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# Regulation of the plasma cell transcription factor Blimp-1 gene by Bach2 and Bcl6

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## Abstract

**B lymphocyte-induced maturation protein 1 (Blimp-1) is a key regulator for plasma cell differentiation. Prior to the terminal differentiation into plasma cells, Blimp-1 expression is suppressed in B cells by transcription repressors BTB and CNC homology 2 (Bach2) and B cell lymphoma 6 (Bcl6). Bach2 binds to the Maf recognition element (MARE) of the promoter upstream region of the Blimp-1 gene (*Prdm1*) by forming a heterodimer with MafK. Bach2 and Bcl6 were found to interact with each other in B cells. While both Bach2 and Bcl6 possess the BTB domain which mediates protein–protein interactions, they interacted in a BTB-independent manner. Bcl6 is known to repress *Prdm1* through a Bcl6 recognition element 1 in the intron 5, in which a putative, evolutionarily conserved MARE was identified. Both repressed the expression of a reporter gene containing the intron 5 region depending on the presence of the respective binding sites in 18-81 pre-B cells. Co-expression of Bach2 and Bcl6 resulted in further repression of the reporter plasmid. Chromatin immunoprecipitation assays showed MafK to bind to the intron MARE in various B cell lines, thus suggesting that it binds as a heterodimer with Bach2. Therefore, the interaction between Bach2 and Bcl6 might be crucial for the proper repression of *Prdm1* in B cells.**

## Introduction

B cells play important roles in the humoral immunity. B cells develop from hematopoietic stem cells in bone marrow and continue to undergo maturation in the spleen in mice. After they encounter an antigen, activated B cells differentiate into plasma cells to secrete IgM or undergo class switch recombination (CSR) and somatic hypermutation (SHM) to secrete other classes of Igs with higher affinity for the antigen (1, 2). This process is regulated by not only antigens but also cytokines and cell–cell signaling via T cells. Such extracellular signals are supposed to converge upon transcription factors to change the gene expression in B cells.

Several transcriptional factors are known to play crucial roles in regulating the differentiation of B cells to plasma cells. The basic region–leucine zipper (bZip) factor BTB and CNC homology 2 (Bach2) is specifically required for CSR and SHM (3). Bach2-deficient B cells become IgM-producing plasma cells but not to class-switched plasma cells. Bach2 expression is high in B cells but low or absent in plasma cells (4). One of the target genes of Bach2 is B lymphocyte-induced maturation protein 1 (Blimp-1) which is required for plasma cell differentiation (5). In contrast to Bach2, Blimp-1 expression is low in B cells but is induced upon the terminal differentiation to plasma cells. Bach2 binds to the promoter region of the Blimp-1 gene (*Prdm1*) with the small Maf proteins to repress its expression (5). *Prdm1* becomes derepressed in Bach2-deficient B cells (3). While it is not clear how the derepression of *Prdm1* relates to the phenotypic changes of *Bach2*-deficient B cells, Bach2 has been shown to be a critical repressor of *Prdm1 in vivo*. Another known repressor of *Prdm1* is B cell lymphoma 6 (Bcl6) (6–8). Bcl6 is required for SHM but not for CSR (9). Therefore, Bach2 and Bcl6 may fulfill overlapping functions in B cells. Interestingly, Bach2 and Bcl6 possess a BTB domain which has been implicated in protein–protein interactions and co-repressor binding (10–12).

This study was designed to determine whether Bach2 and Bcl6 interact to regulate the repression of *Prdm1*. The results showed that the interaction of Bach2 and Bcl6 in B cells was, unexpectedly, independent of their BTB domain. In addition, reporter assays demonstrated that Bach2 and Bcl6 cooperate to repress *Prdm1* through its intron enhancer region. These results indicate that the expression of *Prdm1* is strictly repressed in B cells by the combination of two transcriptional repressors.

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## Methods

### *Cell lines and cell culture*

The 293T human kidney cell line was grown in DMEM (Sigma, St Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS). The 18-81 pre-B and WEHI231 immature B cells were maintained in Isocove's modified Dulbecco's medium (GIBCO BRL, Invitrogen, Carlsbad CA, USA) supplemented with 10% FBS, 50 μM 2-mercaptoethanol and antibiotics.

### *Plasmids*

The reporters containing the promoter Maf recognition element (MARE) of *Prdm1* (promoter-MARE-luc) have been described previously (5). The reporter containing the intron 5 Bcl6 recognition element 1 (BRE1) and MARE of *Prdm1* (intron5-luc) was generated by using *Sac*I site-containing forward primer and *Nhe*I site-containing reverse primer: 5′-AGCGAGCTCGTTAATCTGCTTTCTCGGTTTC-3′ and 5′-ATTCTAGCTAGCTCTTAAATGGCTGTAGGCGGAC-3′. The 193-bp fragment containing MARE and BRE1 was inserted between the *Sac*I and *Nhe*I sites of pGL2-TATA-luc reporter plasmid. The pCMVBach2, pEFMafK, FLAG-tagged Bach2 and Bach2ΔBTB were described previously (13, 14). The expression plasmids for FLAG-tagged Bach2 fragments were constructed as follows. C-terminal deletion fragments (Bach2N1, N2, BTB, N3 and N4) were amplified from Bach2 cDNA (13) by using a *Bam*HI site-containing forward primer and *Hin*dIII site-containing reverse primers. N-terminal deletion fragments (Bach2C1, C2, C3 and C4) were amplified using *Kpn*I site-containing forward primers and a *Hin*dIII site-containing reverse primer. The fragments were blunt ended at the *Kpn*I site. Amplified fragments were cloned in pcDNAFLAG3.1 (15). The primers were as follows: N1, 5′-GTTAAGGACCCATGTCTGTGGATGAGAAGCCT-3′ (F1) and 5′-GCAGCAGGATCCGTAAGACTGCTCACATTT-3′; N2, F1 and 5′-CCCAAGCTTTCACTGAGAGATCCCCTTGTT-3′; N3, F1 and 5′-CCCAAGCTTTCACTCCTCACTGGGGTGGCTC-3′; BTB, F1 and 5′-CCCAAGCTTTCAACAGTCCAGCTTCCTCTT-3′; C1, 5′-GCAGCAGGATCCGACTCTGAGACGGACACG-3′ and 5′-GCAGCAAAGCTTCTAGGCATAATCTTTCCT-3′; C2, 5′-CGGGTACCCACGACATCCGGAGGCGG-3′ and 5′-CCCAAGCTTCTAGGCATAATCTTTCCT-3′ (R1); C3, 5′-CGGGTACCAAGCTGGACTGTATTCAGAACTTG-3′ and R1 and C4, 5′-CGGGTACCAAGGCGTGCATGGGAGAG-3′ and R1 (initiation and stop codons are underlined). The mammalian expression vector encoding human Bcl6, pEFMycBcl6, has been previously described (10). Fragments of Bcl6 were amplified from pEFMycBcl6 (10) by using *Sac*II site-containing forward primers (BTB, ΔBTBΔZF and ZF) or an *Nco*I site-containing forward primer (ΔBTB) and *Bam*HI site-containing reverse primers. Amplified fragments were digested and gel purified for insertion into the pEFMyc mammalian expression vector. The primers were as follows: BTB, 5′-AGTCCCCGCGGATGGCCTCGCCGGCTGAC-3′ and 5′-CGGGATCCCATCTCTGCTTCACTGGCCTTAAT-3′; ΔBTB, 5′-CATGCCATGGCCACGGCTATGTAC-3′ and 5′-TCGGATCCGCAGGCTTTGGGGAGCTC-3′ (R2); ΔBTBΔZF, 5′-AGTCCCCGCGGATGGCCACGGCTATGTACCTGC-3′ and 5′-CGGGATCCGGCCCCGTTCTCACAGCTAGAATC-3′ and ZF, 5′-AGTCCCCGCGGATGTGAGAACGGGGCCTTC-3′ and R2 (initiation codons are underlined).

### *Mutagenesis*

The mutagenesis of the plasmids was performed with the Altered Sites II *in vitro* Mutagenesis System (Promega, Madison, WI, USA) according to the manufacturer's protocols using a mutagenesis primer for the intron 5 MARE (5′-CGAAAATGTGAAAGGGCATAATTAAGCC-3′, mutations underlined). Site-directed deletion of the BRE1 was performed as previously described (8).

### *Co-immunoprecipitation assay*

FLAG-tagged Bach2 plasmids and Myc-tagged Bcl6 plasmids were transfected into 293T cells using GeneJuice Transfection Reagent (Merck, Whitehouse Station, NJ, USA) according to the manufacturer's instructions. Immunoprecipitation assays were performed as described (16). Antibodies used for the immunoblot analysis were anti-Myc antibody (Medical & Biological Laboratories, Nagoya, Japan) or anti-FLAG M2 antibody (Sigma) as primary antibodies and anti-rabbit or anti-mouse Ig–HRP conjugate (Amersham Biosciences, Little Chalfont, UK) as secondary antibodies. To analyze endogenous proteins, whole-cell extracts of WEHI231 cells were pre-cleared with protein G sepharose beads at 4°C for 1 h and immunoprecipitated with anti-Bcl6 (D8) (Santacruz, SantaCruz, CA, USA) for 2 h. Immunoprecipitates were recovered with protein G sepharose beads and were washed four times. Samples were resolved on SDS–polyacrylamide gels, electrotransferred to polyvinylidene difluoride membranes and examined by an immunoblot analysis as previously described (16). Primary antibodies used were anti-Bach2 antiserum [F69-1 (13)] and anti-sentrin antibody [small ubiquitin-like modifier (SUMO)-1] (Alexis, Lausen, Switzerland).

### *Luciferase reporter assay*

The 18-81 cells were transiently transfected with various combinations of reporter and effector plasmids as described above. Each transfection was done in duplicate and the luciferase activity was measured 24 h after transfection with the Dual-Luciferase Reporter Assay Kit (Promega) according to the manufacturer's protocol. Normalized values are reported as the mean ± SD from three independent experiments.

### *Chromatin immunoprecipitation*

Chromatin fixation and purification procedures were carried out as previously described (17). Immunoprecipitations were carried out as previously described (5) using anti-MafK antiserum (A-1) or normal rabbit serum (Jackson ImmunoResearch Co., West Grove, PA, USA). The samples were analyzed by semi-quantitative PCR. The primers were as follows: intron 5 MARE, 5′-GTTAATCTGCTTTCTCGGTTTC-3′ and 5′-TCTTAAATGGCTGTAGGCGGAC-3′. The promoter MARE primers and the *mcm5* primers were the same as previously described (5).

## Results

### *Over-expressed* Bach2 *interacts with Bcl6*

When FLAG-tagged Bach2 and Myc-tagged Bcl6 were co-expressed in 293T cells, Bcl6 was co-immunoprecipitated

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with Bach2 from the 293T cell extracts (Fig. 1a). To determine the region of Bcl6 involved in the Bach2 binding, the deletion derivatives of Bcl6 (Fig. 1b) were examined for Bach2 binding. Whereas Bcl6 lacking both the BTB and Zn-finger domains was co-immunoprecipitated with Bach2, N-terminal and C-terminal fragments containing the BTB domain and Zn-finger domain, respectively, were not co-immunoprecipitated (Fig. 1a). These results indicated that the central region of Bcl6 mediated the Bach2–Bcl6 interaction. The Bcl6 derivative Bcl6ΔBTB showed a very weak interaction with Bach2 even though it contained the central region that interacted with Bcl6. A structural change may have been caused by the deletion of BTB domain, thus masking the Bach2 interaction domain.

To determine the Bach2 region required for the interaction with Bcl6, FLAG-tagged deletion derivatives of Bach2 were expressed (Fig. 2a) with the Myc-tagged central region of Bcl6 (Myc-Bcl6ΔBTBΔZF) which was sufficient for the interaction with Bach2. A deletion of the BTB domain in Bach2 did not affect the Bcl6 binding and, consistent with this, the Bach2 BTB domain did not interact with Bcl6 (Fig. 2b, Bach2BTB and ΔBTB), thus indicating that the Bach2 BTB domain was not involved in the Bach2–Bcl6 interaction. Next, immunoprecipitation assays were performed using other deletion derivatives for the C-terminal (Fig. 2c) and the N-terminal (Fig. 2d) of Bach2. A deletion derivative Bach2N1 interacted with Bcl6, although other C-terminal deletion derivatives of Bach2 failed to interact with Bcl6 (Fig. 2c). These results suggest that the region between amino acids (aa) 378 and 576 of Bach2 was required for the interaction with Bcl6. The results in Fig. 2(d) showed that a deletion derivative Bach2C1 interacted with Bcl6, while further deletion abolished the interaction with Bcl6, indicating that the region between 577 and 646 aa was also involved in the interaction with Bcl6. Taken together, these results indicated that the region between 378 and 646 aa was involved in the interaction region with Bcl6 and that this region contained two separable subregions for Bcl6 binding. No function has been previously assigned to this region.

### *Endogenous Bach2 interacts with Bcl6*

To investigate the interaction between endogenous Bach2 and Bcl6 in B cells, immunoprecipitation was performed with Bcl6 antibody using whole-cell extracts of WEHI231 immature B cells. After Immunoblotting the precipitates with the Bach2-specific antibody, two bands were detected (Fig. 3, upper panel). The weak band at ~150 kDa was determined to be Bach2. Although the predicted molecular weight of Bach2 is ~98 000, Bach2 shows slower mobility in a denaturing protein gel probably due to the presence of clustered basic aa residues on Bach2 protein (13). Interestingly, in addition to this band, a band with much slower mobility was

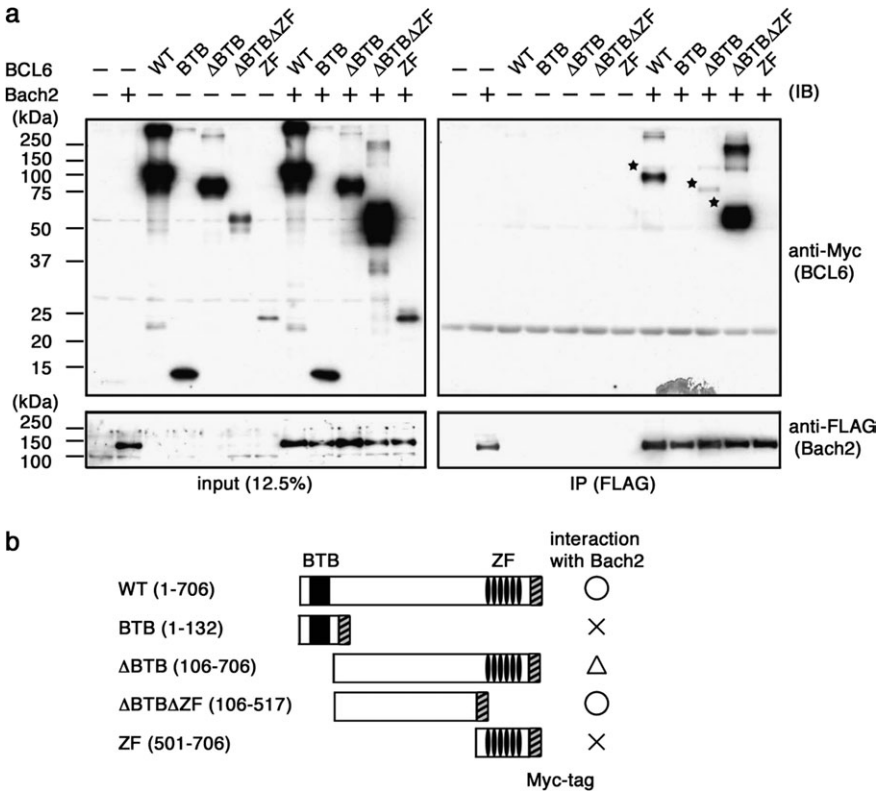


**Fig. 1.** Mapping of the Bach2-binding region on Bcl6. (a) Myc-tagged wild-type Bcl6 (BCL6WT) or mutant derivatives (Bcl6BTB, ΔBTB and ΔBTBΔZF, ZF) were expressed with FLAG-tagged Bach2 in 293T cells. Lysates were immunoprecipitated (IP) with anti-FLAG followed by immunoblotting with anti-Myc (right top) or anti-FLAG antibody (right bottom). Inputs were also analyzed (left). Specific bands are indicated with a star. (b) A schematic representation of Bcl6 deletion derivatives tagged with the Myc epitope is shown. Summary of the results are indicated: circle, clear Bach2 binding; triangle, weak binding and cross, no detectable binding.

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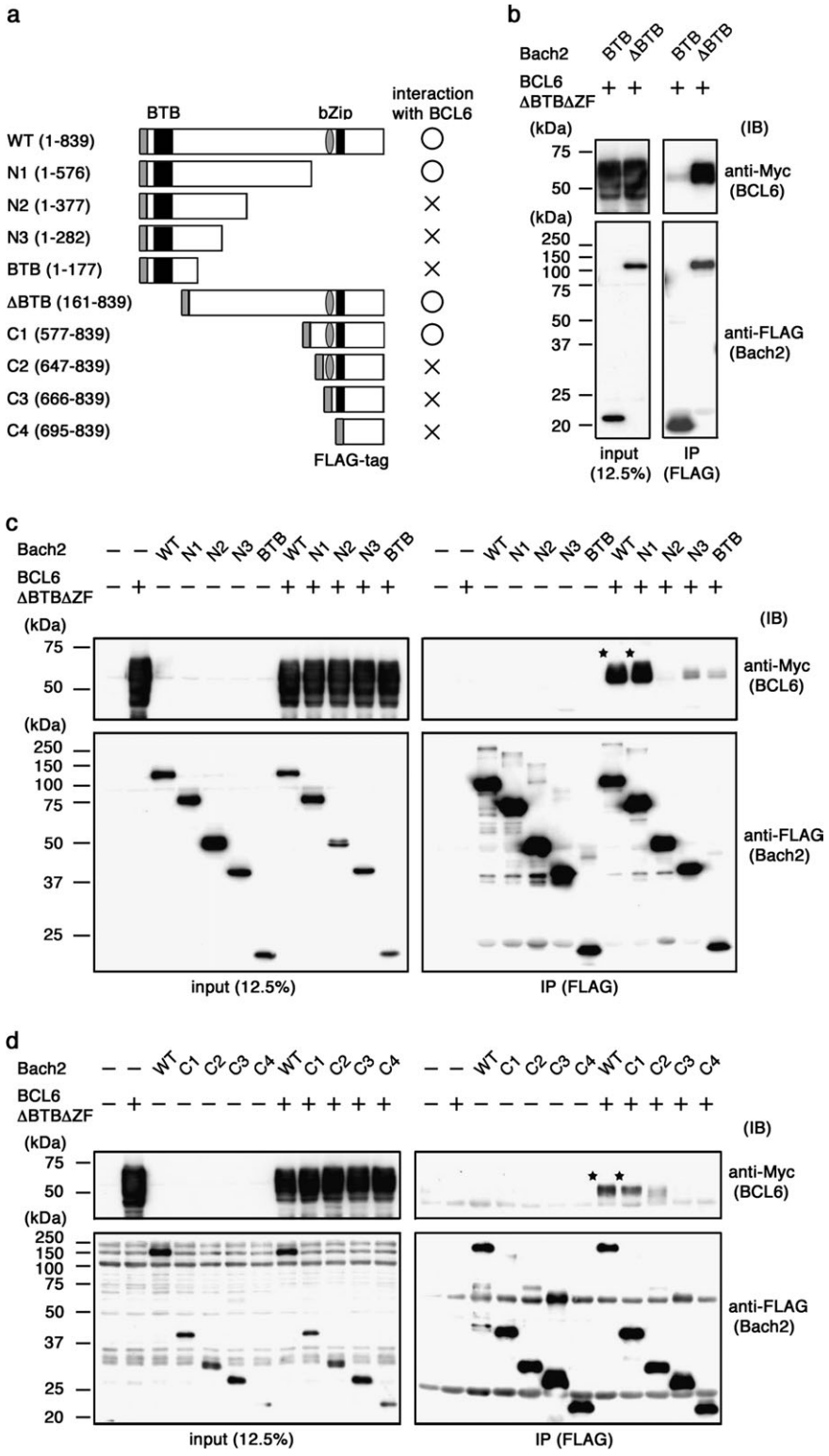


**Fig. 2.** Mapping of the Bcl6-binding region on Bach2. (a) Schematic representations of Bach2 deletion derivatives tagged with the FLAG epitope are shown. Summary of results are indicated: circle, clear Bcl6ΔBTBΔZF binding and cross, no detectable binding. (b) FLAG-tagged Bach2 mutant derivatives (Bach2BTB, ΔBTB) were expressed with Myc-tagged Bcl6ΔBTBΔZF in 293T cells. Lysates were immunoprecipitated with anti-FLAG antibody and analyzed by immunoblotting using anti-Myc (right top) or anti-FLAG (right bottom) antibodies. Inputs were also analyzed (left). (c) FLAG-tagged wild-type Bach2 or C-terminal mutant derivatives (Bach2N1, N2, N3 and BTB) were expressed with Myc-tagged Bcl6ΔBTBΔZF in 293T cells. Immunoprecipitation assays were performed as described in (b). Specific bands are indicated with a star. (d) FLAG-tagged wild-type Bach2 or N-terminal mutant derivatives (Bach2C1, C2, C3 and C4) were expressed with Myc-tagged Bcl6ΔBTBΔZF in 293T cells. Immunoprecipitation assays were performed as described in (b). Specific bands are indicated with a star.

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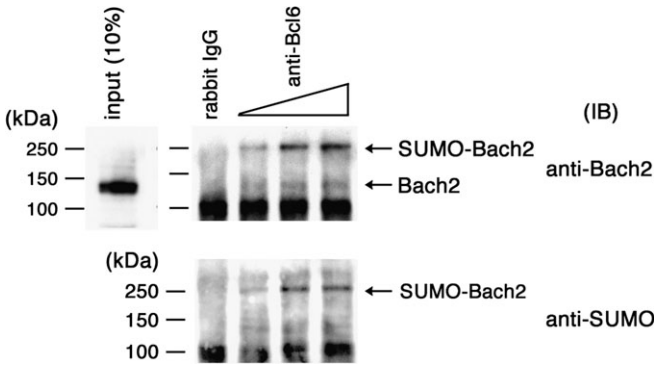


**Fig. 3.** Interaction between endogenous Bach2 and Bcl6. Whole-cell extracts from WEHI231 immature B cells were immunoprecipitated with increasing amounts of anti-Bcl6 antibody (1.5, 3.0 and 4.5 μl) or rabbit IgG and analyzed by immunoblotting using anti-Bach2 antiserum (top) or anti-SUMO antibody (bottom). Input, whole-cell extracts immunoblotted.

also detected at ~250 kDa. Since Bach2 is SUMOylated at several sites (14), this band may represent Bach2 with multiple SUMO modifications. Indeed, this band was also detected with anti-SUMO antibody (Fig. 3, lower panel). The input shows that the majority of Bach2 was not SUMOylated. In contrast, the presumptive SUMOylated Bach2 was enriched relative to non-SUMOylated Bach2 after immunoprecipitation with anti-Bcl6 antibody. SUMOylated Bach2 may bind to Bcl6 more efficiently than non-SUMOylated Bach2, or non-SUMOylated Bach2 may be readily degraded during the experimental procedure. Taken together, these results indicated that Bach2 and Bcl6 physically interact directly or indirectly with each other in B cells and that SUMOylated Bach2 may also interact with Bcl6. Because only a small fraction of Bach2 was co-precipitated with Bcl6, their interaction thus appears to be either transient or unstable.

### *Bach2 represses Prdm1 through MARE-like sequence in intron 5*

Since Bach2 represses *Prdm1* through binding to a MARE present upstream of the promoter (promoter MARE) (5), it is important to determine whether Bcl6 facilitates the repression of *Prdm1* by Bach2. First, reporter assays were performed in the 18-81 cell line representing pre-B cell stage using a reporter carrying the promoter MARE fused to a TATA box [promoter MARE-luc, (5)]. While Bach2 repressed the reporter gene depending on the presence of intact MARE as previously reported, Bcl6 showed no effect (Fig. 4a). Co-expression of Bach2 and Bcl6 did not cause further repression of the reporter gene. It has been reported that Bcl6 binds to a BRE1 in intron 5 to repress *Prdm1* gene (8). A putative MARE was found in intron 5 of *Prdm1*, which was juxtaposed to the BRE1. This intron MARE was found to be conserved between mouse and human along with the BRE1 (Fig. 4b). To investigate whether the putative MARE in the intron 5 is also a target of Bach2, a luciferase reporter was generated which carried both BRE1 and MARE (intron5-luc). For comparison, reporters that carried a deletion in the BRE1 with/without mutation in the MARE (intron5ΔBRE1-luc and intron5ΔBRE1mutMARE-luc) were also generated. Consistent with a previous report (8), the reporter containing both BRE1 and intron 5 MARE (intron5-luc) was repressed by Bcl6 in 18-81 pre-B cells (Fig. 4c). An over-expression of Bach2 also resulted in a reduced reporter gene expression. Co-expression of Bcl6 and Bach2 resulted in further repression of the reporter. The reporter intron5ΔBRE1-luc was repressed by Bach2, while it was not repressed by Bcl6. Finally, the reporter intron5ΔBRE1mutMARE-luc was repressed by neither Bcl6 nor Bach2. Co-expression of Bach2 and Bcl6 did not augment repression of the reporters intron5ΔBRE1-luc or intron5ΔBRE1mutMARE-luc. Similar results were obtained using X63/0 plasma cell line (data not shown). Bach2C2 lacking the Bcl6 interaction region failed to repress transcription of intron5-luc (Fig. 4d). It did not augment the participation of Bcl6 repressor activity, suggesting that the interaction between Bach2 and Bcl6 was important for their collaboration. When taken together, these results suggest that the intron 5 MARE can mediate transcriptional repression by Bach2 and its combination with BRE1 may ensure a further repression by Bach2 and Bcl6.

### *Cooperative repression of Ig 3′ enhancer by Bach2 and Bcl6*

While the transfection reporter assays described above demonstrated an additive effect of Bach2 and Bcl6 in repressing the Blimp-1 reporter gene, it was not clear whether the interaction between Bach2 and Bcl6 played a role in the cooperate repression. To examine the putative role of their interaction, we carried out reporter assays using another Bach2 reporter plasmid. Bach2 represses the 3′ enhancer region [locus control region (LCR)] of Ig heavy-chain gene in B cells (4). When over-expressed in X63/0 cells, Bcl6 did not show any significant repression of the reporter plasmid carrying the IgH 3′ LCR (Fig. 4e). Bach2 repressed the enhancer activity as reported previously (4). Co-expression of Bach2 and Bcl6 showed more strong repression. Synergy in repression is rather poorly understood compared with synergy in activation. However, considering that Bcl6 did not show any effect on its own, the observed effect of co-expression of Bach2 and Bcl6 appeared to reflect synergy between the two repressors. This interpretation was consistent with the idea that Bach2 and Bcl6 interacted with each other to repress target gene expression.

### In vivo *binding of MafK to MARE-like sequence in intron 5*

These results do not necessarily prove that Bach2 regulates the *Prdm1* expression through the MARE-like sequence in intron 5 in B cells. While more direct evidence would be obtained by a chromatin immunoprecipitation (ChIP) assay, there is no anti-Bach2 antibody that could be used in ChIP. Therefore, ChIP was performed using anti-MafK antibodies because previous reports established that Bach2 functions in B cells as a heterodimer with the small Maf proteins including MafK (5). Conversely, majority of MafK forms a heterodimer with Bach2 in B cells (5). Cross-linked chromatin fragments were isolated from 18-81 pre-B cells, WEHI231 immature B cells, BAL17 mature B cells and X63/0 plasma cells, which were immunoprecipitated using antiserum against MafK. Consistent with a previous report, the binding

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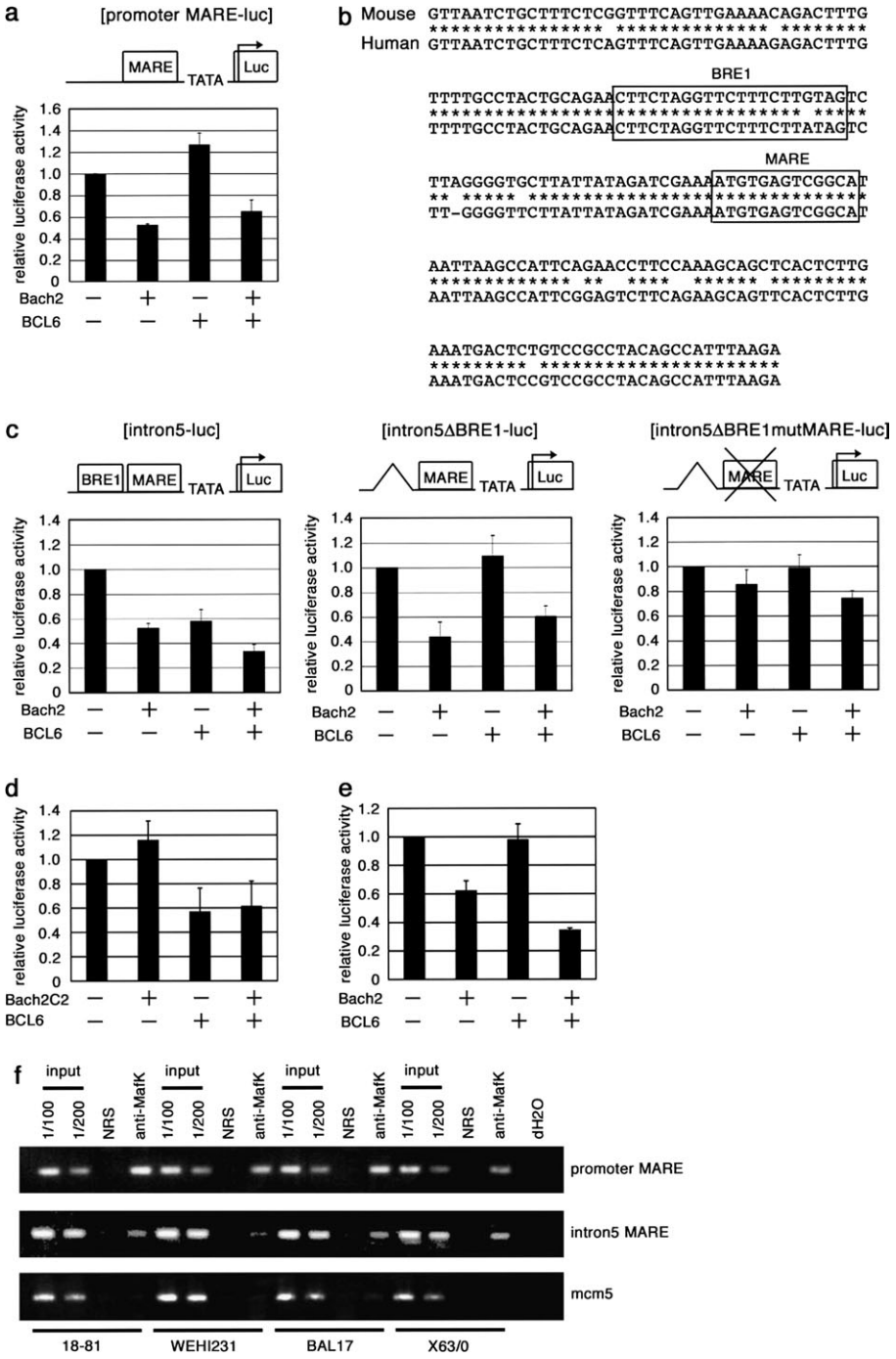


**Fig. 4.** Repression of *Prdm1*reporters by Bach2 and Bcl6. (a) 18-81 cells were transiently transfected with the promoter MARE-containing reporter and the indicated effector plasmids. (b) Comparison of portions of mouse *Prdm1*intron 5 and corresponding human *Prdm1*intron 4 containing MARE and BRE1. (c) 18-81 cells were transiently transfected with indicated reporter and effector plasmids. The amounts of plasmids were as follows: luciferase reporter (1.2 μg), Bach2 (30 ng) and Bcl6 (30 ng). The reporters used are shown above each panel. (d) Reporter assays were carried out as in (c) using the intron5-luc reporter and indicated expression plasmids. (e) Reporter assays were carried out in X63/0 cells using IgH LCR reporter and indicated expression plasmids. (f) ChIP assays of indicated cell lines using anti-MafK. NRS, samples processed with normal rabbit serum; input, PCR amplification of total chromatin fragments and $dH_2O$, distilled water.

of MafK to the promoter MARE was clearly observed in these cell lines (Fig. 4f). In addition, the binding of MafK to the intron 5 MARE was clearly observed in these cell lines irrespective of their differentiation stages, thus indicating that MafK bound to both the promoter MARE and the intron 5 MARE. These results strongly suggest that Bach2 binds to these *cis*-elements together with MafK in B cells. Considering the facts that MafK can either repress or activate transcription depending on the dimerizing partner molecules (18–20) and that Blimp-1 expression is high in X63/0 cells

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(5), MafK may bind to the *Prdm1* MARE sites as an activator by forming a heterodimer with a partner molecule other than Bach2 in X63/0 cells.

## Discussion

Blimp-1 drives plasma cell differentiation by canceling the expression of B cell-specific genes (21). Therefore, the repression of *Prdm1* in B cells appears critical for maintaining B cell differentiation stages. This study demonstrated that two repressors of *Prdm1*, Bach2 and Bcl6, interact in B cells and repress expression of the *Prdm1* reporters. A previous study reported that Bach2 represses *Prdm1* through the promoter MARE (5). In addition to the promoter MARE, the putative MARE was identified in the intron 5 of *Prdm1*. Bach2 repressed the expression of both the promoter MARE-containing reporter and the intron 5 MARE-containing reporter. In contrast, Bcl6 repressed the expression of the intron 5 MARE-containing reporter but not the promoter MARE-containing reporter. Interestingly, Bcl6 represses *Prdm1* through BRE1 at intron 5 (8) which is juxtaposed with intron 5 MARE. Finally, a ChIP assay showed that MafK bound to intron 5 MARE in B cells. Taken together, these results suggest that Bach2, together with MafK and Bcl6, represses the *Prdm1* expression by interacting with two MAREs in the promoter upstream region and at intron 5. Because the intron 5 contains both MARE and BRE1, the protein interaction between Bach2 and Bcl6 may facilitate their cooperation in repression of *Prdm1*.

While negative synergy of transcription repressors is a less well-defined concept than positive synergy of activators, a reasonable index for synergistic inhibition is a comparison of the residual activities when one or both repressors are present (22). When assayed using the intron 5 reporter plasmid (Fig. 4c), these were determined to be 50–60% for Bach2 or Bcl6 alone. When combined, the residual activity was ~30%, which was barely multiplicative. In contrast, their synergistic effect was observed when assayed using the IgH 3′ LCR reporter (Fig. 4e). It is not clear at present how these two reporters showed different responses to Bach2 and Bcl6. More physiological chromatin environment, which may not be recapitulated properly in transient reporter assays, may be necessary to assess their synergy. These results nonetheless raised the possibility that Bach2 and Bcl6 cooperated to repress target gene expression.

Synergy of transcription regulators can result from their cooperative DNA binding or from concerted action by independent mechanisms. The latter possibility can be envisaged based on the following consideration. Although both repressors possess the BTB domain which mediates protein–protein interactions, Bach2 and Bcl6 interacted in a BTB-independent manner (Figs 1 and 2). The BTB domain of Bcl6 forms a homodimer by strand-exchanged domain swapping, which then recruits the silencing mediator for retinoid and thyroid hormone receptor (SMRT)–nuclear receptor co-repressor complex (23). Because it did not involve respective BTB domains, the interaction between Bach2 and Bcl6 does not seem to interfere with the recruitment of the co-repressor complex by Bcl6. Bcl6 also recruits additional co-repressors including metastasis-associated gene 3 (MTA3), Mi-2–nucleosome remodeling and histone deacetylation complex and histone deacetylases (HDACs) (11). Bach2 interacts with the co-repressor SMRT (24). Taken together, these findings suggest that Bach2 and Bcl6 nucleate assembly of a repressive complex on the *Prdm1* locus by recruiting multiple co-repressors to the locus. While these studies do not address the role of these co-repressors in the Bach2-directed *Prdm1* repression, knock down of MTA3 or inhibition of HDAC in B cells are known to cause induction of *Prdm1* (23, 25).

Results of genetic experiments also suggest a cooperation of Bach2 and Bcl6. Both Bach2 deficiency and Bcl6 deficiency in mice result in an impaired germinal center formation (3, 26) and an increased expression of Blimp-1 in B cells. These common defects suggest that they may serve common functions in B cells. Although Bcl6 is expressed in Bach2-deficient B cells, Blimp-1 is derepressed in the absence of Bach2, thus indicating that Bach2 and Bcl6 are not redundant to each other. This supports the contention that the repression of *Prdm1* through intron 5 MARE and BRE1 in B cells requires both Bach2 and Bcl6. The observed mutual dependence can be explained by their synergy in transcription repression of *Prdm1*.

A previous study reported that Bach2 associates with promyelocytic leukemia (PML) bodies when over-expressed (14). Bcl6 is also found in PML bodies (27). PML bodies have been reported to be associated with several nuclear functions including transcriptional regulation. In the present experiments, the majority of Bach2 interacting with Bcl6 was SUMOylated (Fig. 3). Considering the fact that SUMOylation of Bach2 is essential for its recruitment to PML bodies (14), the interaction between Bach2 and Bcl6 and the repression of *Prdm1* may thus be related to the PML bodies function. Further studies are required to understand the regulation of *Prdm1* by Bach2 and Bcl6, and its biological significance, in the process of B cell differentiation.

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## Abbreviations

| | |
|---|---|
| aa | amino acids |
| Bach2 | BTB and CNC homology 2 |
| Bcl6 | B cell lymphoma 6 |
| Blimp-1 | B lymphocyte-induced maturation protein 1 |
| BRE1 | Bcl6 recognition element 1 |
| ChIP | chromatin immunoprecipitation |
| CSR | class switch recombination |
| HDAC | histone deacetylase |
| LCR | locus control region |
| MARE | Maf recognition element |
| MTA3 | metastasis-associated gene 3 |
| PML | promyelocytic leukemia |
| SHM | somatic hypermutation |
| SMRT | silencing mediator for retinoid and thyroid hormone receptor |
| SUMO | small ubiquitin-like modifier |

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