Bob1 (OCA-B/OBF-1) Differential Transactivation of the B Cell-Specific *B29* (Igβ) and *mb-1* (Igα) Promoters[1]

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The *B29* (Igβ) and *mb-1* (Igα) gene products are B cell-specific essential components of the B cell receptor that are coexpressed at all stages of B cell differentiation, with the exception of plasma cells, which lack *mb-1* expression. Transcription of both genes is governed by a similar cassette of interactive transcription factor-binding elements, including octamer motifs, in TATA-less promoters. In this study, we show the B cell-specific *B29* gene promoter is transactivated in B and non-B cells by cotransfection with the B cell-specific octamer cofactor gene, Bob1 (OCA-B/OBF-1). The expression of Bob1 is also sufficient to override the silencing effects of the *B29* silencer. This indicates that Bob1 plays a critical role in B cell-specific *B29* promoter expression. In contrast, coexpression of Bob1 had no effect on *mb-1* promoter activity. Bob1 transactivation only occurs with select octamer sequences that have an adenosine at position 5 (ATGCAAAT). The *B29* promoter conforms to this consensus octamer motif, while the *mb-1* promoter octamer motif does not. Octamer motif swapping between *B29* and *mb-1* promoters renders *B29* unresponsive to Bob1 transactivation and makes *mb-1* competent for Bob1 transactivation, thereby indicating that the *B29* octamer motif is solely responsible for Bob1 interaction. Additionally, the *mb-1* construct containing the *B29* octamer motif is expressed in a plasmacytoma cell line, while the wild-type *mb-1* promoter is not. Bob1 transactivation of *B29* and the lack of this transactivation of *mb-1* account for the differential expression of *B29* and *mb-1* in terminally differentiated plasma cells. ***The Journal of Immunology*, 2002, 168: 3369–3375.**

Bob1 (OCA-B, OBF-1) (1–4) is a B cell-specific coactivator of the octamer family members, Oct-1 and Oct-2 (1, 5). While Oct-1 and Oct-2 bind a variety of octamer motifs, the Bob1-Oct-1/Oct-2 complex binds only a select subset of these motifs. Bob1 interaction with Oct-1/Oct-2 and DNA (Bob1-POU-DNA complex) is dependent on the octamer motif sequence, specifically containing an adenine at position 5 of the octamer motif (ATGCAAAT). Octamer motifs containing a thymine at position 5 do not recruit the Bob1-POU-DNA ternary complex (6, 7). In contrast, octamer motifs that contain an adenine at position 5, but also contain a thymine at position 6 (ATGCATAT) do not recruit the Bob1-POU-DNA ternary complex (8). Furthermore, only a subset of the Bob1-POU-bound promoters is actually transactivated by this interaction. There are conflicting reports as to whether the 5′ and 3′ sequences flanking the octamer motif affect or influence the Bob1-POU-DNA complex (7, 8). Additionally, the TATA element in the promoters of Bob1-transactivated genes has been implicated as a contributing element in the action of the Bob1-Oct-1/2 complex (1).

Bob1 is essential for normal patterns of Ig expression such that $Bob1^{-/-}$ mice are crippled in their Ag-dependent responses, but show normal Ag-independent responses. Hence, Bob1 is dispensable for normal, Ag-independent B cell differentiation and B cell receptor gene expression, but is essential for Ag-dependent maturation of B cells. Specifically, the proliferative response to surface IgM cross-linking is severely impaired, as is the production of secondary Ig isotypes due to the reduced levels of transcription from normally switched Ig H chain loci (9–11).

The *B29* (Igβ) gene is strictly B cell specific and expressed at all stages of B cell differentiation (12). The *mb-1* gene is also B cell specific and is expressed at all stages of B cell development, except the plasma cell stage (13). The products of the *B29* and *mb-1* genes are essential components of the B cell receptor and play critical roles in B cell development (reviewed in Refs. 14–16). Both *B29* and *mb-1* gene transcription is controlled by TATA-less promoters containing almost identical cassettes of interactive transcription factor-binding elements that collectively impart B cell-specific expression, including essential octamer motifs (17–23). Additionally, the *B29* promoter activity is modulated by three upstream silencer elements; FROG, TOAD, and the A + T-rich octamer-binding motif that coordinately act to govern *B29* gene expression (24, 25). In this study, we show that the *B29* promoter is transactivated in B and non-B cells by concomitant expression of the octamer cofactor gene, Bob1. We also show that the *mb-1* promoter is not transactivated by Bob1 under identical conditions. This differential responsiveness was shown to be controlled by the octamer motifs in the two promoters. Together, these data suggest a role for Bob1 in determining the differential expression of *B29* and *mb-1* in plasma cells.

Materials and Methods

Cell culture conditions

All cell lines were propagated in RPMI 1640 supplemented with sodium pyruvate (Life Technologies, Gaithersburg, MD), nonessential amino acids (Life Technologies), 50 μM 2-ME (Sigma-Aldrich, St. Louis, MO), 5 mM glutamine (Sigma-Aldrich), and 5% FCS (Gemini Scientific, Tarzana, CA).

Plasmid construction and mutagenesis

B29 promoter constructs were *B29* promoters introduced into pCAT basic (Promega, Madison, WI), as described (17, 24–26) or *B29* promoters from −164 to +32 in relation to the start of transcription introduced into *Sac*I-*Hin*dIII of pGL3 basic (Promega). *mb-1* promoter constructs were *mb-1* promoters introduced into pCAT basic (Promega), as described (26), or

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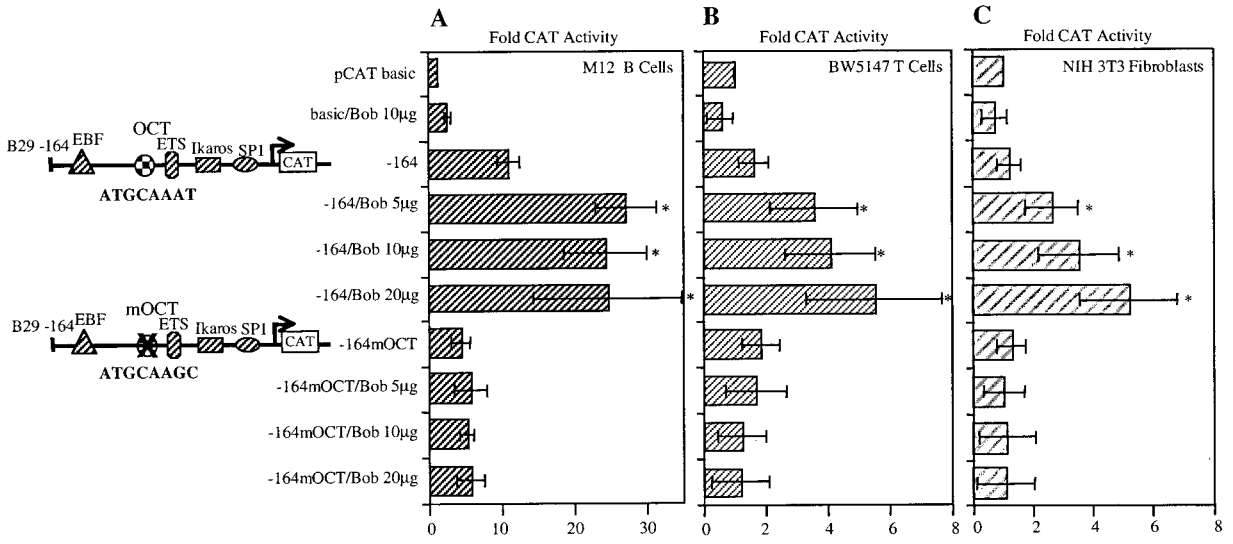

FIGURE 1. Bob1 transactivates the *B29* promoter (−164) in B cells, T cells, and fibroblasts. Transient transfections of *B29* promoter constructs with and without cotransfection of Bob1 were conducted in the M12 B cell line (*A*), BW5147 T cell line (*B*), and NIH 3T3 fibroblast cell line (*C*). The activity of each construct is expressed as the fold activation over the promoterless pCAT basic construct. Increasing amounts of Bob1 expression construct (5, 10, and 20 μg) were added to transient transfections of the *B29* promoter (−164) and the *B29* promoter with a mutated octamer motif (−164 mOCT), as indicated. CAT activities are RSV-luciferase normalized and are the average ± SD of at least four independent transfections using two preparations of DNA. *A*, The −164 value was significantly higher than pCAT basic by the Student two-sided *t* test ($p < 0.001$). Values significantly higher than −164 are denoted by an asterisk. For −164/Bob 5 μg, $p < 0.001$; for −164/Bob 10 μg, $p < 0.002$; and for −164/Bob 20 μg, $p < 0.05$. Values for −164 mOCT/Bob 5 μg, −164 mOCT/Bob 10 μg, and −164 mOCT/Bob 20 μg were not significantly higher than −164 mOCT ($p > 0.05$). *B*, The −164 values were not significantly higher than pCAT basic by the Student two-sided *t* test ($p > 0.05$). Values significantly higher than −164 are denoted by an asterisk. For −164/Bob 5 μg, $p < 0.05$; for −164/Bob 10 μg, $p < 0.05$; and for −164/Bob 20 μg, $p < 0.005$. Values for −164 mOCT/Bob 5 μg, −164 mOCT/Bob 10 μg, and −164 mOCT/Bob 20 μg were not significantly higher than −164 mOCT ($p > 0.05$). *C*, The −164 values were not significantly higher than pCAT basic by the Student two-sided *t* test ($p > 0.05$). Values significantly higher than −164 are denoted by an asterisk. For −164/Bob 5 μg, $p < 0.01$; for −164/Bob 10 μg, $p < 0.01$; and for −164/Bob 20 μg, $p < 0.001$. Values for −164 mOCT/Bob 5 μg, −164 mOCT/Bob 10 μg, and −164 mOCT/Bob 20 μg were not significantly higher than −164 mOCT ($p > 0.05$).

mb-1 promoters from −252 to +48 in relation to the start of transcription introduced into *Hin*dIII of pGL3 basic (Promega). Bob1 construct (27) was kindly provided by M. Peterlin (San Francisco, CA). Mutagenesis of the *B29* and *mb-1* octamer motifs was performed with the Quik Change kit (Stratagene, La Jolla, CA), as described (17, 24–26). Octamer motif swapping between *B29* and *mb-1* was performed by mutagenesis using the Quik Change kit (Stratagene) and the following oligonucleotides and their complements: 5′-GGGTCTCAATTTGCCATGGCAGGAAG-3′-B29 and 5′-GCCCACACATATGCAAATAAAGGGCC-3′-mb-1.

Transfections, CAT assays, and luciferase assays

M12 B cell line was transfected by the DEAE-dextran method, as described (28), using 5 μg chloramphenicol acetyltransferase (CAT)[3] plasmid and 5 μg pRSV-luciferase plasmid, or 10 μg pGL3 plasmid and 5 μg pRL SV40 plasmid. BW5147 T cell and J558L plasmacytoma cell lines were transfected by electroporation, as described (29), using 5 μg CAT plasmid and 5 μg pRSV-luciferase plasmid, or 10 μg pGL3 plasmid and 5 μg pRL SV40 plasmid. NIH 3T3 fibroblast cell line was transfected by the SuperFect (Stratagene) method using 5 μg CAT plasmid, and 5 μg pRSV-luciferase. Bob1 construct (27) was used in transfections as 5, 10, or 20 μg, as described in the figures. Total amounts of DNA transfected were equalized by the addition of pBluescript in samples that do not include Bob1 expression construct DNA. All transfections were harvested 40–48 h post-transfection. For pCAT constructs, extracts were prepared and CAT assays were performed, as described previously (17), with the exception of quantification by PhosphorImager (Molecular Dynamics, Sunnyvale, CA) analysis. Results were normalized to pRSV-luciferase values. For pGL3 constructs, dual luciferase assays were performed as described in the Dual Luciferase Reporter Assay System (Promega). All transfection values are ± SD of at least three transfections using at least two preparations of DNA. Values of *p* were calculated by the Student two-sided *t* test.

[3] Abbreviations used in this paper: CAT, chloramphenicol acetyltransferase; BSAP, B cell lineage-specific activator protein; EBF, early B cell factor; IVT, in vitro translated.

EMSA

Oct-1 and Oct-2 in vitro translate was prepared using the TNT quick coupled reticulocyte lysate system (Promega). EMSA was preformed as described (17) using 2 μl in vitro translate. EMSA probes were double-stranded oligonucleotides 5′ end labeled with [γ-^{32}P]ATP and purified by Microspin G25 columns (Amersham Pharmacia Biotech, Piscataway, NJ). EMSA complementary double-stranded oligonucleotide probes were as follows: 5′-CCTGCCATGCAAATTGAGAC-3′ (B29OCT); 5′-CCTGCCATGCAAcgTGAGAC-3′ (B29mOCT); 5′-CCTGCCATGgCAAATTGAGAC-3′ (B29(mb-1OCT)); 5′-CCACACATATGGCAAATAAAGGGCC-3′ (mb-1OCT); 5′-CCACACATATGGCAAgcAAAGGGCC-3′ (mb-1 mOCT); and 5′-CCACACATATGCAAATAAAGGGCC-3′ (mb-1(B29OCT)). Underlines represent octamer consensus.

Results

Ectopic expression of Bob1 specifically increases activity of the B29 *promoter through the octamer motif in B cells*

B29 promoter activity is dependent on a functional octamer motif, as there is significantly reduced transcriptional activity from the *B29* minimal promoter when this site is destroyed by mutagenesis (17). This critical *B29* octamer motif matches the consensus for Oct-1/2-Bob1-DNA ternary complex formation because it contains the essential "A" in position 5 of the octamer consensus (6, 7).

Transient transfections of M12 B cells with the wild-type *B29* promoter (−164) and the *B29* −164 promoter with a mutated octamer motif (−164 mOCT) were compared with transient transfections of the identical constructs along with increasing concentrations of a Bob1 expression construct. Fig. 1*A* shows that the addition of Bob1 significantly increased (up to 10-fold) the expression of the wild-type *B29* (−164) in B cells ($p < 0.05$) but did

not affect the expression of the variant *B29* (−164 mOCT) construct that cannot interact with octamer factors (17). These data suggest that the Bob1 transactivation effect is exerted specifically through the octamer motif in the *B29* −164 promoter.

Bob1 transactivates the B29 *promoter specifically through the octamer motif in non-B cells*

We next asked whether the concordant expression of Bob1 could transactivate the *B29* promoter in non-B cells and break the B cell specificity of the promoter. Fig. 1, *B* and *C*, shows that the inclusion of the Bob1 expression construct significantly (up to 5-fold) increased expression of the wild-type *B29* (−164) promoter over its negligible expression in transfections of BW5147 T cells and NIH 3T3 fibroblasts. Again, this transactivation was octamer motif dependent, as the *B29* octamer mutant (−164 mOCT) promoter showed no increase in activity with the addition of Bob1 in T cells (Fig. 1*B*) or fibroblasts (Fig. 1*C*).

Bob1 transactivation overrides the silencing effects of the TOAD, FROG, and A + T-rich motifs in the B29 *promoter*

Expression from the *B29* promoter was previously shown to be governed by three independent, but cooperative upstream silencer elements that function in B cells as well as in non-B cells (24, 25). We used our cotransfection system to determine whether Bob1 would transactivate *B29* promoter constructs containing the TOAD, FROG, and A + T-rich silencer elements. Fig. 2 shows the effects of Bob1 expression on the *B29* (−354) construct containing the TOAD silencer element and the *B29* (−565) construct containing the TOAD, FROG, and A + T-rich silencer elements in comparison with these constructs alone in M12 B cells (dark stippled bars). These results are also directly compared with the effects of Bob1 on the wild-type *B29* promoter (−164) and on the *B29* octamer mutant (−164 mOCT) (Fig. 2). Cotransfection of Bob1 with both the *B29* (−354) and (−565) silencer constructs resulted in a significant increase in activity (Fig. 2, compare −354 with −354/Bob; and compare −565 with −565/Bob). These Bob1-transactivated promoter activities (−354/Bob and −565/Bob) are not significantly different from the *B29* (−164) promoter construct (Fig. 2).

One of the 5′ silencers, the *B29* A + T-rich silencer element, is a degenerate octamer motif and is bound by Oct-1 and Oct-2 (25). This degenerate octamer site does not fit the classic octamer consensus motif, and therefore cannot be analyzed by the Bob1 rules for interaction. We tested two different constructs to illustrate that Bob1 did not transactivate through the A + T-rich octamer motif. Fig. 2 shows that when the *B29* (−164) promoter octamer motif was mutated within the *B29* (−565) promoter construct (−565 mOCT), the addition of Bob1 expression construct did not transactivate this construct. These data suggest that the A + T-rich octamer motif alone was not sufficient for Bob1 transactivation of this construct. In contrast, the complementary construct, wild-type *B29* (−164) promoter octamer motif and mutated A + T-rich octamer motif in the context of the *B29* (−565) promoter (−565 mA + T), was transactivated by Bob1 to an extent equivalent to the wild-type *B29* (−565) construct (Fig. 2). These data show that Bob1 transactivation is not mediated through the A + T-rich octamer motif. The *B29* (−565) promoter construct with both octamer sites mutated showed no significant difference from the

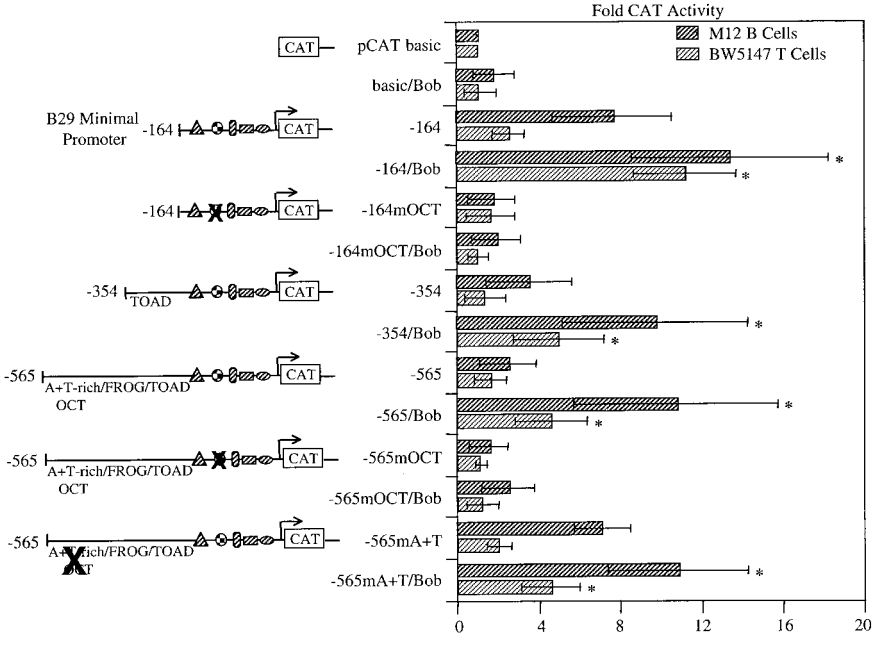

FIGURE 2. Bob1 transactivation overrides the silencing effects of the *B29* silencer elements in B and T cells. Transient transfections of *B29* promoter constructs with and without cotransfection of Bob1 were conducted in the M12 B cell line (dark hatched bars) and BW5147 T cell line (light hatched bars). The activity of each construct is expressed as the fold activation over the promoterless pCAT basic construct. A total of 10 μg Bob1 expression construct was added to transient transfections of the *B29* promoter (−164), the *B29* promoter with a mutated octamer motif (−164 mOCT), the *B29* promoter containing the TOAD silencer (−354), the *B29* promoter containing the TOAD, FROG, and A + T-rich silencers (−565), the *B29* promoter containing the TOAD, FROG, and A + T-rich silencers with a mutated octamer motif (−565 mOCT), and the *B29* promoter containing the TOAD, FROG, and A + T-rich silencers with a mutated A + T-rich motif (−565 mA + T). CAT activities are RSV-luciferase normalized and are the average ± SD of at least six independent transfections using two preparations of DNA. For the M12 B cell line, the −164 value was significantly higher than pCAT basic by the Student two-sided *t* test ($p < 0.001$). Values significantly higher than their counterparts transfected without Bob1 are denoted by an asterisk. For −164/Bob, $p < 0.01$; for −354/Bob, $p < 0.02$; for −565/Bob, $p < 0.001$; and for −565 mA + T/Bob, $p < 0.02$. The value for −565 mA + T was significantly higher than −565 ($p < 0.001$). For the BW5147 T cell line, the −164 value was not significantly higher than pCAT basic by the Student two-sided *t* test ($p > 0.05$). Values significantly higher than their counterparts transfected without Bob1 are denoted by an asterisk. For −164/Bob, $p < 0.001$; for −354/Bob, $p < 0.05$; for −565/Bob, $p < 0.001$; and for −565 mA + T/Bob, $p < 0.02$.

B29 (−164 mOCT) or the *B29* (−565 mOCT) promoter constructs when Bob1 expression constructs were cotransfected (data not shown).

The identical constructs were transfected into BW5147 T cells to determine whether the coexpression of Bob1 was sufficient to override the *B29* silencer element effects in T cells (Fig. 2, light hatched bars). Fig. 2 shows that Bob1 transactivated the *B29* (−354) and *B29* (−565) promoter constructs in T cells, breaking the B cell specificity of the *B29* promoter. These levels of Bob1 transactivation are significantly less than the transactivation seen with the *B29* (−164) promoter (Fig. 2). Again, mutation of the *B29* (−164) octamer motif in the *B29* (−565) promoter (−565 mOCT) did not allow transactivation of this construct by Bob1, and mutation of the A + T-rich octamer motif had no effect on the transactivation potential of the *B29* (−565) promoter (−565 mA + T) in T cells.

Bob1 does not transactivate mb-1 *promoter activity in either B or non-B cells*

The *mb-1* minimal promoter is strikingly similar to the *B29* −164 promoter (see Fig. 7), and is also strongly dependent on its octamer motif for maximal activity (26). Unlike the *B29* octamer motif, the *mb-1* octamer motif does not have the essential sequence required for Bob1 interaction. Specifically, the *mb-1* octamer motif lacks an adenine at position 5 of the octamer consensus (see Fig. 7). We tested the *mb-1* promoter in our Bob1 transactivation system to determine whether the *mb-1* octamer motif would interact with Bob1 and result in transactivation of the promoter. Fig. 3 shows that Bob1 did not transactivate the *mb-1* promoter in either B cells (M12 B cell line; Fig. 3, dark hatched bars) or T cells (BW5147 T cell line; Fig. 3, light hatched bars) when increasing amounts of the Bob1 expression construct were cotransfected with the wild-type *mb-1* promoter (*mb-1*) and mutant octamer *mb-1* promoter (*mb-1* mOCT) constructs. In B cells, the wild-type *mb-1* promoter showed significant activity over pCAT basic, while the addition of Bob1 did not show a significant increase in expression of this construct (Fig. 3). The mutant octamer *mb-1* promoter showed no significant activity above pCAT basic, and the addition of Bob1 did not change the expression level (Fig. 3). In T cells, neither the wild-type *mb-1* promoter nor the mutant octamer *mb-1* promoter showed activity above pCAT basic (Fig. 3). The addition of Bob1 to either of these constructs did not result in any significant transactivation of these promoters (Fig. 3). These data are consistent with the rules for Bob1 interaction and function, and suggest that in our system, Bob1 functions according to its determined specificity.

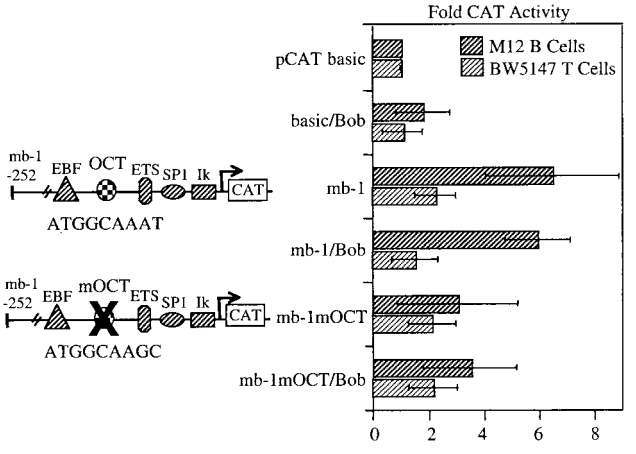

FIGURE 3. Bob1 does not transactivate the *mb-1* promoter (mb-1) in B cells or T cells. Transient transfections of *mb-1* promoter constructs with and without cotransfection of Bob1 were conducted in the M12 B cell line (dark hatched bars) and the BW5147 T cell line (light hatched bars). The activity of each construct is expressed as the fold activation over the promoterless pCAT basic construct. A total of 10 μg Bob1 expression construct was added to transient transfections of the *mb-1* minimal promoter (mb-1) and the *mb-1* minimal promoter with a mutated octamer motif (mb-1 mOCT), as indicated. CAT activities are RSV-luciferase normalized and are the average ± SD of at least six independent transfections using two preparations of DNA. For the M12 B cell line, the mb-1 value was significantly higher than pCAT basic by the Student two-sided *t* test ($p < 0.001$). The value for mb-1/Bob and mb-1 mOCT/Bob was not significantly different from their counterparts transfected without Bob1 ($p > 0.05$ each). For the BW5147 T cell line, the mb-1 value was not significantly higher than pCAT basic by the Student two-sided *t* test ($p > 0.05$). The value for mb-1/Bob and mb-1 mOCT/Bob was not significantly different from their counterparts transfected without Bob1 ($p > 0.05$ each).

Bob1 differential transactivation of the B29 *and* mb-1 *promoters is dictated solely by the octamer consensus sequence*

Based on the inability of Bob1 to transactivate the *B29*-mutated octamer site promoter (−164 mOCT) and the *mb-1* promoter (*mb-1*), we have demonstrated that the Bob1 transactivation activity functions through the octamer consensus sequence (Fig. 1). In this study, we directly show that the *mb-1* 9-bp octamer consensus sequence (ATGGCAAAT), when put into the context of the *B29* octamer site (−164(mb-1OCT)), no longer allows Bob1 transactivation of *B29* in either B or T cells (Fig. 4). In the same way, the *B29* 8-bp octamer consensus sequence (ATGCAAAT), when put into the context of the *mb-1* octamer site (mb-1(B29OCT)), confers Bob1 sensitivity and transactivation to *mb-1* in both B and T cells (Fig. 4). These data also show that even though the *mb-1* octamer site is a functional octamer site based on mutagenesis results (26), the *B29* octamer consensus when swapped with the *mb-1* consensus (mb-1(B29OCT)) confers more overall activity to the *mb-1* promoter (Fig. 4*A*). The opposite is true for *mb-1* octamer site placed in the *B29* promoter (−164(mb-1OCT)) (Fig. 4*A*). In the context of the *B29* sequences (−164(mb-1OCT)), the *mb-1* consensus does not appear to function as an octamer motif, as the activity level is similar to that seen for the *B29*-mutated octamer motif promoter (−164 mOCT) (Fig. 4*A*).

EMSAs comparing the wild-type *B29* octamer motif and the *B29* with the *mb-1* octamer consensus motif (−164(mb-1OCT)) show that the −164(mb-1OCT) oligonucleotide probe shows less binding to in vitro translated (IVT) Oct-1 as compared with wild-type *B29* octamer oligonucleotide probes (Fig. 5*A*), possibly reflecting intrinsic differences in the *B29* and *mb-1* octamer sites. In contrast, the *mb-1* with the *B29* octamer consensus motif (mb-1(B29OCT)) showed greater binding to IVT Oct-1 than the wild-type *mb-1* octamer motif (Fig. 5*B*). This result may explain why the mb-1(B29OCT) construct has higher activity in transient transfections than the wild-type *mb-1* promoter construct (Fig. 4).

Bob1 responsiveness determines promoter activity in terminally differentiated plasma cells

Previous studies have shown that the *mb-1* promoter has no activity in transient transfection of plasma cell lines (19, 23), while B29 has high activity (18). We tested our octamer site swap constructs (−164(mb-1OCT) and mb-1(B29OCT)) in J558L plasmacytoma cell line to determine whether the change in the octamer sites alone would change the expression patterns of the *B29* and *mb-1* promoters. Fig. 6 shows that the Bob1-responsive *B29* octamer site conferred activity onto the *mb-1* promoter (mb-1(B29OCT)) in plasmacytoma cells. Additionally, the Bob1-unresponsive *mb-1* octamer site shut down expression of the *B29* promoter (−164(mb-1OCT)) in

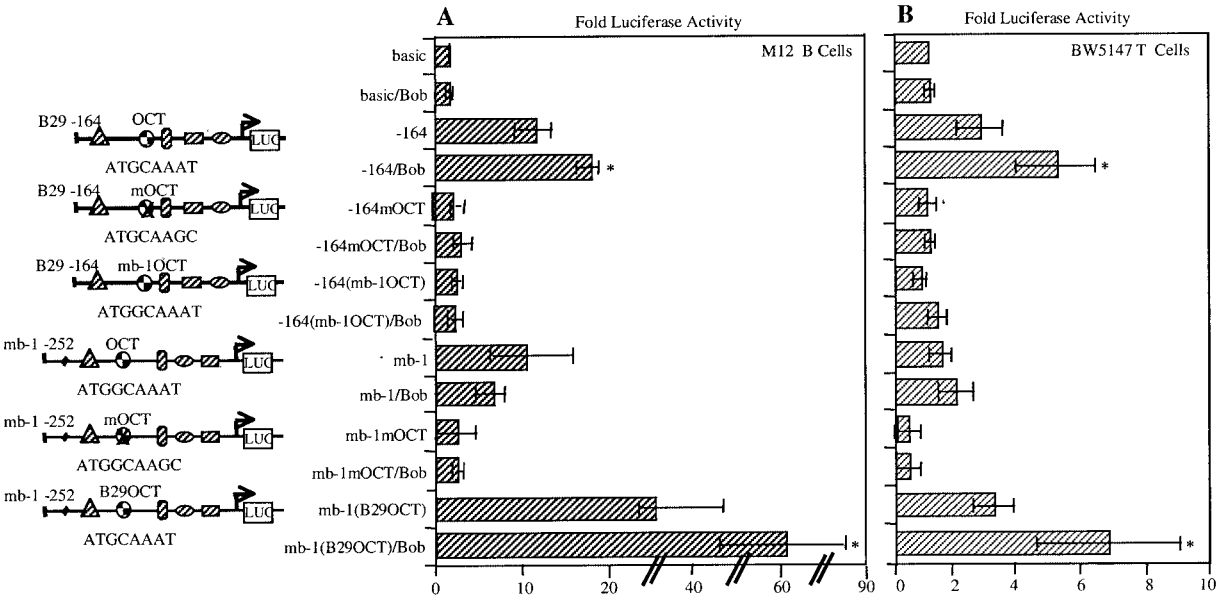

FIGURE 4. Bob1 differential transactivation of the *B29* and *mb-1* promoters is dictated solely by the octamer consensus sequence in B cells and T cells. Transient transfections of *B29* and *mb-1* promoter constructs with and without cotransfection of Bob1 were conducted in the M12 B cell line (*A*) and BW5147 T cell line (*B*). The activity of each construct is expressed as the fold activation over the promoterless pGL3 luciferase basic construct. A total of 10 μg Bob1 expression construct was added to transient transfections of the *B29* minimal promoter (−164), the *B29* minimal promoter with a mutated octamer motif (−164 mOCT), the *B29* minimal promoter with the octamer motif replaced with the mb-1 octamer consensus (−164(mb-1OCT)), the *mb-1* minimal promoter (mb-1), the *mb-1* minimal promoter with a mutated octamer motif (mb-1 mOCT), and the *mb-1* minimal promoter with the octamer motif replaced with the *B29* octamer consensus (mb-1(B29OCT)), as indicated. *B29* and *mb-1* promoter luciferase construct values were pRL SV40 normalized and are the average ± SD of at least four independent transfections using two preparations of DNA. *A*, For the M12 B cell line −164 value and the mb-1 value were significantly higher than pGL3 basic by the Student two-sided *t* test ($p < 0.001$). Values significantly higher than their counterparts transfected without Bob1 are denoted by an asterisk. For −164/Bob, $p < 0.02$; and for mb-1(B29OCT)/Bob1, $p < 0.05$. The values for −164 mOCT/Bob, −164(mb-1OCT)/Bob, mb-1/Bob, and mb-1 mOCT/Bob were not significantly higher than their counterparts transfected without Bob1 ($p > 0.05$ each). The value for mb-1(B29OCT) was significantly higher than mb-1 ($p < 0.001$). *B*, For the BW5147 T cell line, values significantly higher than their counterparts transfected without Bob1 are denoted by an asterisk. For −164/Bob, $p < 0.05$; and for mb-1(B29OCT)/Bob1, $p < 0.02$. The values for −164 mOCT/Bob, −164(mb-1OCT)/Bob, mb-1/Bob, and mb-1 mOCT/Bob were not significantly higher than their counterparts transfected without Bob1 ($p > 0.05$ each). The value for mb-1(B29OCT) was significantly higher than mb-1 ($p < 0.01$).

plasmacytoma cells. These data suggest that the octamer site alone controls differential expression of the *B29* and *mb-1* promoters in terminally differentiated plasma cells. Interestingly, ectopic Bob1 expression had no significant effect on the activity of any of the *B29* or *mb-1* promoter constructs in plasma cells (Fig. 6). The increased level of endogenous Bob1 expression in J558L (4-fold over M12 Bob1 expression) could preclude any further effect by ectopically expressed Bob1 (data not shown) (30).

Discussion

This study shows that the *B29* promoter octamer motif is a target for Bob1 transactivation, while the *mb-1* promoter octamer motif is not. Both *B29* and *mb-1* are TATA-less promoters that rely on a virtually identical cassette of transcription factor motifs for activity (see Fig. 7). Early reports of Bob1 specificity suggested a role for the TATA box in controlling which promoters were targets for Bob1 (1). Our data showing transactivation of the TATA-less *B29* promoter by Bob1 suggest that other criteria must be responsible for selective Bob1 transactivation in the context of TATA-less promoters.

Our study describes a new mechanism for the differential expression patterns of *B29* and *mb-1* in B cell development. The *B29* gene is expressed throughout B cell development (31), while the *mb-1* gene is only expressed through B cell development up to the plasma cell stage (19, 20). An early hypothesis purported that the transcription factor, early B cell factor (EBF), controlled the differential expression of *B29* and *mb-1* (19, 20). This inference was based on the identical expression profiles of *mb-1* and EBF (19, 20, 32, 33), and the apparent lack of interaction of EBF and the *B29* promoter (17). Recently, it was shown that EBF did interact with the *B29* promoter (18), and that *B29* is not expressed in $EBF^{-/-}$ knockout mice (34). In light of these discoveries, EBF cannot account for the differential expression of *B29* and *mb-1*. B cell lineage-specific activator protein (BSAP; Pax5) was shown to interact with the *mb-1* promoter (21, 22). Like EBF, BSAP is not expressed in terminally differentiated plasma cells (35). Unlike EBF, BSAP does not appear to affect *B29* promoter activity because normal levels of B29 expression were seen in $BSAP^{-/-}$ mice (22), and the *B29* promoter sequence does not contain a BSAP binding site (17). The lack of BSAP in plasma cells may contribute to the loss of *mb-1* expression in addition to the unresponsiveness of the *mb-1* promoter to Bob1 transactivation.

The concordant expression of Bob1 and *B29* naturally points to a role for Bob1 in *B29* gene expression. Our evidence for the differential interaction of Bob1 with the *B29* and *mb-1* promoters poses a plausible explanation for the continued expression of *B29* and for the extinction of *mb-1* in the last stage of B cell development. Our study showed that Bob1 transactivated the *B29* promoter, while Bob1 was unable to affect expression from the *mb-1* promoter. These data support our proposal that while a lack of BSAP may contribute to the extinction of *mb-1*, the presence of Bob1 is responsible for the continued expression of *B29* in plasma cells. Data showing that Bob1 promoter expression and Bob1 protein levels are highest in plasma cells (30) support our proposal

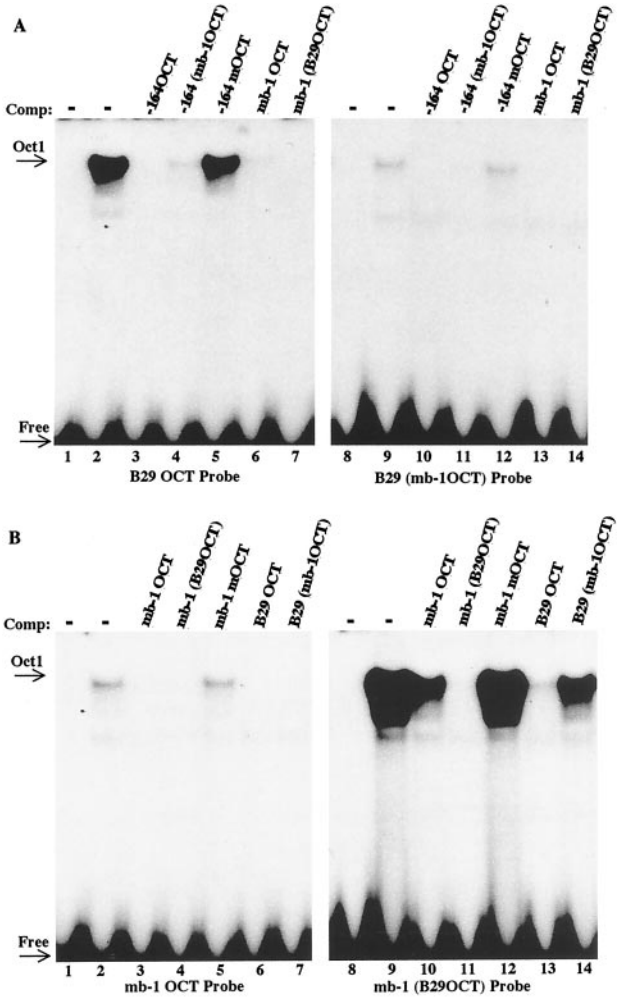

FIGURE 5. Swapping the B29 and mb-1 octamer consensus sites results in altered Oct-1 binding in EMSA. Double-stranded oligonucleotides corresponding to the octamer consensus sequence swaps between B29 and mb-1 from constructs shown in Fig. 4 and their wild-type counterpart double-stranded oligonucleotides were end labeled and used in EMSA. *A*, Wild-type B29 octamer motif probe (−164 OCT, *left panel*) compared with the mb-1 9-bp octamer consensus sequence in the context of the 20-bp B29 octamer motif probe (−164(mb-1OCT), *right panel*) taken from the same exposure of the identical gel. *Lane 1*, The B29 octamer motif probe (−164 OCT) alone; *lanes 2–7*, the B29 octamer motif probe (−164 OCT) incubated with 2 μl IVT Oct-1. Probe was also coincubated with 500-fold molar excess of unlabeled B29 octamer motif (−164 OCT, *lane 3*), the mb-1 octamer consensus sequence in the context of the B29 octamer site probe (−164(mb-1OCT), *lane 4*), mutant B29 octamer motif (−164 mOCT, *lane 5*), mb-1 octamer motif (mb-1 OCT, *lane 6*), and the B29 octamer consensus site in the context of the mb-1 octamer motif (mb-1(B29OCT), *lane 7*). *Lane 8*, The mb-1 octamer consensus sequence in the context of the B29 octamer site probe (−164(mb-1Oct)) alone; *lanes 9–14*, the mb-1 octamer consensus sequence in the context of the B29 octamer site probe (−164(mb-1OCT)) incubated with 2 μl IVT Oct-1. Probe was also coincubated with 500-fold molar excess of unlabeled B29 octamer motif (−164 OCT, *lane 10*), the mb-1 octamer consensus sequence in the context of the B29 octamer site probe (−164(mb-1OCT), *lane 11*), mutant B29 octamer motif (−164 mOCT, *lane 12*), mb-1 octamer motif (mb-1 OCT, *lane 13*), and the B29 octamer consensus site in the context of the mb-1 octamer motif (mb-1(B29OCT), *lane 14*). *B*, Wild-type mb-1 octamer motif probe (mb-1 OCT, *left panel*) compared with the B29 8-bp octamer consensus sequence in the context of the 25-bp mb-1 octamer motif probe (mb-1(B29OCT), *right panel*) taken from the same exposure of the identical gel. *Lane 1*, The wild-type mb-1 octamer motif probe (mb-1 OCT, *left panel*) alone; *lanes 2–7*, the mb-1 octamer motif probe (mb-1 OCT) incubated with 2 μl IVT Oct-1. Probe was also coincubated with 500-fold molar excess of unlabeled mb-1 octamer motif (mb-1 OCT, *lane 3*), B29 octamer consensus sequence in the context of the mb-1 octamer site probe (mb-1(B29OCT), *lane 4*), mutant mb-1 octamer motif (mb-1 mOCT, *lane 5*), B29 octamer motif (−164 OCT, *lane 6*), and mb-1 octamer consensus sequence in the context of the B29 octamer site probe (−164(mb-1OCT), *lane 7*). *Lane 8*, The B29 octamer consensus sequence in the context of the mb-1 octamer site probe (mb-1(B29OCT)) alone; *lanes 9–14*, the B29 octamer consensus sequence in the context of the mb-1 octamer site probe (mb-1(B29OCT) incubated with 2 μl IVT Oct-1. Probe was also coincubated with 500-fold molar excess of unlabeled mb-1 octamer motif (mb-1 OCT, *lane 10*), B29 octamer consensus sequence in the context of the mb-1 octamer site probe (mb-1(B29OCT), *lane 11*), mutant mb-1 octamer motif (mb-1 mOCT, *lane 12*), B29 octamer motif (−164 OCT, *lane 13*), and mb-1 octamer consensus sequence in the context of the B29 octamer site probe (−164(mb-1OCT), *lane 14*). The specifically formed Oct-1 complex is denoted by an arrow. Free, Free probe.

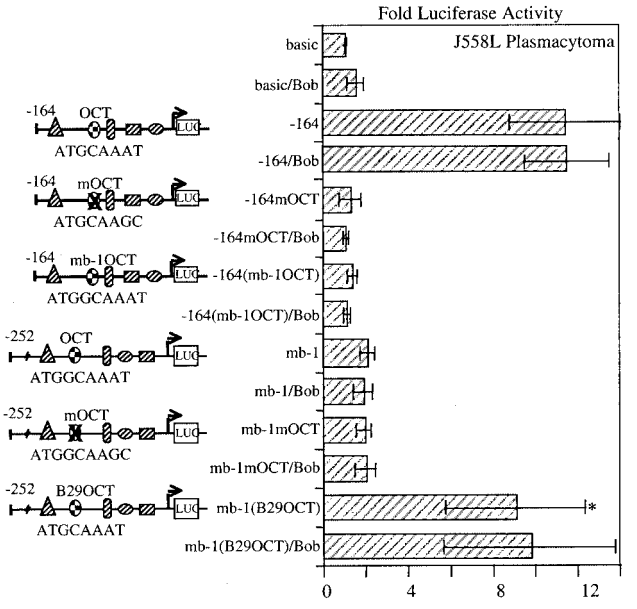

FIGURE 6. Bob1 responsiveness determines promoter activity in terminally differentiated plasma cells. Transient transfections of *B29* and *mb-1* promoter constructs with and without cotransfection of Bob1 were conducted in the J558L plasmacytoma cell line. The activity of each construct is expressed as the fold activation over the promoterless pGL3 luciferase basic construct. A total of 10 μg Bob1 expression construct was added to transient transfections of the *B29* minimal promoter (−164), the *B29* minimal promoter with a mutated octamer motif (−164 mOCT), the *B29* minimal promoter with the octamer motif replaced with the mb-1 octamer consensus (−164(mb-1OCT)), the *mb-1* minimal promoter (mb-1), the *mb-1* minimal promoter with a mutated octamer motif (mb-1 mOCT), and the *mb-1* minimal promoter with the octamer motif replaced with the *B29* octamer consensus (mb-1(B29OCT)), as indicated. *B29* and *mb-1* promoter luciferase construct values were pRL SV40 normalized and are the average ± SD of at least four independent transfections using two preparations of DNA. Values significantly above those for wild-type constructs are denoted with an asterisk. For mb-1(B29OCT), $p < 0.01$. Wild-type B29 promoter construct (−164) was significantly higher than basic ($p < 0.001$).

that Bob1 transactivation supports ongoing *B29*, but not *mb-1* gene expression in plasma cells.

Our data specifically showed that Bob1 was a potent transactivator of *B29* in the lymphoid and nonlymphoid cell types tested, and that this effect was mediated specifically through the octamer motif. Equal amounts of Bob1 expression construct showed no effect on the activity of the *mb-1* promoter, even though the *mb-1* octamer site is functional and has been shown to be required for

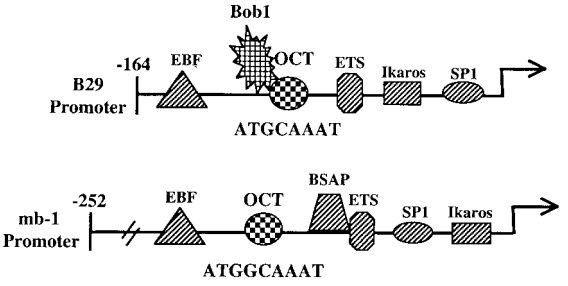

FIGURE 7. *B29* and *mb-1* promoter transcription factor motif comparison. The identical transcription factors control both *B29* and *mb-1* promoter activity, with the exception of the recruitment of BSAP to the ETS site in the *mb-1* promoter and the differential usage of the Bob1 coactivator with the octamer motifs.

maximal *mb-1* promoter activity (26). We directly showed that the sequences responsible for Bob1 transactivation lie within the octamer consensus sequence. The *B29* and *mb-1* octamer consensus site swap constructs showed that Bob1 transactivation is mediated by the *B29* consensus octamer site (ATGCAAAT), regardless of whether the sequences outside this site were derived from *B29* or *mb-1*. Additionally, the octamer swap constructs demonstrated that the *B29* consensus octamer site controlled the plasma cell-specific activity of the promoters; the mb-1 promoter alone had little activity in plasma cells, while the *mb-1* with the *B29* octamer consensus site (mb-1(B29OCT)) had significantly greater activity than the wild-type *mb-1* promoter. In fact, the mb-1(B29OCT) promoter had activity that was not significantly different from the wild-type *B29* promoter in plasma cells. Furthermore, the *B29* with the Bob1-nonresponsive *mb-1* octamer consensus site (B29(mb-1OCT)) had little activity in plasma cells. These data support our proposal that Bob1 expression in plasma cells controls the differential expression of the *B29* and *mb-1* promoters.

Our data showing that Bob1 overrides the effects of the *B29* silencer elements in B cells and non-B cells suggest that Bob1 also plays a critical role in regulating B cell expression of the *B29* gene. The *B29* silencer elements have been shown to govern *B29* expression, but not control cell type specificity because they are equally active in both B and non-B cells (24). The expression of Bob1 may act to negate the effects of the B29 silencers in B cells in which Bob1 and *B29* are expressed. In this mechanism, Bob1 would act as an antisilencer countering the 5′ *B29* silencers, thereby controlling cell type specificity of the *B29* gene and restricting *B29* expression to B cells only. This combination of *cis*-acting silencers and a *trans*-acting transcriptional coactivator functioning as an antisilencer represents a novel mechanism for controlling B cell gene specificity.

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