B Cell-specific Activator Protein Prevents Two Activator Factors from Binding to the Immunoglobulin J Chain Promoter until the Antigen-driven Stages of B Cell Development*

(Received for publication, January 6, 1999, and in revised form, March 11, 1999)

Jeffrey J. Wallin, Julie L. Rinkenberger, Sulekha Rao, Edwin R. Gackstetter, Marian Elliott Kosland, and Patty Zwollo

From the Department of Molecular and Cell Biology, University of California, Berkeley, California 94720 and the Department of Biology, The College of William and Mary, Williamsburg, Virginia 23187

The immunoglobulin J chain gene is inducibly transcribed in mature B cells upon antigen recognition and a signal from interleukin-2 (IL-2). A cell-specific activator protein (BSAP), a transcription factor that silences J chain transcription, has been identified as a nuclear target of the IL-2 signal. The levels of BSAP progressively decrease in response to IL-2 and this change correlates with the differentiation of B cells into antibody secreting plasma cells. Here we report the binding of the upstream stimulatory factor (USF) to an E-box motif immediately upstream from the BSAP site on the J chain promoter. Mutations in the USF binding motif significantly decrease J chain promoter activity in J chain expressing B cell lines. We also show that a functional relationship exists between USF and a second J chain positive-regulating factor, B-MEF2, using co-immunoprecipitation assays and transfections. Finally, we provide evidence that the binding of BSAP prevents USF and B-MEF2 from interacting with the J chain promoter during the antigen-independent stages of B cell development. It is not until the levels of BSAP decrease during the antigen-driven stages of B cell development that both USF and B-MEF2 are able to bind to their respective promoter elements and activate J chain transcription.

During a primary immune response to foreign antigens, mature B cells become activated and differentiate into pentamer IgM-secreting plasma cells. One of the critical events in this process is the synthesis of the immunoglobulin J chain protein required for the assembly and secretion of pentamer IgM antibody (1). Studies of both normal B cells and model B cell lines have shown that J chain synthesis is tightly regulated at the transcriptional level. For efficient J chain transcription to occur, activation signals from both the B cell receptor and the interleukin-2 (IL-2) or IL-5 receptors are needed. Correlating with IL-2/IL-5-induced gene transcription is the appearance of a DNase I-hypersensitive site (bp 170 to +88) on the J chain promoter of activated B cells (2, 3). The full activity of the J chain promoter is contained within this hypersensitive region, as has been shown using 5’ deletion mutants in a CAT reporter system (4).

Three regulatory elements have been found to be present within this region. These include a T-rich positive regulatory element denoted JA (−74 to −60), a purine-rich sequence (−58 to −47) denoted JB, and a repressive motif, JC (−127 to −110) (5–7). The factor interacting with the JA sequence has recently been identified to be a member of the myocyte enhancer factor-2 (MEF-2) family that is expressed in the B cell lineage, denoted B-MEF2 (5). The JB element has previously been shown to interact with transcription factor PU.1, a member of the Ets family of transcription factors expressed in hematopoietic lineages including monocytes, macrophages, and B lymphoid cells (6). Mutational analyses in J chain positive cell lines have indicated positive regulatory roles for both PU.1 and B-MEF2; base changes that prevent either PU.1 or B-MEF2 from binding result in a 95% loss of promoter activity (5, 6). The activity mediated by the JC motif has been shown to be due to the binding of the transcription factor B cell specific activator protein (BSAP), or Pax-5 (7).

BSAP/Pax-5 is a member of the Pax family of transcription factors, which are important regulators of embryonic cell development and differentiation. Targeted gene disruption experiments have shown that BSAP expression is essential both for B cell development as well as development of the nervous system (8, 9). BSAP is highly expressed during the early stages of B cell development, but expression ceases during the antigen-driven stages of B cell development. BSAP is considered a “master regulator” of B cell development, and at least eight B cell-specific putative target genes have been identified so far (10).

Depending on the target gene, BSAP can act either as an activator, a repressor, or a docking protein (9, 11, 12). In the case of the J chain promoter, BSAP acts as a repressor: base changes in the BSAP-binding site result in a relief of repression in J chain negative cell lines (7). An IL-2 or IL-5 signal delivered to mature B cells has been shown to cause a progressive decrease in BSAP transcripts that extends from the presecretor immunoblast through the plasma cell stages (7). This pattern of expression inversely correlates with J chain expression.

In addition to a role in J chain repression, a BSAP repression motif has also been identified in the 3’ a enhancer of the immunoglobulin heavy chain genes. Although the process of BSAP repression is not well understood, a possible mechanism has been suggested by in vivo footprinting studies by Neurath and colleagues (13). This work showed that although two BSAP sites exist in the immunoglobulin 3’ a enhancer, only the most 5’ site is occupied by BSAP in mature B cells. As expected, no
BSAP footprint was detected in plasma cells, due to low levels of BSAP at this cell stage. Importantly, the authors identified a second factor, NF-αp, which bound to a position 50 bp downstream of the 5′ BSAP-binding site in plasma cells, but not in mature B cells. NF-αp, a member of the Ets family of transcription factors, is expressed both in mature B and plasma cells, and is necessary for maximal activity of the 3′ α enhancer. Selective blocking of BSAP binding by triplex-forming oligonucleotides resulted in an NF-αp footprint in mature B cells and an increased level of immunoglobulin gene transcription. Thus, it appears that BSAP prevents NF-αp from activating the 3′ α enhancer until the plasma cell stage.

In the work described here, a new DNA binding motif JF was identified at positions −140 to −132 of the J chain promoter. This sequence resembles the µE3 element of the immunoglobulin heavy chain enhancer and the related κE3 element of the κ light chain enhancer (14). We show that the helix loop helix protein upstream stimulatory factor (USF) binds to the J chain promoter at the JF motif and positively regulates J chain transcription. Recently, it was shown that USF increases the activity of the immunoglobulin heavy chain gene intron enhancer, in combination with two other factors, PU.1 and Ets-1 (15). In addition, USF factors have been shown to interact with members of the basal initiation complex (16, 17). We show here that USF may, at least in part, be mediating its positive effect on J chain transcription through a mechanism which necessitates interaction with B-MEF2. Although these positive-acting factors are both expressed throughout B cell development, it appears that they are only able to bind weakly to the J chain promoter in the presence of BSAP. This may provide a mechanism which ensures repression of J chain transcription until the activated B cell stages. As BSAP levels decrease during that time, USF and B-MEF2 replace BSAP on the J chain promoter and this results in activation of transcription.

**MATERIALS AND METHODS**

**Cell Culture**—PD31, 38C13, K46R, BCL1, and L cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 50 μM 2-mercaptoethanol, 100 units of penicillin/ml, and 100 μg of streptomycin/ml. MOPC315 and SI9 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented as described above.

**Preparation and Assay of Nuclear Proteins**—Large-scale extracts were prepared from 10⁷ cells by the detergent lysis method of Peterson et al. (18) as modified by Lansford et al. (4). All buffers contained the following mixture of protease inhibitors: 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 0.5 mM Na₃VO₄, aprotonin (10 units/ml), leupeptin (5 mg/ml), and pepstatin A (5 mg/ml). Mini-extracts were prepared from 10⁵ cells as described (19).

Probes and DNA competitors used: J1, nt −83 to −9; J2, nt −168 to −84; JA, −74 to −60; JB, nt −58 to −47; JC, nt −127 to −110; JE, nt −140 to −132 of the J chain promoter. For gel mobility shift assays, the specific oligonucleotides were end-labeled with [32P]dCTP and Klenow enzyme (20). The binding reactions with crude nuclear extracts were performed as described previously (19) using 8–10 μg of extract, 4–6 μg of poly(dI-dC) nonspecific competitor, and 10⁴ cpm (0.1–1.0 ng) of probe. For the antibody gel shift assays, crude nuclear extracts were preincubated with 2 μl of a 1:10 dilution of either rabbit preimmune sera or polyclonal rabbit anti-mouse USF-2 antiserum which is specific for one of the two subunits of USF (p44) (Santa Cruz Biotechnology). In each case the protein-DNA complexes formed were resolved from free probe by electrophoresis through glycerol-containing 5% polyacrylamide gels (29:1) containing 0.25 × TBE buffer.

**Footprinting Assays**—Methylation protection footprinting was performed with crude nuclear extracts from the mature B cell line, K46R; 50 μg of nuclear protein was incubated 15 min at 0 °C with 2 μg of poly(dI-dC) as a competitor and 4 μM of DNA probe (one-half n mole of dimethylsulfate was added for 45 s and quenched by the addition of dithiothreitol to a final concentration of 23 mM). The remaining steps in the assay, isolation and sequencing of free and protein-bound DNA, were performed according to the standard protocol for methylation interference footprinting (21). The probe J2 was an 84-bp XbaI/HindIII fragment containing the J chain sequence −168 to −84 that was end-labeled on the top or bottom strand.

**Plasmid Constructions for Linked Promoter Analyses**—In the py42Cas1 vector (24) the CAT gene is under control of a truncated γ-fibrinogen promoter (−54 to +36) that includes a TATA box and a single Sp1-binding site. Fragments from the J chain promoter (J1, bps −83 to −9; J2, bps −168 to −84; J1-J2, bps −168 to −9) and oligonucleotides representing the JE element were synthesized with XbaI linkers and inserted either singly or in multiple copies into the polynucleotide upstream of the γ-fibrinogen promoter. All constructs were sequenced to determine oligomer copy number and orientation.

**Mutagenesis of the py42Cas1 plasmid containing the J1-J2 sequence and the Xβp plasmid was performed with the Transformer™ Site Directed Mutagenesis Kit (CLONTECH Laboratories). The sequence 5′-CTGTAATGTAGACACCTGCTCTTCCAGTGTAAGC-3′ (JE) was used to introduce a 3-bp change in the JE element (the underlined region replaces the wt sequence CATGTTG, see “Results”). The selected plasmid was sequenced to verify the base substitutions. Mutations introduced into the JF motif have been described previously (7).

**Transfections**—Transient transfections of PD31 pre-B cells were performed by the DEAE-dextran technique (22) and transfections of MOPC315 myeloma cells by electroporation (23). In each case, 10⁷ cells in logarithmic growth phase were transfected with 9 μg of supercoiled test plasmid or a combination of plasmids. Cell extracts were prepared 44–48 h after transfection and assayed for CAT activity (4).

**Immunoprecipitations and Western Blots**—Immunoprecipitation reactions using antibody-Sepharose, were performed according to the standard protocol (20). For Western blot analyses, nuclear extract samples were boiled for 5 min, size fractionated by SDS-polyacrylamide gel electrophoresis (10%), and transferred to a nitrocellulose filter. After pretreatment with 5% dry milk in 1 × phosphate-buffered saline, the filters were incubated for 3 h with a 1:10,000 dilution of antibody specific for BSAP, PU.1, MEF-2 (Santa Cruz Biotechnology Inc.), or USF-2 (Santa Cruz Biotechnology Inc.). The filters were then washed 3 times with 1 × phosphate-buffered saline and incubated for 1 h with 1:5,000 dilution of horseradish peroxidase-conjugated goat anti-rabbit IgG. After three 1 × phosphate-buffered saline washes, the filters were developed using an enhanced chemiluminescence kit (Amersham).

**RESULTS**

**Identification of the JE Element by Deletion Analyses**—J chain transcription has been shown to be regulated by at least two positive elements and one repressor element on its promoter (4–7). To investigate whether additional inducible elements are present which are necessary for regulation of J chain transcription, deletion analyses of the 5′ region of the minimal J chain promoter was performed. The constructs used for the deletion analyses have been described previously and were generated through progressive 5′ deletions of the J chain promoter from the hypersensitive region (4, 7). These fragments were tested for their ability to drive expression of the CAT gene, as shown in Fig. 1 (4). In addition to J chain promoter sequences and the CAT gene, each of the constructs also contained the intronic immunoglobulin βE, inserted downstream of the CAT gene in opposite transcriptional orientation.
(24). The μE sequences were included because levels of CAT expression obtained with constructs lacking a heterologous enhancer were too low to yield reliable values. The constructs were transiently transfected into the J-chain-expressing and IgA-secreting myeloma MOPC315, and assayed for CAT activity (Fig. 1). Data obtained from transfection of these constructs into the J chain-silent pre-B cell line PD31 has been shown previously (7) and is shown here for the purpose of comparison.

First we compared the relative CAT activity of MOPC315 cells with the J chain negative and BSAP positive pre-B cell line PD31. Transfection of the −192 construct containing the entire hypersensitive region resulted in approximately 50-fold higher CAT activity in MOPC315 cells compared with PD31 cells, in agreement with activity of the J chain promoter in plasma cell lines, but not in early B cell lines (data not shown).

Deletion of base pairs from −192 to −136 led to an 85% decrease in J chain promoter activity in MOPC315 cells, as shown in Fig. 1. This suggested the presence of a positive regulatory element, denoted JE, located at the 5’ distal portion of the hypersensitive site, upstream from the previously identified JC motif (Fig. 1). Finally, we found that further deletion of the promoter element from −135 to −76 did not cause any additional change in CAT activity (Fig. 1). This is not surprising since the repressive factor BSAP that binds to the JC motif (base pairs −127 to −110), is undetectable in MOPC315 cells (13). However, as had been identified previously, evidence for BSAP repression is observed in the BSAP-containing and J chain negative PD31 cells, where removal of the JC binding motif (nt −136 to −76) resulted in an increased CAT activity (7).

Characterization of a Nuclear Factor Interacting with the JE Element—Next we examined the presence of a putative factor NF-JE that could interact with the JE element on the J chain promoter. Electrophoretic mobility shift assays (EMSAs) were performed using a DNA fragment covering bps −168 to −84 of the J chain promoter (J2) as a probe. This probe contains the JC and JE elements, but not the JA or JB elements (see Fig. 1).

Distinct binding patterns were observed using nuclear extracts from the immature B cell line 38C.13, the mature B cell line K46R, the B presecretor BCL1, the plasmacytoma lines S194 and MOPC315, the fibroblast line L (Fig. 2A), and the pre-B cell line PD31 (not shown). Only S194 and MOPC315 express the J chain.

The EMSA pattern showed three complexes on the gels. The fastest migrating band (complex 1) was detected only in extracts from 38C.13, K46R, BCL1 (Fig. 2A, arrow 1), and PD31 (not shown). This suggested that this complex contained BSAP, based on the presence of a BSAP-binding site on this probe (JC) and its pattern of expression in the B cell lineage (see below).

An intermediate migrating complex (complex 2) was detected in all cell lines of the B cell lineage (Fig. 2A, arrow 2), but not in L cells. The relative amounts of the two complexes varied between early and late B cell lines: early B cell lines had high levels of complex 1, but low levels of complex 2. In contrast, the two plasmacytoma lines had very low or undetectable levels of complex 1, but high levels of complex 2. All cell lines tested showed a slower migrating complex which could not be competed with excess unlabeled J2 DNA and thus represents nonspecific (NS) protein-probe complexes (Fig. 2A, arrow NS).

The complexes 1 and 2 (Fig. 2A, arrows 1 and 2) were analyzed further using competition EMSAs and nuclear extracts from the pre-B cell line PD31. The unlabeled double-stranded (ds) oligonucleotides JB (nt −58 to −47) and JC (nt −127 to −110), were used as competitors and contain the PU.1 and BSAP-binding sites, respectively. As expected, the JB competitor was unable to compete for binding with either complex. The JC oligonucleotide was able to prevent binding of BSAP to the J2 probe (Fig. 2B, arrow 1), but could not compete with the second, slower migrating complex (Fig. 2B, arrow 2). In contrast, this second complex was specifically competed by a second double-stranded oligonucleotide JE, which covers −140 to −132 of the J chain promoter.

To analyze the JE region further, we performed copper phenanthroline and methylation protection footprinting using the J2 probe (Fig. 3). The retained band corresponding to the JE region gave an extended footprint on the noncoding strand (Fig. 3A) from bp −138 through the BSAP-binding site to bp −124 (which also contained a footprint). The coding strand showed protection in the 5’ region of the noncoding strand footprint (Fig. 3B) in the region between bp −138 through −134. These results are in agreement with a putative factor NF-JE binding to this region of the J chain promoter.

Identification of NF-JE as the Helix Loop Helix Transcription Factor USF—To search for possible candidates for NF-JE, known consensus DNA-binding motifs were compared with the

![Figure 2: EMSA analyses of NF-JE using the J2 probe which covers base pairs −168 to −84 of the J chain promoter. Arrow 1 indicates the complex containing BSAP, arrow 2 the complex containing NF-JE. NS indicates nonspecific probe binding. A, nuclear extracts from five B cell lines representing different stages of development: 38C.13 (J−), immature B; K46R (J−), mature B; BCL1 (J−), presecretor B; S194 (J+) and MOPC315 (J+), plasmacytoma; and one fibroblast cell line, L (J−). B, competition EMSA using 100× excess unlabeled double-stranded oligonucleotides JB, JC, and JE and nuclear extracts from the pre-B cell line PD31.](image)
sequence in the JE region. One motif that contained high homology to the sequence recognized by NF-JE (CATGTG) was one recognized by E-box family proteins, particularly the μE3 (CAGATG) and κE3 (CATGTG) motifs present on the immunoglobulin μ heavy chain intron enhancer and κ light chain enhancer, respectively (14, 25, 26).

To test whether NF-JE represents an E-box factor, EMSAs were performed using a polyclonal rabbit antiserum against one of the two subunits of USF, namely USF-2. When tested with PD31 nuclear extracts this antiserum was able to block NF-JE binding to the JE oligonucleotide probe (Fig. 4, lane 4). Next, we tested whether mutation of the core binding sequence for E-box proteins would result in prevention of binding of the factor NF-JE, using competition EMSAs. A mutant JE oligonucleotide (mJE) was synthesized containing a 3-base pair mutation in the region corresponding to the consensus E motif (Fig. 4, lane 2). In Fig. 2B we already showed that a wt JE oligonucleotide could compete with this complex. DNA-protein complexes were not observed when the mJE oligonucleotide was used as a labeled probe (data not shown). Together, these findings provide evidence that NF-JE represents the helix loop helix transcription factor USF and will be referred to as such hereafter.

USF Needs Downstream Regulatory Elements to Affect J Chain Transcription—To determine the contribution of the JE element to J chain transcription in vivo, we performed transient transfections in the J chain-expressing plasma cell line MOPC315, using either a wild-type (wt) or JE-mutated J chain promoter (bp −1150 to +88) driving expression of a CAT reporter gene. The mutated J chain promoter contains a 3-nt replacement mutation of the JE sequence (indicated by an "X"). CAT reporter constructs containing different combinations of wt or mutated fragments of the J chain promoter, linked to a minimal γ fibrinogen promoter. See text for details.

To determine whether the JE element can act as an independent activator of transcription, the truncated rat γ-fibrino-
Regulation of J Chain Expression

**Fig. 6. Detection of protein interactions between USF and B-MEF2.** Nuclear extracts from PD31 or MOPC315, and antibodies to USF were used in co-precipitation reactions, and resulting complexes analyzed by Western blot analysis using an anti-MEF2 antibody (right two lanes). As a negative control, immunoprecipitations were performed with preimmune serum (left two lanes). Molecular weight markers are indicated on the right.

py42CassI vector upstream of the minimal γ-fibrinogen promoter: the J1-J2 fragment (bp −168 to −9, which contains all four regulatory motifs JA, JB, JC, and JE), the J1-J2 fragment with a mutant JE-binding site (J1-J2mE), J1-J2 with a mutant JC site (J1-J2mC), the J2 fragment alone (bp −168 to −84, which contains the JC and JE motif only), the J2 fragment with a mutant JE site (J2mE), and the J2 fragment with a mutant JC site. A 4-bp mutation (underlined sequences) changes CAGGTGACATGACGT to CAGGTGAGTGCAGT in the JC motif and this results in the absence of BSAP binding to this region (7). These constructs were then transfected into MOPC315 cells. The constructs containing J1 as well as both JC and JE (J1-J2 and the J1-J2mC) induced a 5- and 5.5-fold increase in the basal level of CAT expression, respectively (Fig. 5B). Alternatively, the J1-J2mC construct which contained a 3-bp mutation in the JE motif, exhibited a 62% loss of activity in comparison to the other J1-J2 constructs. In contrast to the results obtained with the J1-J2 constructs, the wild-type J2 fragments alone (which did not contain JB or JA), did not show detectable changes in basal CAT activity (Fig. 5B). These results suggest that USF can act as an activator of J chain transcription only if in the context of other promoter elements present on both the J1 and J2 regions.

Based on the above results, we hypothesized that USF may be mediating its positive influence on J chain transcription by interacting with downstream activating elements PU.1 and/or B-MEF2, both of which bind to the J1 region of the J chain promoter (to JB and JA, respectively). To test this possibility, we performed co-immunoprecipitation reactions using antibodies to B-MEF2, PU.1, and USF-2. We were able to co-precipitate B-MEF2 with USF-2 antibody (Fig. 6) and vice versa (data not shown), both in the J chain negative pre-B cell line PD31 as well as in the J chain positive line MOPC315. In contrast, no evidence was found for interactions between either PU.1 and B-MEF2 or PU.1 and USF using this approach (data not shown). These results are in agreement with the above deletion analyses data of the J chain promoter (Fig. 5B) and suggest that promoter activity involves functional interactions between B-MEF2 and USF. This interaction may be necessary to assemble a functional transcription complex necessary for efficient J chain transcription.

**BSAP Decreases USF and B-MEF2 Binding to Their Regulatory Motifs in the J Chain Promoter—**Transcription of the J chain gene is initiated when a mature immunocompetent B cell receives both an antigen signal and a cytokine signal from IL-2 or IL-5. It has been shown previously that the transcription factor BSAP is responsible for repressing J chain transcription until this time (7, 10). The concentration of BSAP present in activated B cells decreases over the course of a primary immune response until it becomes almost undetectable at the plasma cell stage (7). Overexpression of BSAP in plasma cell lines, however, reverses this trend and results in BSAP-mediated repression of J chain transcription (7, 10).

To further our understanding of the regulation of J chain transcription, we analyzed the in vitro DNA binding characteristics of all four factors that influence J chain promoter activity, including BSAP, USF, B-MEF2, and PU.1. DNA binding was analyzed by EMSA using nuclear extracts from the mature B cell line K46R. Two different probes were used, either the wild-type J1-J2 segment (bp −168 to −9) which contains binding sites for all four factors, or the same probe with a mutated BSAP-binding site (mJC).

In none of the EMSAs did we observe the binding of all four factors at the same time, using either the wild-type or the BSAP mutant probes (Fig. 7, A and B). This was surprising, especially since we could show by Western blot analysis that all four factors were present in the nuclear extracts of the K46R cells (Fig. 7C). For example, B-MEF2 was unable to bind to the J chain probe (Fig. 7A, left three lanes) except when the BSAP motif was mutated (Fig. 7, A, right three lanes, and B). In the presence of BSAP binding, some USF was able to bind to the JE motif (using the wild-type probe), but USF binding increased greatly in the absence of BSAP (using the BSAP mutant probe), as shown in Fig. 7, A and B. Interestingly, the binding of USF to the JE motif was reduced by competition with B-MEF2 oligos (Fig. 7B). In contrast, PU.1 binding was unaffected by the presence of bound BSAP (Fig. 7, A and B). Together, these results imply that BSAP mediates its repression on the J chain gene by preventing two activator factors, USF and BMEF-2, from binding to their regulatory elements.

**DISCUSSION**

Regulation of gene expression during B cell development is extremely complex and involves combinatorial binding activities of multiple transcription factors at each target gene. In this study we sought to further explore the transcriptional regulation of the immunoglobulin J chain gene promoter. This promoter is relatively well defined, and the nucleosome hypersensitivity region between base pairs −170 and +85 has been shown to be sufficient for full activity in J chain positive cells (4). Earlier studies defined three regulatory elements on the J chain promoter, JA, JB, and JC, which were shown to associate with the transcription factors B-MEF2, PU.1, and BSAP, respectively (5–7). This report defines a fourth binding motif denoted JE, with a consensus E-box factor binding sequence. The protein binding to this motif was identified as USF, a member of the E box family of basic helix loop helix transcription factors. Since we have not observed the binding of other nuclear factors within the nucleosome hypersensitivity region, we believe that USF represents the fourth and final major inducible DNA-binding protein necessary for activation of the J chain promoter.

**USF Interacts with the MADS Box Family Member B-MEF2—**Results from the 5′ deletion analyses and co-immunoprecipitation assays suggest that USF interacts with a second, downstream binding transcription factor, B-MEF2. The ubiquitously expressed factor USF commonly consists of two subunits, USF-1 and USF-2 (26), and has been shown to be a transcriptional activator in combination with other factors (27–30). For example, in the immunoglobulin μ heavy chain enhancer, activity is detected only in the presence of a three protein-DNA complex consisting of USF, PU.1, and Ets-1 (15). In this case, the restricted expression pattern of PU.1 and Ets-1 ensures that its activity is limited to lymphoid cells. The observed association between USF and B-MEF2 is supported by studies on protein–protein interactions between MEF2 family
members and E box proteins, and it has been proposed that such interactions may lead to increased assembly of the basal machinery (29, 30).

**BSAP Prevents B-MEF2 and USF from Binding to the J Chain Promoter**—The expression pattern of BSAP throughout B cell development has been well established and has been found to be inversely correlated with expression of the J chain (7, 9, 12, 13). In addition, it has been shown that BSAP is responsible for repression of the J chain gene during the antigen-independent stages of B cell development (7). In the present study, the mechanism of BSAP repression of the J chain gene was explored further. EMSAs were used to analyze the DNA binding characteristics of BSAP as well as the three other factors that interact with the J chain promoter, using nuclear extracts from the mature B cell line K46R. The data suggest that the binding of BSAP to the J chain promoter reduces binding of the ubiquitously expressed USF to the JE motif and simultaneously prevents B-MEF2 from binding to its JA motif. In contrast, BSAP binding does not influence the binding of the fourth factor, PU.1. Since both B-MEF2 and USF are activators of J chain transcription, the presence of BSAP may provide an efficient mechanism to prevent activation of the J chain gene during the antigen-independent stages of B cell development. This would also be in agreement with the observation (in Fig. 2A) that extracts from early B cell lines show relatively low amounts of USF bound to the J2 probe, but plasmacytoma lines (which have no BSAP) show significantly higher amounts of bound USF.

The exact mechanism by which binding of BSAP prevents B-MEF2 and USF binding to their respective motifs on the J chain promoter is not clear. Perhaps the easiest explanation is that the failure of USF to bind to its JE motif is a direct result of its close proximity to the BSAP binding motif JC. In this scenario, BSAP would prevent the access of USF to its binding motif by either occluding its binding site, or changing the structure of the DNA within this region. A local unwinding of the DNA helix has been proposed as a mode of repression in the major histocompatibility complex class II promoter (31).

The mechanism by which B-MEF2 binding is inhibited in the presence of BSAP, which binds 35 bp upstream of this activating factor, is also not clear. The situation is, however, analogous to what has been observed in the 3' α enhancer, where BSAP has been shown to prevent the binding of an activator factor whose binding motif is 50 bp downstream (13). One explanation would be that USF and B-MEF2 each need to interact with their DNA-binding sites as well as with each other, to enable formation of a stable complex on the J chain promoter.

The analyses of J chain gene regulation presented here supports a model where the key regulatory switch for J chain expression is determined entirely by nuclear levels of BSAP protein. The three other regulatory factors that can bind to the J chain promoter are unable to influence J chain transcription despite their expression throughout B cell development. When BSAP concentrations decrease as a result of B cell activation, all three activator factors are now able to rapidly bind to the J chain promoter and presumably interact with each other to promote efficient transcription of the J chain gene.

**Regulation of J chain expression provides a good example of combinatorial regulation:** although all four factors are expressed during B cell development, a unique combination of three is necessary to activate J chain transcription. The fourth factor, BSAP, not only prevents J chain activation until the final stages of B cell development, but plays other, essential roles on distinct target genes during earlier stages of development as well. USF, unable to activate transcription of the J chain gene until the final stages of B cell development, plays essential roles in the expression of the immunoglobulin μ heavy chain gene during early B cell development (15). Thus, although many different transcription factors are expressed throughout B cell development, the interactions of unique combinations of factors with their target promoters will largely determine the level of specific gene expression during each developmental stage.

**Acknowledgments**—We thank members of the Sha laboratory for useful comments and suggestions. We also thank Joan Fujita for technical assistance.
REFERENCES

1. Koshland, M. E. (1985) Annu. Rev. Immunol. 3, 425–453
2. Blackman, M. A., Tigges, M. A., Minie, M. E., and Koshland, M. E. (1986) Cell 47, 609–617
3. Minie, M. E., and Koshland, M. E. (1986) Mol. Cell. Biol. 6, 4031–4038
4. Lusnford, R. D., McFadden, H. J., Siu, S. T., Cox, J. S., Cann, G. S., and Koshland, M. E. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5866–5870
5. Rao, S., Karrey, S., Gackettetter, E. R., and Koshland, M. E. (1998) J. Biol. Chem. 273, 26123–26129
6. Shin, M. K., and Koshland, M. E. (1993) Genes Dev. 7, 2006–2015
7. Rinkenberger, J. L., Wallin, J. J., Johnson, K. W., and Koshland, M. E. (1996) Immunity 5, 377–386
8. Strachan, T., and Read, A. P. (1994)Curr. Opin. Genet. Dev. 4, 427–438
9. Busslinger, M., and Urbanek, P. (1995) Curr. Opin. Genet. Dev. 5, 595–601
10. Wallin, J. J., Gackettetter, E. R., and Koshland, M. E. (1998) Science 279, 1961–1964
11. Neurath, M., Stuber, E. R., and Strober, W. (1995) Immunol. Today 16, 564–569
12. Michaelson, J. S., Singh, M., and Birshstein, B. K. (1996) J. Immunol. 156, 2349–2351
13. Neurath, M., Max, E. E., and Strober, W. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5336–5340
14. Staudt, L. M., and Lenardo, M. J. (1991) Annu. Rev. Immunol. 9, 373–398
15. Rao, E., Dang, W., Tian, G., and Sen, R. (1997) J. Biol. Chem. 272, 6722–6732
16. Kokubo, T., Takada, R., Yamashita, S., Geng, D. W., Roeder, R. G., Horikoshi, M., and Nakatani, Y. (1993) J. Biol. Chem. 268, 17554–17558
17. Chiang, C. M., and Roeder, R. G. (1995) Science 267, 531–536
18. Peterson, C. L., Orth, K., and Calame, K. L. (1986) Mol. Cell. Biol. 6, 4168–4178
19. McFadden, H. J., and Koshland, M. E. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11027–11031
20. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987) Current Protocols in Molecular Biology, Wiley-Interscience, New York
22. Grosschedl, R., and Baltimore, D. (1985) Cell 41, 885–897
23. Neurath, M., Strober, W., and Wakatsuki, Y. (1994) J. Immunol. 153, 730–742
24. Durand, D. B., Bush, M. R., Morgan, J. G., Weiss, A., and Crabtree, G. R. (1987) J. Exp. Med. 165, 395–407
25. Beckmann, H., Su, L.-K., and Kadesch, T. (1990) Genes Dev. 4, 167–179
26. Gregor, P. D., Sawadogo, M., and Roeder, R. (1990) Genes Dev. 4, 1730–1740
27. Navunkasitthas, S., Sawadogo, M., van Bijlen, M., Dang, C. V., and Chien, K. R. (1994) Mol. Cell. Biol. 14, 7331–7339
28. Halle, J.-P., Stelzer, G., Goppelt, A., and Meisterernst, M. (1995) J. Biol. Chem. 270, 21307–21311
29. Molkentin, J. D., Black, B. L., Martin, J. F., and Olson, E. N. (1995) Cell 83, 1125–1136
30. Pollock, R., and Treisman, R. (1991) Genes Dev. 5, 2327–2341
31. MacDonald, G. H., Itoh-Lindstrom, Y., and Ting, J. P-Y. (1995) J. Biol. Chem. 270, 3527–3533