections were prepared. Formation of GCs was monitored by staining of B cells with an anti-B220 antibody (green) together with PNA (red). Spi-B expression was detected by immunohistochemistry with a specific riboprobe (antisense, blue), and nuclei were counterstained with Nuclear Fast red (red). A control with a sense Spi-B probe is shown. (B) Upper) Spi-B expression in GC and follicular B cells of immunized WT mice. Splenic GC (GL7 PNA+ B220+) and non-GC (GL7 PNA+ B220+) B cells of WT mice were FACs-sorted 10 days after immunization, and Spi-B mRNA expression was measured by semiquantitative RT-PCR with primers specific for RNA originating from P1 or P2. GAPDH expression was used for normalization. Three experiments were performed with identical results. (Lower) RT-PCR analysis of AID expression, a GC-specific enzyme, demonstrated the purity of the sorted GC B cell population (lane 1) compared with the non-GC B cells (lane 3). Nontemplate controls (without reverse transcription) are shown in lanes 2 and 4, respectively.
T and B cells. Expression of OB1-F-1 from the lck promoter in transgenic mice did not lead to major changes in T cell development or function but modulated expression of specific genes (data not shown). One of them, encoding the transcription factor Spi-B, was identified as a physiologically relevant target.

Although ectopic overexpression might represent an artificial situation, the comparison with OB1-F-1-deficient thymocytes strongly enhances the relevance of our findings. In the absence of OB1-F-1, a general reduction of Spi-B mRNA levels was observed in thymocytes, mainly reflecting a reduction in the use of P2 (Fig. 1). The normal physiological significance of these findings still remains to be elucidated, because OB1-F-1 protein is undetectable in total thymic extracts, and the absence of OB1-F-1 (and of Spi-B) does not notably affect T cell development. It is possible, however, that low levels of endogenous OB1-F-1 protein exist in a subset of thymic populations, be it T or thymic B cells. It was recently shown that overexpression of Spi-B in DN3 thymocytes can reverse T lineage commitment and block β-selection due to impaired expression of Egr proteins (42), which, however, was not observed in our mice.

A number of in vitro experiments showed that OB1-F-1 can bind to Spi-B P2 and transactivate transcription from that promoter (Fig. 2). For this phenomenon, the perfect octamer site present in Spi-B P2 is essential. Furthermore, ChIP assays clearly showed a specific association of the ectopic OB1-F-1 with Spi-B promoter P2 in vivo (Fig. 3).

Although it is still possible that OB1-F-1 also regulates an additional gene(s) required for proper expression of Spi-B, our data demonstrate that OB1-F-1 directly controls Spi-B promoter activity through a consensus octamer site important for its expression. Other putative low-affinity OB1-F-1 sites in the Spi-B promoter may explain why our mutated reporter constructs (P2-S-loc and P2-M-loc) do not completely abolish transcription activation in transient assays.

Because OB1-F-1 expression is known to be critical for the function of B lymphocytes, we directed our focus to the physiologically relevant B cell compartment. It was recently shown that Spi-B is induced upon treatment of v-Ab1-transformed pro-B cells with the v-Ab1 kinase inhibitor STI571 (40). We show here that OB1-F-1 is absolutely crucial for this induction, which is caused mainly by a strong increase in transcription from Spi-B P2 (Fig. 4A). Although necessary, the presence of OB1-F-1 is in itself not sufficient for induction of Spi-B transcription in these cells, and the OB1-F-1 protein or RNA levels in v-Ab1-transformed B cells are not noticeably altered by STI571 treatment (data not shown). This finding indicates that inhibition of the v-Ab1 kinase does not influence OB1-F-1 expression but, rather, promotes Spi-B transcription through a different mechanism. For example, v-Ab1 activity could repress Spi-B transcription by directly phosphorylating OB1-F-1, thereby leading to inhibition of ternary complex formation or coactivation, or by phosphorylating other targets, such as Oct-1 or Oct-2. In the case of Oct-2, it has been proposed that phosphorylation of a residue in the POU domain interferes with binding of Oct-2 to the noncanonical octamer sequence in the murine BLR-1 promoter (43).

Although v-Ab1-transformed pro-B cells are useful to establish the requirement for OB1-F-1 in Spi-B transcription in B cells, these cells do not correspond to the developmental stage, namely mature B cells, at which the main phenotype has been described in mice lacking either Spi-B or OB1-F-1. Neither of these two factors appears to be essential at this early stage of B cell development, at which they might be redundant. We hence investigated expression of SpiB in mature B cells and showed that it is reduced ≥2-fold in mature splenic B cells of OB1-F-1-deficient mice as compared with WT (Fig. 4B), demonstrating that Spi-B levels critically depend on OB1-F-1 in B cells as well. This decrease in Spi-B levels is primarily caused by a reduction in transcripts initiated from the octamer-dependent promoter P3.

It is interesting to note that Spi-B levels are also reduced in the bone marrow of OB1-F-1 knockout mice (data not shown) and that the loss of OB1-F-1 expression has effects on early B lymphopoiesis that have been recognized earlier. There is a reduction of transitional B cells in the spleen of OB1-F-1-deficient mice (15, 16), possibly caused by apoptosis during the normal development of self-reactive B cells occurring at this stage (44, 45). Interestingly, OB1-F-1 deficiency also has an impact on the Ig k repertoire (46) and leads to a reduction of a subset of Ig k promoters (47), suggesting the involvement of OB1-F-1 in the selection of transitional B cells. This effect of OB1-F-1 deficiency might be independent of Spi-B and could be caused by direct binding of OB1-F-1/Oct-1 or -2 complexes to specific octamer sites in some Ig k promoters. In contrast, constitutive overexpression of OB1-F-1 in B cells from a μ enhancer-V region promoter results in a block of B cell development in the bone marrow of transgenic mice (our unpublished data), further supporting the notion that tight control of the OB1-F-1 level is critical for early B cell development.

There are several lines of evidence for the involvement of OB1-F-1 in modulating BCR signaling strength. First, OB1-F-1-deficient B cells are impaired in BCR-triggered Ca2+ mobilization; this defect was restored in CD22+/− OB1-F-1−/− double-deficient animals (48). CD22 is a negative regulator of BCR signaling, and CD22-deficient mice display a lowered activation threshold for BCR cross-linking and increased Ca2+ mobilization upon BCR stimulation (49). Second, OB1-F-1−/− B cells also showed a reduced proliferation after anti-IgM stimulation (50). It is thus tempting to assume that the B cell differentiation defect in the bone marrow of OB1-F-1−/− mice is BCR signal-dependent. Third, Spi-B itself, together with PU.1, was implicated in signaling downstream of the BCR, possibly by controlling the transcription of a component coupling Syk to downstream targets, such as PLCγ and BLNK (34). One direct Spi-B and PU.1 target gene in this cascade was recently identified as being GmhpA2, a hematopoietic adenosine transporter that, when overexpressed in A20 B cells, inhibited inductive BLNK phosphorylation and its recruitment to Igα (51). In addition, increased apoptosis in the bone marrow, possibly caused by the increased sensitivity of OB1-F-1−/− transitional B cells to BCR cross-linking observed in vitro (46) could explain the receptor-specific block at the T1 stage of B cell development in OB1-F-1 mutant mice. These effects, but also the reduced numbers of MZ B cells found in one OB1-F-1-deficient mouse strain (52), can be explained by a model in which OB1-F-1 is required for setting the normal threshold for immature B cell development and selection in the bone marrow.

The crucial role of OB1-F-1 for the development of GCs in the spleen reflects a different mechanism, because OB1-F-1−/−/CD22+/− double-deficient mice were still unable to mount humoral immune responses or to form GCs, although their BCR activation threshold was similar to the WT situation (48). The data presented here indicate that the expression of Spi-B, controlled by OB1-F-1 protein in the GC B cells may be a key element for the GC formation. Importantly, expression of Spi-B from the OB1-F-1-independent promoter P1 is normally down-modulated in GC B cells, so that Spi-B mRNA expression becomes highly dependent on the OB1-F-1-driven P2 (Fig. 5B); this explains mechanistically how OB1-F-1 can be crucial for Spi-B expression in GC B cells.

To conclude, we have identified Spi-B as a physiologically relevant in vivo target of OB1-F-1. These findings greatly help to extend our understanding of how OB1-F-1 is involved in B cell activation and GC formation, and place it directly upstream of Spi-B in a transcriptional hierarchy.

**Materials and Methods**

**Mouse Strains and Cell Lines.** Mouse strains and cell lines used are described in Supporting Methods, which is published as supporting information on the PNAS web site.
PCR. The sequence of all primers used is presented in Supporting Methods.

Immunohistochemistry and in Situ Hybridization. Paraformaldehyde-fixed thyresses were paraffin-embedded, and 6–µm sections were prepared for histochrometry. For in situ hybridization, a 450-bp probe from the 3’ UTR of the murine Spi-B cDNA and the corresponding antisense probe were digoxigenin-labeled (Roche).

ChIP. ChIP was performed as described in ref. 53 with 10 µg of chromatin from murine thymocytes or 3 µg of chromatin from STI571-treated Abelson B cells. Immunoprecipitation was performed with an anti-HA antibody (12CA5) and with the monoclonal OBF-1 antibody C-20 (SC-955 X; Santa Cruz Biotechnology).

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