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OCA-B was identified as a B cell-specific coactivator that functions with either Oct-1 or Oct-2 to mediate efficient cell type-specific transcription via the octamer site (ATGCAAAT) both in vivo and in vitro. Mice lacking OCA-B exhibit normal Ag-independent B cell maturation. In contrast, Ag-dependent functions, including production of secondary Ig isotypes and germinal center formation, are greatly affected. To better understand OCA-B expression and, ultimately, the defects observed in the OCA-B knockout mice, we have cloned the OCA-B promoter and examined its function in both transformed and primary B cells. We show here that the OCA-B promoter is developmentally regulated, with activity increasing throughout B cell differentiation. Through physical and functional assays, we have found an activating transcription factor/cAMP response element binding protein binding site (or cAMP response element) that is crucial for OCA-B promoter activity. Furthermore, we demonstrate that IL-4 and anti-CD40 induce both the OCA-B promoter and octamer-dependent promoters, thus implicating OCA-B in B cell signaling events in the nucleus. The Journal of Immunology, 2000, 164: 6372–6379.

The octamer site (5'-ATGCAAAT-3') is a conserved sequence motif found in a number of eukaryotic promoters. For Ig genes, the octamer site is critical for the B cell specificity of the heavy and light chain variable region promoters, as well as the heavy chain gene intrinsic enhancer, as shown by in vitro transcription, transfection, and transgenic experiments (1–4). The importance of the octamer site in B cell-specific activation is further reinforced by data showing that octamer function is conserved evolutionarily (5).

A large variety of octamer-binding transcription factors have been cloned, and these include the ubiquitous Oct-1 and B cell-restricted Oct-2, which are related through a conserved DNA-binding protein structure motif called the POU domain (6, 7). Initially, the cell-specific expression of Oct-2 and various in vivo and in vitro functional assays led to the idea that the B cell-enriched Oct-2 was responsible for octamer-dependent activation of Ig genes in B lymphocytes, but more detailed biochemical analyses clearly demonstrated that this was not the case. In fact, in vitro transcription data indicated that Ig promoters function equally well with either the B cell-restricted Oct-2 or the ubiquitous Oct-1 and that B cell-specificity is actually due to a novel Oct-interacting cofactor, designated OCA-B, found only in B lymphocytes (Ref. 8; reviewed in Ref. 9).

The biochemical identification of OCA-B was followed by cDNA cloning using both biochemical purification and protein-protein interaction screening methods (9–12). In agreement with previous biochemical data, recombinant OCA-B was shown to interact physically with either Oct-1 or Oct-2 through their highly related POU domains and to activate transcription from Ig promoters in combination with either Oct-1 or Oct-2. Although the isolated POU domains of Oct-1 or Oct-2 are sufficient to recruit OCA-B to the octamer site, the POU-OCA-B complex is unable to stimulate transcription significantly in the absence of Oct-1 or Oct-2 activation domains (9). Similarly, structure/function analyses have demonstrated that OCA-B possesses an intrinsic activation domain that is distinct from the POU interaction domain and functions synergistically with the Oct activation domain (9, 13–15).

The role of OCA-B in vivo has been examined using OCA-B-deficient mice created by gene targeting (9, 16–18). These studies show 1) that OCA-B is dispensable for Ag-independent B cell differentiation, with no observable defects in early stage B cell-specific gene expression; 2) that OCA-B is crucial for Ag-dependent B lymphoid development; although class switching itself appears unaffected, OCA-B−/− mice exhibit a severe deficiency in production of secondary Ig isotypes; 3) that OCA-B-deficient B cells show a reduced rate of proliferation in response to surface IgM cross-linking; and 4) that germinal center formation in the secondary lymphoid organs is dependent upon OCA-B. In more detailed analyses (19), histochemical studies have shown that OCA-B protein is expressed specifically in germinal center B cells, suggesting a more direct role for OCA-B in germinal center formation. Furthermore, OCA-B protein levels are nearly undetectable by Western blot in resting primary B splenocytes, but can be strongly increased by treatment with a combination of IL-4 and anti-CD40 (9, 19). Taken together, these data indicate that OCA-B is more important for later stages of B cell differentiation in vivo and that OCA-B is a target of crucial cell signaling pathways.

Although there is a significant amount of data regarding the molecular function of OCA-B, relatively little is known about the factors that regulate its B cell-specific expression. This information is especially important in light of the apparent function of OCA-B in late B cell differentiation and its induction by important lymphoid signals. In this study, we have cloned the OCA-B promoter and characterized its function throughout B cell development to better assess the regulation of OCA-B in vivo.
Materials and Methods

Cloning and sequencing of the OCA-B promoter

Murine genomic DNA was digested, circularized by ligation, and used in PCR with tail-to-tail primers designed against known OCA-B sequences (20). PCR fragments were cloned and sequenced at the Rockefeller University Protein DNA Technology Center. Resulting sequences were searched for transcription factor binding sites using TFSearch ver. 1.3 (Yutaka Akiami, Kyoto University, Kyoto, Japan). Site-directed mutagenesis of the OCA-B promoter was carried using the “overlap PCR” method (21). Oligonucleotides used for mutagenesis are as follows: NF-AT mutant top, 5'-CAGGACAGGTCTAAGTTTTTATTGACACGCCT-3' NF-AT mutant bottom, 5'-GTGACAGGGGTGTGCTCTAATCAAAAAAGTACAGCTC-3'; cAMP response element (CRE) mutant top, 5'-TCGAGAAGGGCCCGACGTACCTCCCTCAAGCCATG-3'; CRE mutant bottom, 5'-GAAGAAAAAGTACAGCTTCGCTGAGCTTGAAG-3'; Plasmids constructs

Vp1 LUC3. The IgH promoter, Vp1, was removed from pGL2 BV (described in Ref. 22) using KpnI and BglII and inserted into the identical sites in pGL3 Basic (Promega, Madison, WI).

Minimal promoter constructs. The LBK minimal promoter (23, 24) from the LBK44 CAT construct was removed with BamHI and HindIII and inserted into the BglII and HindIII sites upstream of the luciferase gene in pGL3 Basic (Promega). Oligonucleotides bearing a single copy of either an intact or mutated octamer sequence were subsequently cloned between the SacI and XhoI sites upstream of the minimal promoter. The oligonucleotide sequences are as follows: octamer top, 5'-CAGTACAGTTATACAAATCGATCTAGAC-3' octamer bottom, 5'-TCGAGTTAGCTACTGAGTTGC-3' octamer mutant top, 5'-TCGAGTTAGCTACTGAGTTGC-3'; octamer mutant bottom, 5'-TCGAGTTAGCTACTGAGTTGC-3'; Cell lines

The murine pre-B cell lines 18-8 and 18-81 and murine B cell line M12.4.1 were supplied by Dr. L. A. Eckhardt (Huner College, New York, NY). The murine cell line B20, human B cells Namalwa and Raji, the murine plasmacytoma cell line P3X63Ag8, and the murine fibroblastic cell line NIH3T3 were all obtained from American Type Cell Culture (Manassas, VA). The pre-B and B cell lines were grown in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% FBS (JRH Biosciences, Lenexa, KS), 1% penicillin-streptomycin (Life Technologies, Grand Island, NY) supplemented with 10% FBS (JRH Biosciences, Lenexa, KS), 1% penicillin-streptomycin (Life Technologies), and 2 mM l-glutamine (Life Technologies). Plasma and fibroblast cells were grown in DMEM (Life Technologies) supplemented with 10% FBS, 1% penicillin-streptomycin, and 2 mM l-glutamine. Primary B cells were cultured in RPMI 1640 (Sigma) containing 10% FBS (JRH Biosciences), 2 mM l-glutamine (Sigma), 1 mM sodium pyruvate (Sigma), 1% nonessential amino acids (Sigma), 5 × 10^{-5} M 2-ME (Sigma), and 1% penicillin-streptomycin (Life Technologies). Cells were maintained in 8% CO₂ at 37°C.

Primary B cell isolation

Spleens were freshly isolated from 4- to 6-wk-old C57BL/6 wild-type mice and OCA-B-/- mice. For each experiment, splenocytes from each genotype were isolated concurrently. After washing in serum-free media, the spleens were crushed between the frosted ends of microscope slides, releasing the cells. RBCs were depleted by lysis in buffer containing 0.16 M NH₄Cl, 10 mM potassium bicarbonate, and 1 mM EDTA. The remaining cells were pelleted, washed, and resuspended in serum-free media and incubated at room temperature for 20 min with anti-mouse Thy1.2 monoclonal antibody (Sigma) diluted 1:1000. The T cells were then depleted by incubation with guinea pig complement (Sigma), diluted 1:400, for 45 min at 37°C. The cells were washed several times and resuspended at 1 × 10^9 cells/mL in complete media containing 50 μg/mL LPS (Sigma) for 48 h. Macrophages and other adherent cell types were depleted by subsequent decanting of suspension cells. The B cells isolated via this method were shown by FACS to be >90% pure (data not shown). The activated B cells were pelleted, resuspended in PBS, and layered on a pad of 60% Percoll (Sigma). The cells remaining at the PBS/Percoll boundary after centrifugation were isolated and washed several times with PBS and counted. A total of 1 × 10^7 cells were used for each transfection sample, and cells were transfected using the DEAE-dextran method (Promega).

Transient transfections

Primary B, pre-B, and B cells were transfected by the DEAE-dextran method (25), plasmacytoma cells were transfected with Lipofectin (Life Technologies) (26), and fibroblasts were transfected with Superfect (Qiagen, Chatsworth, CA). In experiments in which samples were further treated with both cell lines and primary B cells, the inducer(s) was added to the media immediately after transfection and left until harvesting of the samples 48 h later. For cell lines, 2 × 10^6 cells were used per transfected sample, while for primary B cells, 1 × 10^6 cells were used per transfectant sample. The amounts of inducers added to the media are as follows: dibutyryl cAMP (Sigma) 1 mM, IL-4 (Sigma) 5 ng/mL, anti-CD40 (PharMingen, San Diego, CA) 10 μg/mL.

Transfection harvesting and reporter gene assays

Transfections of cell lines and primary B cells were harvested using 1 × reporter lysis buffer after 48 h (Promega). Luciferase assays were conducted using Promega’s luciferase assay system. β-galactosidase control vector assays were conducted (27), and resulting values were used to normalize the luciferase data for differences in transfection efficiency.

Western blotting

Cells were washed once in serum free media, then resuspended in cold buffer (20 mM HEPES, 400 mM KCl, 0.5 mM EDTA, 0.5 mM DTT, 0.5% nonidet P-40, 20% glycerol, pH 7.5) at 5 × 10^5 cells/50 μL and subjected to three rounds of freezing and thawing on dry ice before use. Then, 10 μl of 1 × reporter cell equivalents of whole-cell extract were electrophoresed and transferred to Immobilon P (Millipore, Bedford, MA). The filter was blocked in TBST containing 5% nonfat dry milk, incubated with rabbit anti-OCA-B antisera (supplied by Dr. Y. Luo, The Rockefeller University), washed with TBST, then incubated with HRP-conjugated goat anti-rabbit antisera (Promega) and visualized using chemiluminescence (NEN, Boston, MA).

EMSA

The DNA probe for EMSA was made by digestion of plasmid DNA and labeling with Klenow (28). Binding reactions (15 μl) contained 10 mM Tris, 40 mM NaCl, 1 mM EDTA, 1 mM 2-ME, 4% glycerol (final concentrations), 1000 ng nonspecific competitor (poly dIdC or salmon sperm DNA), 2 × 10^6 cpm probe, and either 4 μg Namalwa or 5 μg HeLa nuclear extract prepared as described (28). Nuclear extracts were previously titrated for optimal binding; data not shown.) For the antisera supershift experiments, 2 μl of NF-AT-, BCL6-, or activating transcription factor (ATF)/CRE binding protein (CREB)-specific antisera (Santa Cruz Biotechnology, Santa Cruz, CA) were added 5 min after initiating the binding reaction and further incubated for 10 min on ice before electrophoresis. Then, 0.5× TGE (12.5 mM Tris, 95 mM glycine, 0.5 mM EDTA, pH 8.5 final concentration) was used as both gel and chamber buffer.

Results

Cloning of the OCA-B promoter

Murine genomic DNA was partially digested with the restriction enzyme EcoRI, and the resulting fragments were circularized with T4 DNA ligase. Based upon the genomic sequences generated during the previous cloning of the OCA-B locus for gene targeting, we designed tail-to-tail oligonucleotides for use as PCR primers. Employing the circularized genomic DNA as a template, we amplified a series of DNA fragments that were subcloned and then sequenced. These fragments were inserted upstream of the luciferase reporter gene in the pGL3 Basic vector and tested for their ability to express luciferase in plasma cells. One of these clones, which contained 1.5 kb of DNA upstream of the start codon of the OCA-B gene (see Fig. 1), was the smallest construct that retained all of the promoter activity and thus was used for further analyses (GenBank accession no. AF241257).

Sequence analysis of the OCA-B promoter

Comparison of the OCA-B promoter sequence to databases of known transcription factor binding sites indicated a large number of matches, some of which are shown in Fig. 1. There is a TATA sequence ~30 bp upstream of the major start site (see Fig. 1). Relatively close to the TATA sequence are near-perfect matches to

Abbreviations used in this paper: CRE, cAMP response element; ATF, activating transcription factor; CREB, CRE binding protein.
the CREB/ATF (−64 to −57 bp) and NF-AT (−105 to −98 bp) consensus binding sites. Further upstream, there are strong matches to binding sites for YY-1, Myb, and Sox (or SRY-related) factors.

Prime extension analysis shows transcription initiation from one major site (indicated as 1 in Fig. 1) and several minor sites within the promoter in B cells (data not shown). These latter sites are consistent with previously published data (13, 14) and therefore were not further investigated.

**Developmental regulation of the OCA-B promoter**

To test whether the putative promoter region of OCA-B has B cell-specific activity, we conducted a series of transfections with cell lines representative of the pre-B, B cell, and plasma cell stages of B cell differentiation and with fibroblasts as a negative control. Each cell line was transfected with a luciferase reporter containing either the full-length −1.5 kb OCA-B promoter or a truncated OCA-B promoter extending from −40 bp through the TATA homology and transcription start site. This minimal construct shows a low constitutive activity in all the cell lines tested (data not shown) and therefore was used to establish a baseline value; the values for the full-length promoter are expressed as fold activity relative to the value for the minimal promoter. The data are shown in Fig. 2A. In NIH3T3 fibroblasts, which were used as representative non-B cells, there is little activity of the full-length OCA-B promoter above basal promoter levels. In contrast, the full-length OCA-B promoter is 17-fold above the basal promoter level in 18-81 pre-B cells, 44-fold above the basal level in M12.4.1 B cells, and 240-fold above the basal level in P3X63Ag8 plasma cells. Similar values were obtained in transfections of other representative non-B, pre-B, and plasma cell lines (data not shown).

**Promoter activity reflects actual protein levels**

To ensure that the progressive increase in promoter activity observed throughout B cell development reflects an actual increase in endogenous OCA-B expression in vivo, whole-cell extracts were made from the cell lines representative of each stage in B cell differentiation and checked for OCA-B protein levels via Western blotting. Equal cell equivalents were loaded onto each lane, and transfer efficiency was checked using Ponceau S staining (data not shown). OCA-B is normally detectable in Western blots as a doublet of 34 and 35 kDa (10, 19). As shown in Fig. 2B, the OCA-B doublet is not at all detectable in the NIH3T3 fibroblasts and only weakly visible in the pre-B cells, 18-81. The OCA-B protein levels are further increased in B cells and highest in the plasmacytoma cells. Because each lane contains equal cell equivalents of whole-cell extract, the amounts of protein detected represent the relative levels of OCA-B expression in each of the cell lines.

**Functional analysis of the OCA-B promoter**

To delineate the functional regions of the promoter sequence, a series of deletion mutants of the OCA-B promoter were generated via PCR and cloned into the pGL3 Basic vector. Deletion endpoints were selected, in part, on the basis of the positions of various transcription factor binding sites, as indicated in Fig. 1.
The data derived from the promoter deletion studies indicated that the CREB/ATF and NF-AT sites may be important for OCA-B promoter function. Therefore, we used PCR-based mutagenesis to generate site-specific mutants in the CREB/ATF and NF-AT binding site matches. The sequences of the mutations were designed on the basis of previously published reports of mutations that eliminate NF-AT or CREB/ATF DNA binding. The mutations were made in the full-length (~1.5 kb) OCA-B promoter, subcloned into the luciferase reporter pGL3 Basic, and assayed by transient transfection. Interestingly, as shown in Fig. 4, mutation of the NF-AT binding site match has absolutely no effect upon OCA-B promoter function in plasma cells. More significantly, disruption of the CREB/ATF binding site reduces overall promoter activity >90% in plasma cells. It is important to note that the mutation of the CREB/ATF site is in the context of the entire ~1.5 kb OCA-B promoter, indicating a crucial role for this sequence in overall promoter function and no significant redundancy with other elements.

Multiple members of the CREB/ATF family bind to the OCA-B promoter

To test whether any factors bind to the region of the OCA-B promoter bearing both the NF-AT and CREB/ATF binding site homologies, we conducted a series of EMSAs with a probe comprising the ~270 to ~40 region of either the wild-type OCA-B promoter or an OCA-B promoter bearing a mutation in the CRE region, with nuclear extracts from non-B cells (HeLa) and B cells (Namalwa). As shown in Fig. 5A, multiple complexes are generated with wild-type probe in both HeLa extract (lane 1) and Namalwa extract (lane 3). Nearly all of the complexes are eliminated with mutated probe in HeLa extract (lane 2) and Namalwa extract (lane 4), indicating that they depend upon the presence of the CRE for binding.

To investigate the nature of the complexes, reactions were incubated with antisera to specific proteins. As shown in Fig. 5B, the HeLa extract yields three major complexes of intermediate mobility (lane 2), and the Namalwa extract yields two major complexes of intermediate mobility (lane 6). However, there are qualitative differences in the mobilities of the intermediate complexes produced with each extract, perhaps suggesting that different protein complexes are binding to the promoter in B cell vs non-B cell extracts. We found that additions of an antisera that specifically recognizes a region common to many NF-AT family members (lanes 3 and 7) and an antisera to BCL6, which was used as a negative control (lanes 5 and 9), have no effect upon the observed complexes. In contrast, an antisera that recognizes a region common to many of the CREB/ATF family members almost quantitatively eliminates and/or supershifts the major intermediate complexes in both HeLa (lane 4) and Namalwa (lane 8) extracts. These data indicate that all the major intermediate complexes (three in the case of HeLa extracts, two in the case of Namalwa extracts) must contain at least one member of the CREB/ATF family,
whether derived from B cell or non-B cell extracts, and provide evidence for the specificity of the complexes. However, the data do not exclude the possibility that the complexes also contain other DNA elements or to ATF/CREB factors.

Dibutylryl cAMP stimulates the OCA-B promoter via the CREB/ATF site

Another hallmark of functional CREB/ATF sites and cognate factors is their ability to be stimulated with dibutylryl cAMP, which activates the cAMP-dependent protein kinase signaling pathway and results in activation of CREB/ATF family members (29). We tested the OCA-B promoter for dibutylryl cAMP responsiveness by transiently transfecting B cells in duplicate with either the full-length —1.5 kb OCA-B promoter, the minimal —0.04 kb OCA-B promoter, or the full-length —1.5 kb OCA-B promoter with a mutated CREB/ATF binding site and then stimulating one set with 1 mM dibutyryl cAMP. We chose to use B cells because the promoter is less active in these cells than in plasma cells and thus can be further stimulated.

Data from several transfection assays are summarized in Fig. 6A. While the full-length OCA-B promoter is significantly stimulated by dibutylryl cAMP treatment, both the minimal OCA-B promoter and the full-length OCA-B promoter with a mutated CREB/ATF site are unaffected by dibutylryl cAMP addition. These data indicate that the CREB/ATF site in the OCA-B promoter is indeed functional.

Dibutylryl cAMP stimulates octamer-dependent promoters

Because the OCA-B promoter is stimulated by dibutylryl cAMP, and because OCA-B is important for octamer-dependent promoter function in B cells, we examined whether dibutylryl cAMP has any effect upon octamer-dependent promoters. We transiently transfected B cells with a minimal promoter alone or with a minimal promoter linked either to an intact octamer sequence or to a mutant octamer sequence; consistent with previous reports (30) the minimal construct bearing a single octamer exhibits B cell-specific activity (data not shown). We also analyzed an intact Vh promoter containing functional octamer and TATA elements. The cells were transfected in duplicate with these constructs in the absence or presence of 1 mM dibutyryl cAMP.

The data from several independent experiments indicate that dibutylryl cAMP treatment can stimulate octamer-dependent promoter function in B cells (Fig. 6B). Thus, a single copy of the wild-type octamer sequence linked to the minimal promoter, as well as the natural octamer-dependent Vh promoter, are significantly stimulated by the addition of dibutylryl cAMP. In contrast, the minimal TATA-containing promoter, as well as the minimal promoter linked to a single copy of a mutated octamer site, are unaffected by dibutylryl cAMP treatment. The fact that the mutant and minimal promoters are not stimulated by dibutylryl cAMP shows that the observed activities are due to the octamer sequences themselves and not the minimal promoter sequences.

Stimulation of the OCA-B promoter by IL-4 and anti-CD40 treatment

OCA-B protein levels in resting primary B splenocytes are greatly increased by treatment with IL-4 and anti-CD40 (19). CREB/ATF sites are known to be targets of cell signaling pathways in a variety of systems, including B cells (31). Therefore, we examined whether the OCA-B promoter, and, more specifically, the CREB/ATF site within the promoter, was a target of IL-4 and anti-CD40 stimulation. We transiently transfected either the full-length —1.5 kb OCA-B promoter, or the full-length —1.5 kb OCA-B promoter with a mutated CREB/ATF binding site and then stimulating one set with 1 mM dibutyryl cAMP added immediately after transfection.

The results in Fig. 7A indicate that while IL-4 and anti-CD40 treatment has little or no effect upon the minimal promoter construct, it strongly activates the intact OCA-B promoter. This stimulation of the OCA-B promoter is largely abrogated by mutation of the CREB/ATF site, suggesting that this site is a target of the IL-4 and anti-CD40 signaling pathways in primary B cells.

IL-4 and anti-CD40 stimulation of octamer-dependent promoters

Because treatment of primary B splenocytes with IL-4 and anti-CD40 clearly elevates both OCA-B promoter activity and protein levels (19), we tested whether these increases resulted in changes
in the activity of octamer-dependent promoters. We transiently transfected primary B cells in duplicate with the intact VH promoter, a minimal TATA-containing promoter alone, and a minimal promoter linked either to an intact octamer site or to a mutant octamer site (see above). One set of samples was treated with IL-4 and anti-CD40 immediately after transfection.

As shown in Fig. 7B, IL-4 and anti-CD40 treatment activates both the VH promoter and the synthetic promoter bearing a single copy of the intact octamer site. In contrast, there is no significant stimulation of either the minimal TATA-containing promoter or the minimal promoter linked to the mutant octamer sequence, clearly showing that the stimulation is specific to the octamer site.

Discussion
In this report, we have defined the activity of the OCA-B promoter region through a variety of physical and functional methods. This is the first study to examine the regulation of OCA-B at the transcriptional level in B lineage cells and thus provides a better understanding of the molecular basis of the B cell-specificity of the
octamer site. The data presented here make several important points regarding the function of the OCA-B promoter, and of OCA-B itself.

The OCA-B promoter is B cell-specific and developmentally regulated

The OCA-B promoter is clearly more active in B cells than non-B cells, as indicated by transient transfection analysis. More importantly, the OCA-B promoter shows increasing activity throughout B cell development, consistent with data from gene targeting studies indicating that OCA-B is more crucial at later stages of B cell development (16–18). While the OCA-B promoter is relatively weak in pre-B cells, it shows an increased activity in B cells and the highest activity in plasma cells. The increase in OCA-B promoter activity throughout B cell differentiation is not due to variations of individual cell lines, because transfection of other cell lines representative of similar stages in B cell development exhibited equivalent levels of OCA-B promoter function (data not shown).

The observed increases in OCA-B promoter activity during B cell differentiation are paralleled by endogenous OCA-B protein levels, as shown by Western blot. This correlation suggests that OCA-B is regulated, at least in part, at the transcriptional level.

The role of the CREB/ATF site in OCA-B promoter function

Deletion and site-directed mutations of the CREB/ATF binding site show that it is crucial for overall OCA-B promoter function, and mobility shift analysis suggests that multiple complexes are able to form at this site. These complexes contain genuine CREB/ATF family members, because most are supershifted by antisera that recognize CREB/ATF family members but not by control antisera. These data do not eliminate the possibility that other factors are present, but simply indicate that one component of any putative multiprotein complex is a member of the CREB/ATF family. It is important to note that complexes were observed with both B cell and non-B cell nuclear extracts. This is to be expected, because some members of the CREB/ATF family are expressed in all cells (32–34). More importantly, however, published reports have already demonstrated a role for CREB/ATF factors both in B cell-related signaling and in expression of B cell-specific genes such as the Ig κ light chain (29, 31). There also is evidence for differential expression of specific spliced forms of ATF-1 and CRE modulator over the course of B cell development (29, 35). Furthermore, a B cell-enriched ATF factor, designated B-ATF, has recently been cloned (36). Therefore, there is a significant amount of data implicating the CREB/ATF family of factors in B cell-specific signaling and gene expression.

Induction of OCA-B promoter activity

An important functional aspect of CREB/ATF binding sites is promoter inducibility through these sites. Reagents such as dibutyryl cAMP stimulate the activity of factors binding to the CREB/ATF site by inducing cell signaling pathways leading to activation of CREB/ATF family members via phosphorylation (29, 31). We find that the OCA-B promoter is strongly activated by dibutyryl cAMP treatment and that this stimulation is dependent upon the CREB/ATF site. These data are in accord with previously published data suggesting that OCA-B is the target of multiple cell signaling pathways in vivo (19). Even more significantly, dibutyryl cAMP treatment is able to stimulate the activity of octamer-dependent promoters. Mutation of the octamer element abolishes the dibutyryl cAMP effect, demonstrating that factors binding directly (e.g., Oct-1) or indirectly (e.g., OCA-B) to the octamer site itself are being induced. In toto, these data strongly suggest that the endogenous OCA-B is being induced by dibutyryl cAMP, which in turn activates the octamer-dependent promoters.

In primary B cells, the OCA-B promoter is a target for stimulation by IL-4 and anti-CD40, a treatment that previously has been shown to increase OCA-B protein levels in primary B splenocytes (19). Activation of the OCA-B promoter by IL-4 and anti-CD40 appears to be mediated at least in part by the CREB/ATF site, because mutation of the site eliminates the stimulatory effect. These data suggest that CREB/ATF factors are at least one target of the IL-4 and anti-CD40 signaling pathways, further establishing the role of CREB/ATF factors in B cell-specific gene expression. More importantly, IL-4 and anti-CD40 treatment specifically activates octamer-dependent promoters in primary B cells, implicating the octamer site, and the interacting Oct-1 (or Oct-2) and OCA-B, in B cell-specific signaling events.

Taken together, the results presented here show that the OCA-B promoter is regulated throughout B cell development and is the target for cell signaling events via one or more members of the CREB/ATF family. OCA-B promoter activation appears to result in increased activity of octamer-dependent promoters, implicating OCA-B in B cell signaling events in the nucleus.

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References


