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An essential octamer motif in the *mb-1* (Igα) promoter

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Abstract

The *mb-1* (Igα) gene is B cell-specific and expressed throughout B cell maturation. In combination with *B29* (Igβ) and surface immunoglobulin *mb-1* comprises the B cell receptor complex (BCR). The murine *mb-1* promoter has been characterized to depend on the trans-acting transcription factors; Sp1, ets, lkaros, and EBF for full promoter activity. These trans-acting factors are also involved in the regulation of *mb-1*'s closely related heterodimeric partner, *B29*. However, octamer transcription factors 1 and 2 (Oct-1 and Oct-2) are also necessary for full *B29* promoter activity while they are not known to be required for *mb-1* promoter activity. Here, we show that the octamer transcription factors bind a degenerate octamer consensus sequence within the *mb-1* promoter. Like *B29*, the *mb-1* octamer-binding, motif interacted with both ubiquitously expressed Oct-1 and the B cell-specific Oct-2 transcription factors. Furthermore, the interaction of Oct-1 and Oct-2 contributed to the regulation of the *mb-1* promoter as site-directed mutations within the octamer motif substantially reduced its activity. These data confirm that octamer factor interactions and function contribute to the full transcriptional activity of the *mb-1* promoter.

Keywords: *mb-1*; Igα; B29; Igβ; Oct-1; Oct-2; Transcription; Gene regulation; Promoter

1. Introduction

The B cell receptor complex (BCR) is composed of the Ig molecule associated with the heterodimeric complex between *mb-1* (Igα) and *B29* (Igβ) (reviewed in Cambier et al., 1993; Gong and Nussenzweig, 1996; Roth and DeFranco, 1996). The *mb-1* and *B29* gene products are similar in both structure and function. Both mb-1 and B29 are cell surface glycoproteins with single extracellular immunoglobulin-like domains, transmembrane domains, and immunoreceptor tyrosine activation motif (ITAM)-containing intracellular cytoplasmic tails. The mb-1/B29 heterodimer is responsible for all the signaling activities associated with the BCR (reviewed in Cambier, 1992). Additionally, several events in B cell development have been attributed to mb-1/B29 heterodimer activity such as allelic exclusion (Manz et al., 1988; Papavasiliou et al., 1995) and surface translocation of the BCR (Hombach et al., 1988; Campbell and Cambier, 1990; Venkitaraman et al., 1991; Papavasiliou et al., 1995).

B cells are dependent on *Ig* μ, *Ig* κ, *B29* and *mb-1* gene expression for differentiation from multipotential progenitor cells to antibody-secreting plasma cells (reviewed in Blackwell and Alt, 1988; Cooper and Burrows, 1989). The *mb-1* and *B29* genes, unlike the *Ig* genes, do not contain a TATA box to determine the start site of transcription, and consequently, initiate transcription at multiple sites (Hermanson et al., 1989; Travis et al., 1991). Additional lymphoid genes that lack a TATA box and initiate transcription from multiple start sites include *λ5* (Kudo et al., 1987; Yang et al., 1995), *VpreB* (Kudo and Melchers, 1987), *Ets-1* (Chen et al., 1993), *bcl-2* (Young and Korsmeyer, 1993), *pp52* (Omori et al., 1997), *CD11a* (Shelley et al., 1993), *CD18* (Agura et al., 1992), *CD19* (Zhou et al., 1992; Kehrl et al., 1994) *CD20* (Rieckmann et al., 1991; Kehrl et al., 1994), and *CD22* (Wilson et al., 1993; Kehrl et al.,

Abbreviations: BCR, B cell receptor; BSAP, B cell specific activator protein; CAT, chloramphenicol acetyltransferase; EBF, early B cell factor; EMSA, electrophoretic mobility shift assay; Ig, immunoglobulin; ITAM, immunoreceptor tyrosine activation motif.

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1994). The promoters of these genes share many of the same cis-acting transcription factor binding sites that interact with factors such as NF-κB, AP-1, Sp1, Ets, Ikaros, early B cell factor (EBF), B cell specific activator protein (BSAP) (Pax-5), and Octamer factors. Interestingly, *mb-1* and *B29* share identical transcription factor motifs within their minimal promoters with two exceptions. Sp1, Ets, Ikaros, and EBF have been shown to be important for both *mb-1* (Hagman et al., 1991; Travis et al., 1991; Feldhaus et al., 1992) and *B29* (Omori and Wall, 1993; Akerblad et al., 1999) B cell-specific gene regulation. Octamer factors have only been shown to interact with and be critical for *B29* promoter activity (Omori and Wall, 1993) and BSAP has only been sown to interact with and be critical for mb-1 promoter activity (Fitzsimmons et al., 1996; Nutt et al., 1998). The *B29* gene and the *Ig* μ and *Ig* κ gene promoters have been shown to require the octamer factor binding motif for activity (Singh et al., 1986; Wirth et al., 1987; Muller et al., 1988; Scheidereit et al., 1988; Staudt et al., 1988; Omori and Wall, 1993). *mb-1*, *Ig* μ, and *Ig* κ require BSAP for expression as shown by the lack of these gene transcripts in BSAP$^{-/-}$ mice while *B29* gene expression is uneffected by a lack of BSAP expression (Nutt et al., 1998). No single control element has been shown to solely direct B cell specificity in any of these promoters (reviewed in Ernst and Smale, 1995).

Because of the striking similarities between the structures, functions, and promoters of the *mb-1* and *B29* genes, we investigated whether the degenerate octamer consensus sequence (ATGCAAAT) at -113 of the *mb-1* minimal promoter functions as an octamer-binding motif in regulating *mb-1* promoter activity. Oligomers encompassing the *mb-1* octamer motif formed complexed with B cell unclear extracts in electrophoretic mobility shift assay (EMSA). The identities of the interacting proteins at this site within the *mb-1* promoter were confirmed to be Oct-1 and Oct-2 with antibodies and in vitro translated Oct-1 and Oct-2 proteins. This Oct-1 and Oct-2 binding motif was determined to be important for *mb-1* promoter activity. Mutations within the *mb-1* octamer site that abrogated Oct-1 and Oct-2 binding also significantly decreased *mb-1* promoter activity. These data demonstrate that the *mb-1* promoter contains a functional octamer factor-binding motif that significantly influences *mb-1* promoter activity.

2. Materials and methods

2.1. Plasmid construction and mutagenesis

The *mb-1* promoter fragment -252 to $+48$ (kind gift of Dr Rudolf Grosschedl) was inserted into the pCAT basic plasmid (Promega, Madison, WI, USA) upstream of the chloramphenicol acetyltransferase (CAT) gene. The endogenous *mb-1* ATG was destroyed by site-directed mutagenesis, leaving the translation start site as the first methionine codon of the CAT gene. The *mb-1* construct with a mutated octamer motif (mb-1 mOct) was created using the quick-change site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) and the complimentary oligonucleotides, 5′-CCACACATATGGCAAgcAAAGGGCCAGGAG-3′.

2.2. DNA transfections and CAT assays

The B cell line M12 was transfected by the DEAE-dextran method (Grosschedl and Baltimore, 1985). Cells were cotransfected with 5 μg of pCAT reporter plasmid and 2 μg of pRSV-luciferase. Extracts were prepared and assayed as described (Omori and Wall, 1993) and were quantitated by Phosphorimager analysis (Molecular Dynamics, Sunnyvale, CA, USA). Results were normalized to luciferase activity and are the averages of at least three independent transfections using at least two preparations of DNA.

2.3. Nuclear extracts and DNA-binding assays

Preparation of crude nuclear extracts from M12 cells was previously described (Lo et al., 1991). EMSA was performed as described (Omori and Wall, 1993) with modifications of 2 μg poly(dl-dC) and analysis in 4.5% of 60:1 polyacrylamide:bis-acrylamide; 0.25X TBE (22-mM Tris base, 7-mM boric acid, 0.5-mM EDTA pH 8) gels at 125 V for 2.5 h at room temperature. Oct-1 and Oct-2 in vitro translate was prepared using the TNT coupled reticulocyte lysate system (Promega). Oct-1, Oct-2, and Bob1 antibodies and Oct-1 and Oct-2 blocking peptides (Santa Cruz Biotechnology, Santa Cruz, CA, USA) used in EMSA were incubated in the binding reaction at 4°C overnight. EMSA probes were double stranded oligonucleotides 5′ end-labeled with [γ^{32}P]ATP and purified by G25 sephadex (Sigma Chemicals, St. Louis, MO, USA) spin column chromatography. EMSA complimentary double-stranded oligonucleotide probes were as follows,

5′-CCACACATATGGCAAATAAAGGGCC-3′ (mb-1 Oct);
5′-CCACACATATGGCAAgcAAAGGGCCAG-GAG-3′ (mb-1 mOct);
5′-GAGAGAGACTCAAGGGAATTGTG-GCCAGCC-3′ (mb-1 EBF);
5′-TGTCGAATGCAAATCACTAGAA-3′ (Octamer, Santa Cruz Biotechnology);
5′-TGTCGAATGCAAgcCACTAGAA-3′ (mOctamer, Santa Cruz Biotechnology).

3. Results

3.1. The mb-1 promoter contains a degenerate octamer-binding motif

Both the *mb-1* and *B29* gene promoters are regulated through the activity of an almost identical cassette of transcription factor elements including Ets, Ikaros, Sp1, and EBF (Hagman et al., 1991; Travis et al., 1991; Omori and Wall, 1993). The EBF gene was originally thought only to regulate the *mb-1* gene, and not the *B29* gene, but recent evidence has demonstrated that EBF is also involved in *B29* gene regulation (Akerblad et al., 1999). This discovery narrowed the differences between the trans-acting factors affecting the *B29* gene promoter and the *mb-1* gene promoter. Upon further inspection of the *mb-1* gene promoter sequence, we noted a degenerate octamer-binding site sequence was found at −113 from the major start site of transcription +1 (Fig. 1A). This putative *mb-1* octamer sequence differed from the octamer consensus sequence, ATGCAAAT, by one additional base pair at position 4, ATGGCAAAT.

Oligonucleotides were designed to encompass the *mb-1* putative-octamer-binding site to determine the ability of this site to specifically interact with B cell nuclear extract proteins. Fig. 2 shows EMSA using M12 B cell nuclear extracts and the putative *mb-1* octamer motif oligonucleotides (Fig. 2A) or the consensus octamer motif oligonucleotide (Fig. 2B). Fig. 2A clearly shows a specific *mb-1* DNA-protein interaction as the putative *mb-1* octamer motif competes protein complex formation (lane 3) and the mutated form of the putative *mb-1* octamer motif did not compete complex formation (lane 4), even when used as 500-fold

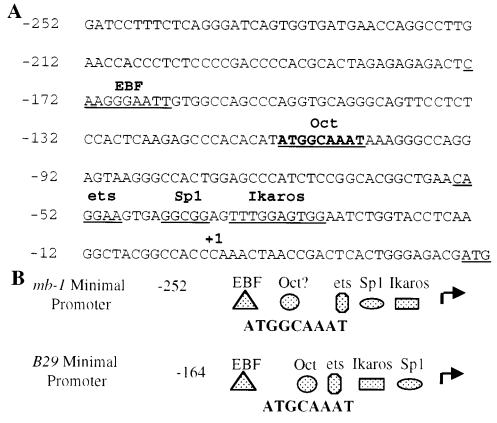

Fig. 1. The *mb-1* promoter sequence contains a putative-octamer-binding motif. (A) Sequence of the promoter region of the *mb-1* gene from −252 to +31. The major start site of transcription is denoted as (+1) and transcription-factor-binding sites are underlined. The putative-octamer-binding motif is underlined and bolded at position −113. (B) Comparison of the transcription-factor-binding motifs and octamer motif sequences in the *mb-1* and *B29* promoter regions.

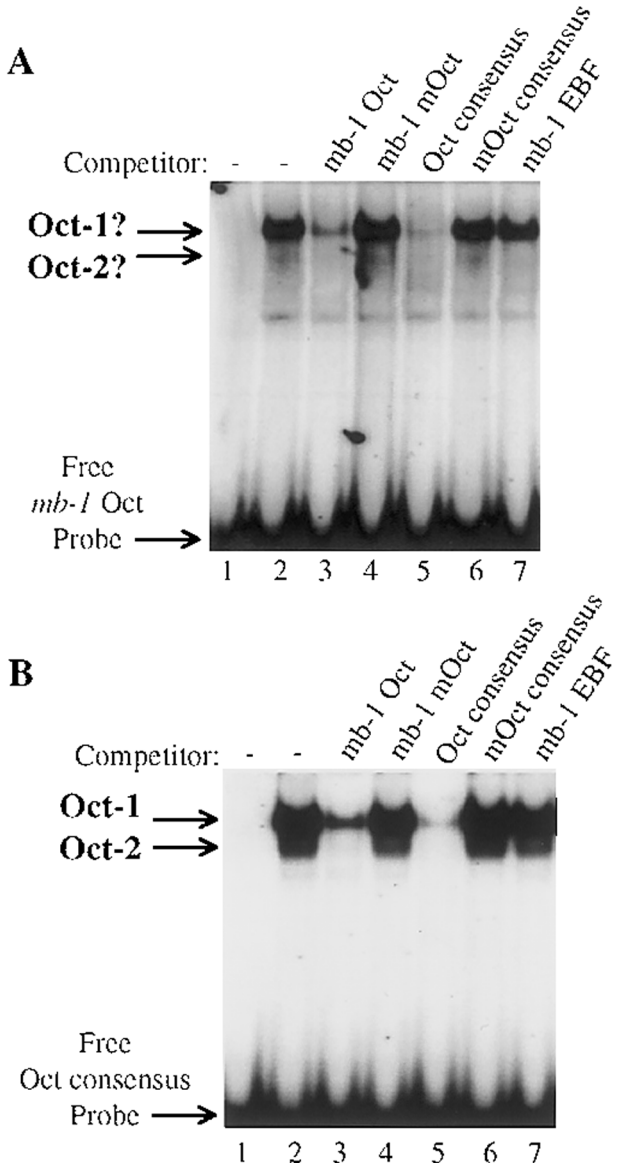

Fig. 2. The *mb-1* promoter putative-octamer-binding motif interacts with DNA-binding proteins. (A) Double-stranded oligonucleotides corresponding to the *mb-1* promoter sequence from positions −121 to −97 (mb-1 Oct motif) were end-labeled and used in EMSA. Lane 1 contains *mb-1* octamer motif probe alone while lanes 2–7 contain *mb-1* octamer motif probe incubated with 20 μg M12 mature B cell nuclear extracts. Probe was also co-incubated with 500-fold excess of unlabeled *mb-1* octamer motif (mb-1 Oct, lane 3), mutant *mb-1* octamer motif (mb-1 mOct, lane 4), consensus octamer motif (Oct consensus, lane 5), mutant consensus octamer motif (mOct consensus, lane 6), and *mb-1* early B cell factor motif (mb-1 EBF, lane 7). Specifically formed Oct-1 and Oct-2 complexes are as indicated by arrows. (B) Double-stranded oligonucleotides corresponding to the consensus octamer motif (Oct consensus motif) were end-labeled and used in EMSA. Lane 1 contains consensus octamer motif probe alone while lanes 2–7 contain consensus octamer motif probe incubated with 20 μg M12 nuclear extract. Probe was also co-incubated with 500-fold excess of unlabeled *mb-1* octamer motif (mb-1 Oct, lane 3), mutant *mb-1* octamer motif (mb-1 mOct, lane 4), consensus octamer motif (Oct consensus, lane 5), mutant consensus octamer motif (mOct consensus, lane 6), and *mb-1* early B cell factor motif (mb-1 EBF, lane 7). Specifically formed Oct-1 and Oct-2 complexes are as indicated by arrows. Results in A and B are representative of at least three independent experiments.

excess cold competitors. Additionally, the *mb-1* EBF motif was used as a nonspecific 500-fold cold competitor to further illustrate the specificity of this protein complex (Fig. 2, lane 7).

To determine if octamer transcription factors were interacting with this putative *mb-1* octamer-binding motif, we used the consensus octamer-binding motif and the mutant form of the consensus octamer motif as 500-fold cold competitors in EMSA. Consensus octamer competitor abrogated the putative *mb-1* octamer motif DNA-protein complex while the mutant form of the octamer consensus had no effect on the complex formation (Fig. 2A, lanes 5 and 6, respectively). These data suggest that an octamer family member(s) may be responsible for the DNA-protein complex seen by EMSA with the putative *mb-1* octamer motif.

For comparison of the *mb-1* octamer motif, we used the consensus octamer motif oligonucleotide as a probe in EMSA using M12 B cell nuclear extracts. Fig. 2B shows the identical pattern of binding and competition using the consensus octamer motif probe for what appears to be Oct-1 and Oct-2 in the *mb-1* octamer

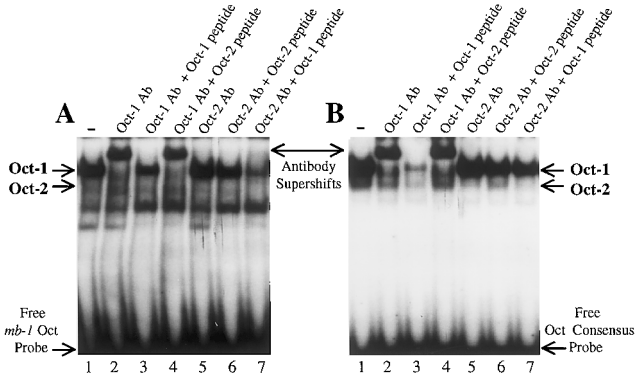

Fig. 3. Antibodies specific for Oct-1 and Oct-2 supershift complexes using the *mb-1* octamer motif probe in EMSA. (A) Double-stranded oligonucleotides corresponding to the *mb-1* promoter sequence from positions −121 to −97 (mb-1 Oct motif) were end-labeled and used in EMSA. Lanes 1–7 contain *mb-1* octamer motif probe incubated with 20 μg M12 nuclear extract. Reactions were co-incubated in the presence of 2 μg anti-Oct-1 antibody (lane 2); 2 μg anti-Oct-1 antibody and 2 μg Oct-1 blocking peptide (lane 3); 2 μg anti-Oct-1 antibody and 2 μg Oct-2 blocking peptide (lane 4); 2 μg anti-Oct-2 antibody (lane 5); 2 μg anti-Oct-2 antibody and 2 μg Oct-2 blocking peptide (lane 6); 2 μg anti-Oct-2 antibody and 2 μg Oct-1 blocking peptide (lane 7). Specifically formed complexes are indicated by arrows. (B) Double-stranded oligonucleotides corresponding to the consensus octamer motif (Oct consensus motif) were end-labeled and used in EMSA. Lanes 1–7 contain consensus octamer motif probe incubated with 20 μg M12 nuclear extract. Reactions were co-incubated in the presence of 2 μg anti-Oct-1 antibody (lane 2); 2 μg anti-Oct-1 antibody and 2 μg Oct-1 blocking peptide (lane 3); 2 μg anti-Oct-1 antibody and 2 μg Oct-2 blocking peptide (lane 4); 2 μg anti-Oct-2 antibody (lane 5); 2 μg anti-Oct-2 antibody and 2 μg Oct-2 blocking peptide (lane 6); 2 μg anti-Oct-2 antibody and 2 μg Oct-1 blocking peptide (lane 7). Specifically formed complexes are indicated by arrows. Results in A and B representative of at least three independent experiments.

motif probe. These specific complexes seen in Fig. 2A were retarded to the same degree as the Oct-1 and Oct-2 complexes using the consensus octamer probe when the both *mb-1* and consensus octamer probes were run side-by-side in EMSA (data not shown). Competition experiments were also performed with both *mb-1* octamer motif probe and consensus octamer motif probe using 100- and 1000-fold molar excess cold oligonucleotides. These experiments indicated similar levels of competition as the 500-fold competitors showed in the above experiments; the 100-fold specific competitor (mb-1 Oct and Oct consensus) showed slightly less competition and the 1000-fold specific competitor showed slightly greater competition while nonspecific competitors (mb-1 mOct, mOct consensus, and mb-1 EBF) did not compete binding at any molar excess tested (data not shown).

3.2. The mb-1 octamer motif binds both Oct-1 and Oct-2 transcription factors from B cell nuclear extracts

Comparison between the *mb-1* octamer motif probe and the consensus octamer motif probe suggested that the *mb-1* octamer motif may interact with Oct-1 and Oct-2 (Fig. 2A and B, respectively); Antibodies specific for Oct-1 and Oct-2 were used in supershift EMSA in order to confirm these factor interactions. EMSA using the *mb-1* octamer motif probe and M12 B cell nuclear extracts with the addition of Oct-1 antibody shows a supershifted complex (Fig. 3A, lane 2). This complex was specifically competed by Oct-1 blocking peptide (Fig. 3A, lane 3) but not by Oct-2 blocking peptide (Fig. 3A, lane 4), illustrating the specificity of the antibody for Oct-1. The addition of Oct-2-specific antibody resulted in less distinct results but suggests the presence of Oct-2 in the complex as shown by a modest supershifted complex in Fig. 3A, lanes 5 (Oct-2 antibody) and 7 (Oct-2 antibody and Oct-1 blocking peptide). The addition of Oct-2 blocking peptide in Fig. 3A, lane 6 did not result in any super shifted complex suggesting a specific Oct-2 interaction.

Consensus octamer motif probe was used again for comparison and illustrated the nature of the Oct-2 antibody effect. Fig. 3B shows identical antibodies and blocking peptides in order to corroborate the interaction of Oct-2 with the *mb-1* octamer motif probe. Here, the faster migrating Oct-2 complex was more easily discernible; however, the addition of Oct-2-specific antibody resulted in not only a less distinct supershifted complex, but also a more apparent competition of the Oct-2 complex than was seen using the *mb-1* octamer motif probe (Fig. 3B, lanes 5 and 7; Fig. 3A, lanes 5 and 7, respectively). These data suggest that using Oct-2 antibodies in EMSA under these conditions resulted in a slight supershifted complex regardless of the probe used.

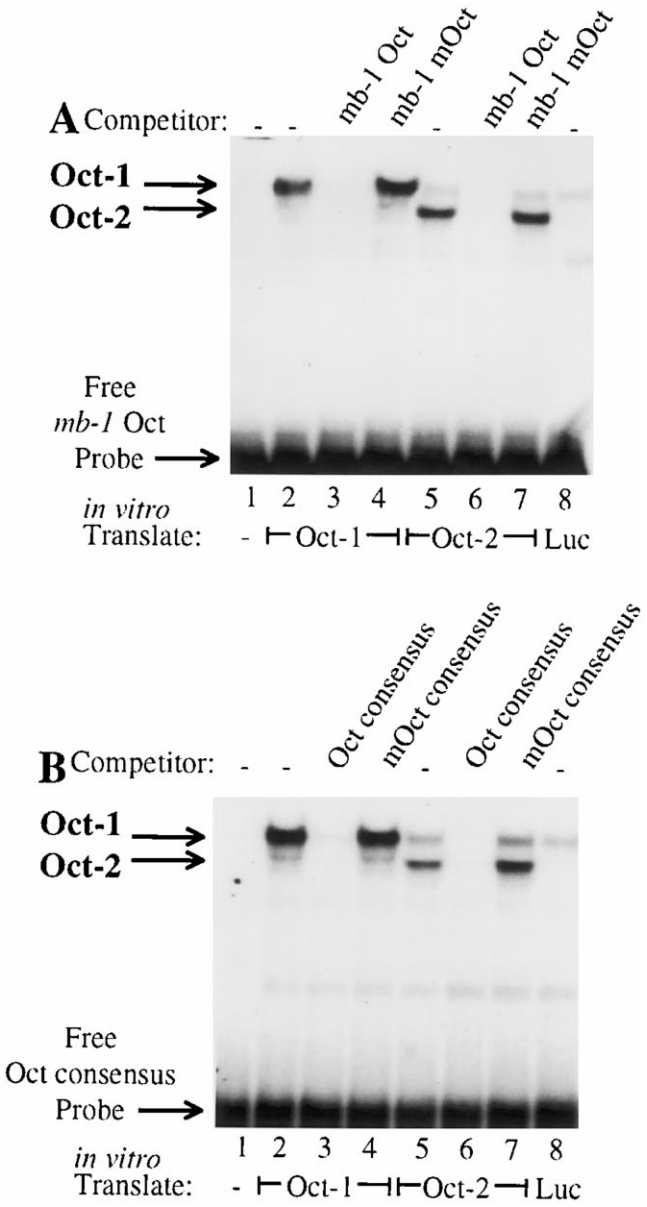

Fig. 4. (*Continued*)

3.3. mb-1 Promoter degenerate octamer site binds in vitro translated Oct-1 and Oct-2 protein

To further confirm the interaction of Oct-1 with the *mb-1* octamer motif probe and to clarify the apparent Oct-2 interaction with the *mb-1* octamer motif probe, we used in vitro translated Oct-1 and Oct-2 in EMSA. A specific protein-DNA interaction using in vitro translated Oct-1 and Oct-2 with the *mb-1* octamer motif probe was identical to the specific protein-DNA complexes seen in EMSA using the consensus octamer motif probe (Fig. 4A and B). Oct-1 complexes formed between the in vitro translated Oct-1 and the *mb-1* octamer motif probe were specifically competed by 500-fold cold *mb-1* octamer motif competitor oligonucleotides (Fig. 4A, lane 3) while this complex was not competed by the cold mutated form of the *mb-1* octamer motif competitor oligonucleotides (Fig. 4A, lane 4). Oct-2 complexes were clearly formed between the in vitro translated Oct-2 and the *mb-1* octamer motif probe (Fig. 4A, lane 5) in a specific manner, as shown by the specific competition using 500-fold cold *mb-1* octamer motif competitor oligonucleotides (Fig. 4A, lane 6) and no competition when suing the cold mutated form of the *mb-1* octamer motif competitor oligonucleotides (Fig. 4A, lane 7). These data are consistent with the results seen using the consensus octamer motif probe in EMSA with the in vitro translated Oct-1 and Oct-2 proteins (Fig. 4B). Faint bands seen in Oct-2 in vitro translate lanes migrating at equivalent distance as Oct-1 in the Oct-1 in vitro translate lanes were Oct-1 protein found in the rabbit reticulocyte extracts used in the in vitro transcription and translation of the Oct-1, Oct-2, and control luciferase proteins (Fig. 4A, lanes 5, 7, and 8; Fig. 4B, lanes 5, 7, and 8). These rabbit reticulocyte Oct-1 complexes were specifically competed both by the *mb-1* octamer motif competitor oligonucleotides (Fig. 4A, lane 6), and the consensus octamer motif competitor oligonucleotides (Fig. 4B, lane 6). The appearance of these rabbit reticulocyte Oct-1 complexes have been reported previously (Strubin et al., 1995).

3.4. mb-1 Octamer site is essential for full mb-1 promoter activity in B cells

We next tested the contribution of the octamer-factor-binding motif to *mb-1* promoter activity. The two

Fig. 4. The *mb-1* promoter octamer motif interacts with in vitro translated Oct-1 and Oct-2 proteins. (A) Double-stranded oligonucleotides corresponding to the *mb-1* promoter sequence from positions −121 to −97 (mb-1 Oct motif) were end-labeled and used in EMSA. Lane 1 contains *mb-1* octamer motif probe alone while lanes 2–4 contain *mb-1* octamer motif probe incubated with in vitro translated Oct-1 lanes 5–7 contain *mb-1* octamer motif probe incubated with in vitro translated Oct-2, and lane 8 contains *mb-1* octamer motif probe with in vitro translated luciferase control (Luc). Probe was also co-incubated with 500-fold excess of unlabeled *mb-1* octamer motif (mb-1 Oct, lanes 3 and 6) and mutant *mb-1* octamer motif (mb-1 mOct, lanes 4 and 7). Specifically formed Oct-1 and Oct-2 complexes are as indicated by arrows. (B) Double-stranded oligonucleotides corresponding to the consensus octamer motif (Oct consensus motif) were end-labeled and used in EMSA. Lane 1 contains consensus octamer motif probe alone while lanes 2–4 contain consensus octamer motif probe incubated with in vitro translated Oct-1, lanes 5–7 contain consensus octamer motif probe incubated with in vitro translated Oct-2, and lane 8 contains consensus octamer motif probe with in vitro translated luciferase control (Luc). Probe was also co-incubated with 500-fold excess of unlabeled consensus octamer motif (Oct consensus, lanes 3 and 6) and mutant consensus octamer motif (mOct consensus, lanes 4 and 7). Specifically formed Oct-1 and Oct-2 complexes are as indicated by arrows. Results in A and B are representative of at least three independent experiments.

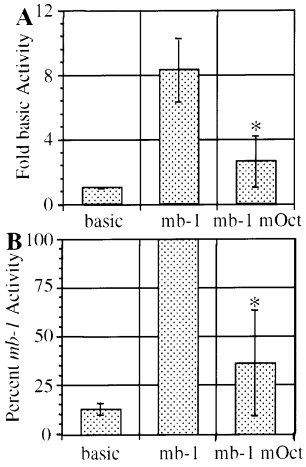

Fig. 5. Site-directed mutagenesis of the *mb-1* octamer motif affirms that this site is essential for maximal *mb-1* promoter activity in B cells. Transient transfections of the *mb-1* promoter with introduced point mutations in the *mb-1* octamer motif were carried out in the M12 B cell line. Mutagenized nucleotides are indicated by lowercase script. Wild type sequence of the *mb-1* octamer motif within the *mb-1* promoter -252 to $+48$ is 5′-CCACACATATG-GCAAATAAAGGG-3′ (mb-1 construct). Mutated sequence of the *mb-1* octamer motif within the *mb-1* promoter -252 to $+48$ is 5′-CCACACATATGGCAAgcAAAGGGCCAGGAG-3′ (mb-1 mOct construct). CAT activities are RSV-luciferase normalized and are the average $\pm$ standard deviation (S.D.) of four independent transfections using at least three different preparations of DNA. (A) The activity of each construct is expressed as fold increase over the CAT activity obtained with the promoter-less pCAT basic construct (basic). (B) The activity of each construct is expressed as a percentage of the CAT activity obtained with the *mb-1* promoter -252 to $+48$ with wild type octamer motif (mb-1) set at 100%. *P value ≤ 0.005.

engineered point mutations in the *mb-1* degenerate octamer-binding motif that prevented Oct-1 and Oct-2 binding in EMSA were tested for functional activity in transient transfection of M12 B cells in the context of the entire *mb-1* minimal promoter (-252). These mutations (mb-1 mOct construct) resulted in a significant 5-fold reduction (P value ≤ 0.005) in *mb-1* minimal promoter activity (Fig. 5A). The remaining activity of the *mb-1* octamaer motif mutant account for only 35% of the wild type *mb-1* minimal promoter, suggesting an important role for the Oct-1 and Oct-2 binding motif in the regulation and activity of the *mb-1* minimal promoter (Fig. 5B). Clearly, full *mb-1* promoter activity is dependent strongly on the *mb-1* octamer motif.

4. Discussion

In the present study, we show that the *mb-1* gene promoter is dependent on a previously unrecognized octamer-binding motif for maximal gene expression. We tested the degenerater octamer motif in the *mb-1* promoter and showed that it is functional and interacts with Oct-1 and Oct-2 factors. The *mb-1* promoter octamer-binding motif differs from the consensus octamer-binding site by a one base pair insertion at position 4 (ATGGCAAAT *mb-1* promoter versus ATGCAAAT consensus octamer motif). EMSA using B cell nuclear extracts, specific octamer competitors, Oct-1 and Oct-2-specific antibodies, and in vitro translated Oct-1 and Oct-2 proteins established the interaction of Oct-1 and Oct-2 with the *mb-1* octamer-binding motif. These Oct-1 and Oct-2 *mb-1* octamer motif interactions were consistently less robust than octamer consensus motif interactions in EMSA using equivalent amounts of probe and extracts (data not shown). Considering that the *mb-1* promoter octamer motif is not a perfect octamer consensus site, it is plausible that Oct-1 and Oct-2 would not interact with this site as strongly as with a consensus octamer site. However, the *mb-1* promoter octamer motif was shown to be essential for maximal promoter activity as evidenced by a 65% reduction in *mb-1* promoter activity with mutations that eliminated *mb-1* octamer factor binding. These results are similar to the reductions reported for mutated *B29* promoter octamer motif constructs (Omori and Wall, 1993), indicating that the octamer motifs appear to be equally important in the context of both promoters. These data, confirming a role for Oct-1 and Oct-2 in the regulation of the *mb-1* promoter, bring *mb-1* into the circle of BCR genes regulated by octamer transcription factors.

Oct-1 and Oct-2 are known to interact with a specific co-factor, Bob1 (OCA-B, OBF-1), that accentuates Oct-1 and Oct-2 trans-activation when these interact with certain octamer-binding motifs (reviewed in Graef and Crabtree, 1997; Matthias, 1998). The addition of the octamer cofactor Bob1 (OCA-B, OBF-1)-specific antibodies to EMSA using the *mb-1* octamer-binding motif had no effect on complex formation and did not result in any supershifted complexes (data not shown). These data are consistent with the specific sequence requirements for Bob1 interaction with Oct-1 and/or Oct-2. Bob1 trans-activation requires a specific octamer factor binding motif sequence where the fifth nucleotide in the motif must be an A (Cepek et al., 1996; Gstaiger et al., 1996). The *mb-1* octamer-binding motif has a C at position 5 and, therefore, does not qualify as a likely candidate for Bob1 interaction, consistent with the lack of interaction using Bob1-specific antibody in EMSA. In addition, ectopic expression of Bob1 in transient transfections of the *mb-1* promoter in M12 B cells did not increase in promoter activity (data not shown). In contrast, we have found that Bob1 is a potent trans-activator in conjunction with Oct-1 and Oct-2 binding to the canonical octamer motif in the minimal *B29* promoter (Malone et al., manuscript in preparation).

This study demonstrates that the *mb-1* promoter contains an essentially identical complement of cis-acting promoter regulatory elements as the *B29* promoter (i.e. Oct, Ets, Ikaros, Sp1, and EBF). The minor differences in promoter binding elements, BSAP recruited by ets in the *mb-1* promoter (Fitzsimmons et al., 1996) and Bob1 interaction with Oct-1 and Oct-2 in the B29 promoter (Malone et al., manuscript in preparation), may contribute to the subtle developmental regulatory differences between these two genes; namely, the extinction of *mb-1* expression and the continuation of *B29* expression in terminally differentiated plasma cells. The differential regulation of these genes in plasma cells was attributed originally to the lack of the EBF factor which is expressed throughout B cell maturation and is, then, extinguished at the plasma cell stage (Hagman et al., 1991, 1993; Feldhaus et al., 1992; Milatovich et al., 1994), mirroring the expression of *mb-1* (Sakaguchi et al., 1988) but not *B29* (Hermanson et al., 1988). The cis-acting elements originally defined in the *mb-1* promoter (Ets, Ikaros, Sp1, EBF) and the *B29* promoter (Oct, Ets, Ikaros, Sp1) were consistent with the proposition that EBF controlled the difference in developmental expression of *mb-1* and *B29*. In this proposition, the *mb-1* promotor would be active only in B cell developmental stages that express EBF, whereas the *B29* promotor could remain active after EBF shut down in plasma cells. However, $EBF^{-/-}$ mice do not express either mb-1 or B29, consistent with an EBF requirement for both genes (Lin and Grosschedl, 1995). Additionally, the *B29* promoter was recently shown to be a direct target of EBF (Akerblad et al., 1999). The lack of expression of both BSAP (Pax-5) in fully differentiated plasma cells may account for the loss of *mb-1* expression in these cells. Like EBF, BSAP is not expressed in terminally differentiated plasma cells (Barberis et al., 1990). BSAP has been shown to recruit ets proteins to the cis-acting ets binding site within the *mb-1* promoter (Fitzsimmons et al., 1996) and to be necessary for *mb-1* expression (Nutt et al., 1998). Unlike EBF BSAP does not appear to affect *B29* promoter activity since normal levels of *B29* expression were seen in $BSAP^{-/-}$ mice (Nutt et al., 1998), and the *B29* promoter sequence does not contain a BSAP binding motif (Omori and Wall, 1993). The differential interactions of the *mb-1* and *B29* octamer motifs with Bob1 also provides another plausible explanation for the continued expression of the *B29* gene, but not the *mb-1* gene, in plasma cells. Bob1-mediated transactivation of the *B29* promoter may support continued *B29* expression in plasma cells by compensating for the lack of EBF factor activity. Without Bob1 binding and interaction, the *mb-1* promoter may be unable to overcome the loss of the critical transcription factors, EBF and BSAP.

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