Plasmacytic Transcription Factor Blimp-1 Is Repressed by Bach2 in B Cells*

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Bach2 is a B cell-specific transcription repressor whose deficiency in mice causes a reduced class switch recombination and a reduced somatic hypermutation of immunoglobulin genes. Little is known about the direct target genes of Bach2 in B cells. By analyzing various B cell and plasma cell lines, we showed that the expression patterns of Bach2 and Blimp-1 (B lymphocyte-induced maturation protein 1), a master regulator of plasma cell differentiation, are mutually exclusive. The reporter gene of the Blimp-1 gene (Prdm1) was repressed by the overexpression of Bach2 in B cell lines. The heterodimer of Bach2/MafK bound to the Maf recognition element located upstream of the Prdm1 promoter in an electrophoretic mobility shift assay. The binding of MafK in B cells to the Prdm1 Maf recognition element was confirmed by chromatin immunoprecipitation assays. When MafK was purified from the BAL17 B cell line, a significant portion of it was present as a heterodimer with Bach2, with no apparent formation of MafK homodimer. These results strongly suggest that Bach2 represses the expression of Blimp-1 together with MafK in B cells prior to plasma cell differentiation. Accordingly, the knockdown of Bach2 mRNA using short hairpin RNA in BAL17 cells resulted in higher levels of Prdm1 expression after the stimulation of B cell receptor by surface IgM cross-linking. Induction of Prdm1 was more robust and faster in primary Bach2-deficient B cells than in wild-type control B cells upon lipopolysaccharide stimulation. Therefore, the Prdm1 regulation in B cells involves the repression by Bach2, which may be cancelled upon terminal plasma cell differentiation.

The process of cell differentiation is usually composed of multiple steps, each of which is characterized by a distinct gene expression pattern. Because the gene expression patterns are defined by transcription factors that are active in particular differentiation stages, transition from one stage to the next may thus involve reprogramming of transcription factor networks. B lymphoid cells provide an excellent model system to understand the transcription factor networks that regulate cell differentiation, because each step of B cell differentiation is well defined and can be monitored using various molecular markers. B cells develop from the hematopoietic stem cells in the bone marrow and continue to undergo maturation in the spleen in mice. After encountering antigen, activated B cells differentiate into plasma cells to secrete IgM or, after undergoing class switch recombination (CSR) and somatic hypermutation (SHM), into plasma cells that secrete other classes of immunoglobulins with a higher affinity (1, 2). It has been well established that this process is regulated by not only antigen but also by cytokines and cell-cell signaling emitted by T cells (2). However, nuclear events that are generated in response to these signals are less well characterized. Because differentiation processes are often choreographed by a network of transcription factors, the plasma cell differentiation may thus be associated with a rewiring of the transcription factor network.

Among the several transcription factors known to regulate the differentiation of B cells into plasma cells (3, 4), Blimp-1 (B lymphocyte-induced maturation protein 1) appears to be the key regulator, which thus provides a feasible bridgehead to investigate the gene network for plasma cell differentiation (5, 6). An overexpression of Blimp-1 induces mature B cells to differentiate into plasma cells (7–9). On the other hand, Blimp-1-deficient B cells fail to become either plasma cells or memory B cells (10). Since Blimp-1 down-regulates a large number of the genes required for germinal center formation and/or activated B cells including Pax5 (paired box gene 5) and Bcl6 (B cell lymphoma 6) (11, 12), Blimp-1 may therefore promote plasma cell differentiation by canceling the B cell identities (11). Blimp-1 also represses c-Myc and inhibits cell proliferation (13), thus facilitating the cell cycle arrest associated with terminal differentiation. These previous studies have thus established that Blimp-1 is the master regulator of plasma cell differentiation. The Blimp-1 expression is high in plasma cells but either low or absent in B cells (10, 14), thus indicating that its

The abbreviations used are: CSR, class switch recombination; MARE, Maf recognition element(s); SHM, somatic hypermutation; TRE, 12-O-tetradecanoylphorbol-13-acetate response element; IL, interleukin; RT, reverse transcription; EMSA, electrophoretic mobility shift assay; kbp, kilobase pairs; ChiP, chromatin immunoprecipitation; siRNA, small interfering RNA.
expression is strictly regulated during plasma cell differentiation. Bcl6 has been suggested to be a repressor of the Blimp-1 gene (Prdm1) (15). Whereas AP-1 binds to 12-O-tetradecanoylphorbol-13-acetate response element (TRE) in the promoter upstream region of Prdm1 to activate its expression, Bcl6 binds to AP-1 to inhibit DNA binding (16). In addition, Bcl6 represses Prdm1 through binding to Bcl6 response element 1, located in intron 5 of mouse Prdm1 (17). Finally, Bcl6 inhibits the STAT3-driven Prdm1 expression by competing for the STAT3-binding sites (18).

The basic region-leucine zipper factor Bach2 (BTB (broad complex, tramtrak, and bric-a-brac) and CNC homology 2) functions as a transcription repressor by heterodimerizing the small Maf proteins (MafK, MafG, and MafF) and then binding to the Maf recognition elements (MARE) (19–22). Bach2 is functions as a transcription repressor by heterodimerizing the small Maf proteins (MafK, MafG, and MafF) and then binding to the Maf recognition elements (MARE) (19–22). Bach2 is expressed from pro-B cell to mature B cell stages, but it is absent in plasmacytic cell lines. Genetic ablation of Bach2 in mice severely abrogates both T-cell-independent and T-cell-dependent CSR and SHM of the immunoglobulin genes (24). In contrast, the production of IgM is apparently not defective in the absence of Bach2. These phenotypes indicate that Bach2 is somehow required for CSR and SHM. A straightforward prediction would be that Bach2 regulates the proper expression of the genes that are required either directly or indirectly for these events. Interestingly, the expression of Blimp-1 and XBP-1, which are both essential for plasmacytic differentiation (10, 12, 25), is up-regulated in Bach2-deficient B cells. In this study, we ask whether Bach2 regulates the expression of Blimp-1. We identified MARE on the locus. The MARE was shown to function as a target site of Bach2/MafK. These observations suggest that Bach2 is a direct repressor of Prdm1 in B cells.

**Experimental Procedures**

**Cell Lines and Cell Culture**—38B9 pro-B, 18-81 pre-B, WEHI231 immature B, BAL17 mature B, and X63/0 plasmacytoma cell lines were maintained in Iscove’s modified Dulbecco’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 50 μM 2-mercaptoethanol, and antibiotics. BCL1 presc statutory factor 2 cell line (CWR13.20–3B3, ATCC CRL–1669) was maintained in RPMI 1640 medium (RPMI; Sigma) supplemented with 10% fetal bovine serum, 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 0.1 mM nonessential amino acids, and antibiotics. The BCL1 cells were stimulated with IL-2 and IL-5 for differentiation (26). The sources of recombinant IL-2 and IL-5 were supernatants of X63/0 BCMG-neo-mIL-2 and X63/0 BMG-neo-IL-5 (27).

**Plasmids**—The reporter containing the promoter MARE of Prdm1 (promoter-MARE-luc) was generated by PCR from C57/B16 genomic DNA using KpnI site-containing primers, 5′-GGGTACCATGACTTACATGTATCGTG- GCCC-3′ and 5′-GGGTACCAAATCCAGCCTCTGCAG- AGG-3′. The 192-bp PCR fragment containing the promoter MARE was inserted into the blunt-ended Smal site of pGL2-TATA-luc. Both pCMV Bach2 and pEF MafK have been described previously (21, 28).

**Mutagenesis**—The mutagenesis of plasmids was performed using the Altered Sites II in vitro mutagenesis system (Promega) according to the manufacturer’s protocol using mutagenesis primers for the promoter MARE (5′-TG GCC CATAGGTGGTGCAG- TCACGATCGGTATA-3′, mutations underlined).

**Immunoblot Analysis of Whole Cell Extracts from B Cell Lines**—The proteins were extracted with radioimmune precipitation buffer from each cell line and then were resolved on SDS-polyacrylamide gels, electrotransferred to polyvinylidene difluoride membrane, and examined by immunoblot analysis as described (29). The primary antibodies used were anti-Bach2 antiserum (F69–1) (21) and anti-MafK antiserum (A-1) (30). Anti-Blimp-1 antiserum raised against 535–661 amino acids of mouse Blimp-1 was provided by Prof. Yoichi Shinkai (Kyoto University). The antiserum was collected and used for the experiment. Anti-rabbit Ig-horseradish peroxidase conjugate (Amersham Biosciences) was used as a secondary antibody.

**RT-PCR**—RNA was purified from B cell lines by using the TRIzol method (Invitrogen). The cDNA was generated by Omniscript Reverse Transcriptase (Qiagen) using the random priming method, and cDNA was used in the RT-PCR. The primers have all been described previously (24). Quantitative RT-PCR was carried out using LightCycler Fast Start DNA Master SYBR Green I and LightCycler 1.5 with HPRT expression as the normalization control.

**Luciferase Reporter Assay**—18–81 pre-B cells or X63/0 plasma cells were transiently transfected with various combinations of reporter and effector plasmids using the GeneJuice Transfection Reagent (Merck) according to the manufacturer’s instructions. Each transfection was done in duplicate, and the luciferase activity was measured 24 h after transfection using the dual-luciferase reporter assay kit (Promega) according to the manufacturer’s protocol. The normalized values are reported as the mean ± S.D. from three independent experiments.

**Electrophoretic Mobility Shift Assay**—Nuclear extracts from WEHI231, BAL17, and X63/0 cells were isolated, and 1 μg of nuclear protein was used for electrophoretic mobility shift assay (EMSA) as described previously (22). The double-stranded probes were as follows: promoter MARE, 5′-ATCGAAAATGTGAAAGGGCATAATTAAGC-3′; promoter mut-MARE, 5′-ATCGAAATGTGAAAAAGGCTATATAGC-3′. For a competition analysis or a supershift analysis, nuclear extracts were incubated with a competitor, rabbit IgG, Bach2 (F69–2), Bach1 (1–5), MafK (A-1) antisera, or anti-Bcl6 (D8; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 30 min before the addition of radiolabeled probes.

**Constructed Cell Lines Derived from Mouse Mature B Cells**—The retrovirus vector pOZ-FHH-N-MafK was generated by PCR from pmMafk15 (31) using the SalI site-containing forward primer and NotI site-containing reverse primer: 5′-CCCCGGTACGGTTATAGCAGTCAATTCCCCGCCCA- ACA-3′ and 5′-GGTTAAGCCGCGCGCTAGGAGCGGGCG- TGAGAAAGGTATA-3′. The fragment was inserted into the XhoI and NotI sites of pOZ-FHH-N (32). Phoenix Ecotropic packaging cells (33) were transfected with pOZ-FHH-N-MafK using Genejuice Transfection Reagent, and then the viral supernatants were harvested 2 days after transfection to infect...
BAL17 mature B cells. The FLAG-hemagglutinin-His-tagged MafK (eMafK)-expressing cells were sorted by anti-human IL-2 receptor beads.

**Protein Purification**—Nuclear extracts were purified as described previously (34). The cells were collected by centrifugation for 8 min at 1,865 \( \times \) g and then were washed with phosphate-buffered saline. After centrifugation for 10 min at 1,190 \( \times \) g, pellets were suspended in six volumes of hypotonic buffer (10 mM Tris-HCl (pH 7.3), 10 mM KCl, 1.6 mM MgCl\(_2\), 2 mM 2-mercaptoethanol, 40 \( \mu \)M phenylmethylsulfonyl fluoride) and collected by centrifugation for 5 min at 1,190 \( \times \) g. Next, the cells were resuspended in the same volume of hypotonic buffer, and the suspensions were homogenized and centrifuged for 30 min at 2,330 \( \times \) g to collect nuclei. The obtained crude nuclei were suspended in a half-volume of low salt buffer (0.02 M KCl, 20 mM Tris-HCl (pH 7.3), 25% glycerol, 1.5 mM MgCl\(_2\), 0.2 mM EDTA (pH 8.0), 2 mM 2-mercaptoethanol, 40 \( \mu \)M phenylmethylsulfonyl fluoride) for homogenization. The resulting suspension was dropped with a half-volume of high salt buffer (1.2 M KCl, 20 mM Tris-HCl (pH 7.3), 25% glycerol, 1.5 mM MgCl\(_2\), 0.2 mM EDTA (pH 8.0)) and then stirred gently for 30 min and centrifuged for 60 min at 48,384 \( \times \) g. The supernatants were dialyzed against 50 volumes of BC-0 buffer (20 mM Tris-HCl (pH 7.3), 20% glycerol, 0.2 mM EDTA (pH 8.0), 2 mM 2-mercaptoethanol, 40 \( \mu \)M phenylmethylsulfonyl fluoride) until the conductivity was dropped to 65–70 \( \mu \)S/cm. The dialysate was centrifuged for 20 min at 23,700 \( \times \) g, and the supernatant was used as nuclear extracts. eMafK complexes were immunoprecipitated from nuclear extracts by incubating with M2 anti-FLAG-agarose (Sigma) for 4 h with rotation. After an extensive wash with 0.1 M KCl-containing buffer B, the bound proteins were eluted from FLAG M2-agarose by incubating for 60 min with the FLAG peptide in the same buffer (0.5 mg/ml). The eluates were then applied to Ni\(^{2+}\)-nitrilotriacetic acid resin (Qiagen). After 60 min of rotation, the resin was collected and washed with 1 ml of 20 mM imidazole-containing NTN buffer (50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% Nonidet P-40). The bound proteins were eluted from Ni-nitrilotriacetic acid by incubating for 30 min with 200 mM imidazole-containing NTN buffer.

**Mass Spectrometric Analysis**—Purified proteins were subjected to SDS-PAGE on 4–20% gel, and then the gel was stained with Coomasie Brilliant Blue. The stained bands were excised from the gel, and the proteins therein were subjected to in-gel reduction, S-carboxymethylmethylation, and digestion with trypsin (Promega). The molecular masses of the tryptic peptides were determined using LC-HCT plus (Bruker Daltonics), and protein identification was performed using the Mascot search engine (Matrix Science).

**Chromatin Immunoprecipitation Assay**—Chromatin fixation and purification procedures were carried out as previously described (35). B cells were fixed by adding formaldehyde to 1% final concentration for 20 min at 4 °C. Cells were then sonicated to prepare chromatin suspensions of 300–1,000 bp DNA in length. Immunoprecipitations were carried out as previously described (35) using anti-MafK antiserum (A-1) or normal rabbit serum (Jackson ImmunoResearch). The samples were analyzed by quantitative real time PCR using LightCycler Fast Start DNA Mas-
Upon examining the DNA sequence of mouse Prdm1, we found a MARE at 1.8 kbp upstream of the transcriptional start site (Fig. 2A). This sequence was reported previously as TRE (16). This region was conserved between the mouse and human Prdm1 (Fig. 2A). To examine whether Bach2 regulates Prdm1 expression through this MARE, we generated a luciferase reporter plasmid that carried the aforementioned promoter MARE/TRE in front of a TATA-box (Fig. 2B). When transfected into 18-81 pre-B cells, the wild-type promoter MARE reporter was repressed by Bach2 in a dose-dependent manner. A reporter containing mutations in the MARE (promoter-mutMARE-luc) was not significantly repressed by Bach2 (Fig. 2C), thus indicating that the promoter MARE sequence is required for the Bach2-mediated repression of the reporter plasmid. Bach2 failed to repress the promoter MARE reporter in X63/0 plasma cells (Fig. 2D), thus raising the possibility that the repressor activity of Bach2 is regulated in a stage-specific manner. These results suggest the possibility that Bach2 repressed Prdm1 through the promoter MARE in B cells.

MARE-binding Complex in B Cells Contains Bach2 and MafK—To identify proteins that bind to the promoter MARE in B cells, we performed an EMSA with nuclear extracts from WEHI231 immature B cells, BAL17 mature B cells, and X63/0 plasma cell lines. A retarded complex was observed with extracts from WEHI231 and BAL17 that was absent in the reactions

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containing nuclear extracts from X63/0 cells (Fig. 3A, lanes 2–4). The formation of this complex was ablated by antiserum against Bach2 (lanes 5–7) or MafK (lanes 8–10) but not by a control rabbit IgG (lanes 11 and 12), thus demonstrating that it contained both Bach2 and MafK. Whereas this complex was apparently not reduced by antiserum against Bach1, a band with a much slower mobility (i.e. supershift) was observed (Fig. 3B, lane 4). These results showed that Bach2 contributed to the majority of specific binding activity and that Bach1 also contributed to some extent. The Bach2 complex was not ablated by an unrelated antibody against Bcl6 (Fig. 3B, lane 6), thus verifying the antibody specificity. Although MafK can also function as a repressor by binding DNA as a homodimer, the homodimeric binding of MafK (i.e. band that is reactive with MafK antibody but not with other antibodies) was not observed in the extracts from the B cell lines (Fig. 3B).

To verify the DNA sequence specificity of Bach2 binding to the Prdm1 promoter MARE, we carried out competition assays. As shown in Fig. 3C, the wild-type oligonucleotide inhibited the formation of Bach2-DNA complex (lanes 4 and 5). In contrast, an oligonucleotide containing mutations in the MARE failed to compete with the bound complex (lanes 6 and 7). Furthermore, the mutated DNA did not generate a protein-DNA complex as efficiently as that of the wild-type probe when the radiolabeled mutated DNA was incubated with the nuclear extracts (lanes 8 and 9). Taken together, these results suggested that the Bach2/MafK heterodimer was the major factor that bound to the promoter MARE in B cells.

Purification of MafK Complex from BAL17 B Cells—The small Maf proteins (MafK, MafG, and MafF) play dual functions as activators or repressors by changing their heterodimer partners (20). They also function as repressors by forming homodimers when overexpressed in various cells. To examine whether Bach2 is the major partner of the small Maf proteins in B cells, we purified the MafK complex from BAL17 mature B cells. FLAG-hemagglutinin-His-tagged MafK (eMafK) was stably expressed in BAL17 mature B cells by using a retrovirus infection system. We first checked the expression levels of eMafK. As shown in Fig. 4A, the expression level of eMafK was similar to endogenous MafK. eMafK was purified from the nuclear extracts by using anti-FLAG antibody column followed by a nickel column. Samples thus purified were analyzed by SDS-polyacrylamide gels along with samples that were purified with the same procedures from control cells. The purified fraction of eMafK contained several proteins, including a prominent band around 115 kDa (Fig. 4B). Mass spectrometry analysis confirmed that the major band was eMafK. Mass spectrometry analysis of the second major band revealed that it was Bach2 (Fig. 4C). These results were further confirmed by an immunoblotting analysis of the purified fractions with respective antibodies (Fig. 4D). Interestingly, it appeared that endogenous...
MaFK was not co-purified with eMaFK (Fig. 4B). An immunoblotting analysis of the purified fractions with anti-MaFK antibody showed that endogenous MaFK was almost completely absent in comparison with eMaFK (Fig. 4D). These observations suggest that the majority of MaFK existed in either heterodimeric or monomeric forms and that the homodimer of MaFK either was not abundant in B cells or was very unstable. Dimerization is required for binding of basic region-leucine zipper proteins to DNA (38, 39). Considering the fact that purified recombinant Bach2 and MaFK form a heterodimer for DNA binding (21), we conclude that MaFK bound to the Prdm1 MARE as a heterodimer with Bach2 in cells expressing Bach2. Binding of MaFK to the Prdm1 MARE in X63/0 cells was unexpected, since we did not detect specific MARE-binding activity in EMSA using X63/0 cell extracts (Fig. 3A). MaFK may bind to the MARE as a heterodimer with an activator whose DNA binding is relatively unstable by EMSA. Nonetheless, these results indicated that MaFK bound to the promoter MARE of Prdm1 in pro-B to plasma cell stages.

Impairment of Bach2 Causes Induction of Prdm1 Expression in B Cells—To determine the role of Bach2 in the Prdm1 repression in B cells, we employed an RNA interference of endogenous Bach2. Expression of Bach2 small interfering RNA (siRNA) in BAL17 mature B cells reduced effectively the expression of Bach2 compared with control cells (mock) at both mRNA and protein levels (Fig. 6, A and B). We found that the expression of Prdm1 was slightly elevated but reproducibly in both of the Bach2 siRNA1 and siRNA2 cell lines (Fig. 6A). Because BAL17 cells can be activated by the signaling via B cell receptor (40), we next stimulated these cells using anti-IgM antibody, which mimics B cell receptor signaling. As shown in Fig. 6C, the levels of Bach2 mRNA were not affected by anti-IgM stimulation. Prdm1 was highly and quickly induced in the Bach2 knockdown cell lines in comparison with the mock cells, thus indicating that the impairment of Bach2 caused a derepression of Prdm1.

To confirm that Bach2 is required for Prdm1 repression, we stimulated mouse spleen B220-positive B cells from wild-type
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FIGURE 5. MafK binds to the promoter MARE of Prdm1 in vivo. A, MafK binding to the promoter MARE region. ChIP assays were performed by using anti-MafK antiserum with BAL17 mature B cells. The samples were analyzed by quantitative real time PCR. Primers were designed 2.0 or 4.0 kbp of upstream/downstream from the promoter MARE; a (−4.0 kbp), b (−2.0 kbp), c (promoter MARE), d (+2.0 kbp), and e (+4.0 kbp). NRS, samples proceeded with normal rabbit serum. B, ChIP assays of the indicated cell lines using anti-MafK antiserum. The samples were analyzed as described in A using primer set c.

or Bach2-deficient mice with LPS to induce plasma cell differentiation and also examined the expression of Prdm1 as a function of time. As described in previous report (24), Prdm1 was expressed at higher levels in Bach2-deficient B cells than wild-type B cells without stimulation (Fig. 7). When B cells were stimulated with LPS, the induction of Prdm1 expression was more robust in Bach2-deficient B cells than in the control cells. These results suggested that Prdm1 was therefore a target gene of the Bach2 in B cells.

DISCUSSION

Although Bach2 plays a critical role in the execution of CSR and SHM of immunoglobulin genes, its direct target genes still remain elusive. In this study, we showed that Bach2 represses expression of Blimp-1 in B cells. Because Blimp-1 is one of the key transcriptional regulators of plasma cell differentiation, our findings pave the way toward detailed understanding of the transcriptional network governing B-to-plasma cell differentiation. The expression of Blimp-1 is kept low, if it is not induced after terminal differentiation into plasma cells (41) (Fig. 1). The induction of Blimp-1 appears to play a decisive role in plasma cell differentiation because Blimp-1 is not only essential but also sufficient, at least in certain contexts, for the terminal differentiation process (3). Several lines of evidence indicate that Bach2 represses Prdm1 through binding to MARE located in the promoter. First, the overexpression of Bach2 repressed the Prdm1 reporter expression. Second, the heterodimer of Bach2 and MafK was found to bind to the promoter MARE in EMSA. Third, the purification of MafK from BAL17 cells revealed that the heterodimer of Bach2 and MafK was the major MARE-binding form of MafK in these cells. Fourth, ChIP assays revealed that MafK bound to the Prdm1 MARE in several B cell lines. Finally, the knockdown of Bach2 in BAL17 cells resulted in higher expression levels of Blimp-1, especially after stimulation via B cell receptor. These results therefore suggest that one of the functions of Bach2 and MafK in B cells is to repress Prdm1 prior to the plasma cell stage. This model is further fueled by our previous observation that the absence of repressor Bach2 is not sufficient for induction of Prdm1 in BCL1 cells. Previous reports indicate that Bcl6 is a critical repressor of the Prdm1 gene (17). Both Bach2 and Bcl6 may be essential for the proper repression of Blimp-1 in B cells. The deregulation of Prdm1 in the Bach2-deficient B cells indicates that Bcl6 does not suffice to achieve proper regulation in the absence of Bach2 (24).

The identification of Blimp-1 as a direct target gene of Bach2 poses an interesting question regarding the phenotype of deficiency for Bach2. Does the increased expression of Blimp-1 in Bach2-deficient B cells cause the defects observed in CSR and SHM? Interestingly, mRNA of activation-induced cytidine deaminase (AID), an essential enzyme for CSR and SHM (42), is observed to dramatically decrease in Bach2-deficient B cells (24). At present, the results of several experiments, such as reporter assays, negate direct activation of the AID gene by Bach2.4 Taken together with the fact that Blimp-1 represses expression of AID (12), our results suggest a transcriptional regulatory circuit consisting of Bach2, Blimp-1, and AID. In this double-negative circuit, Bach2 represses Blimp-1 that represses AID. Bach2 is expected to maintain AID expression in activated B cells via inhibiting Blimp-1 gene expression. In the absence of Bach2 or reduced Bach2 activity, an increased expression of Blimp-1 in activated B cells is expected to suppress the AID expression, thus resulting in a reduced CSR and SHM.

4 K. Ochiai, A. Muto, and K. Igarashi, unpublished observations.
model does not rule out the possibility that Bach2 regulates additional genes that are involved in CSR and/or SHM. It has been pointed out that, in order to generate plasma cells with a high affinity antibody having secondary isotype, it is critical that plasma cell differentiation does not occur before affinity maturation and isotype switching are completed (41). Bach2 may provide a window of opportunity for these reactions by repressing Blimp-1 in B cells.

The results in our current studies place Bach2 upstream of Blimp-1. The expression of a limited number of genes is dramatically changed during differentiation from mature B cells to plasma cells (43). Interestingly, both Bach2 and Blimp-1 are among such genes. Considering the facts that Bach2 and Blimp-1 tend to show an exclusive expression, that both of these factors function as transcriptional repressors, and that Bach2 represses Blimp-1, it appears that the differentiation process of B to plasma cells is regulated by the balance of these two repressors. In this model, Bach2 skews cells away from the terminal differentiation, whereas Blimp-1 pushes the cells toward terminal differentiation. This model predicts that the regulation of Bach2 activity is crucial in plasma cell differentiation. Because the terminal differentiation of plasma cells is regulated by several signals, it is considered important to investigate how Bach2 responds to such signals when B cells are activated.

A logical extension of the above consideration is that transcriptional activators may bind to the Prdm1 MARE to induce its expression during the terminal differentiation process. Because the promoter MARE has been reported to be an AP-1 binding site (16), AP-1 may function as an activator of the promoter MARE. Indeed, c-Fos has been implicated in the activation of Prdm1 in B cells (44). The small Maf proteins are dual function factors that either repress or activate transcription, depending on their partners (20). Although we did not detect any Maf-containing MARE binding activity in the X63/0 cell extracts (Fig. 3), we did detect the binding of MafK in ChIP (Fig. 5). It is possible that the conditions of EMSA in this study failed to capture the Maf-containing activator in the plasma cell line. The switching of small Maf partners may take place as differentiation along plasma cell proceeds, as is the case for the /H9252-globin gene regulation in erythroid cells (36, 45). Further studies are required to investigate the possibility that MafK may also function as an activator of Prdm1.

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FIGURE 6. Derepression of Prdm1 in Bach2 knockdown or knock-out cells. A, BAL17 mature B cells stably expressing Bach2 siRNA1 or Bach2 siRNA2 or control cells (Mock) were generated. The transcript levels for the indicated gene products were determined by quantitative RT-PCR. B, an immunoblot analysis of Bach2 protein in the Bach2 knockdown and control cell lines. C, the cells were cultured in the presence of 10 μg/ml anti-IgM antibody for the indicated periods. The levels of Bach2 and Prdm1 mRNAs were determined by quantitative RT-PCR.

FIGURE 7. A derepression of Prdm1 in Bach2-deficient B cells. Purified spleen B cells from wild-type (WT) or Bach2-deficient (KO) mice were stimulated with 20 μg/ml of LPS for the indicated periods. The transcript levels of Prdm1 were analyzed by quantitative RT-PCR.
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