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A B-Cell-Specific Nuclear Protein That Binds to DNA Sites 5′ to Immunoglobulin Sα Tandem Repeats Is Regulated during Differentiation

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Immunoglobulin heavy-chain switching is effected by recombination events between sites associated with tandemly repeated switch sequences located 5′ to immunoglobulin heavy-chain genes. Using the band mobility shift assay, we have identified two distinct sites 5′ to the α heavy-chain switch sequence with affinity for a single B-cell-specific DNA-binding protein, Sα-BP. Sα-BP was present in nuclear extracts from pre-B and B cells but was not detected in extracts from plasmacytomas, B-cell hybridomas, T-cell lymphomas, or a macrophage cell line. It was also not detectable in other nonlymphoid cells tested. Evidence suggests there are Sα-BP-binding sites near other immunoglobulin switch sequences. As with the Sα sites, these sites appear to be distinct from the consensus tandem repeats characteristic of immunoglobulin switch sequences. The possible functions of Sα-BP on contacting its binding sites are discussed in the context of immunoglobulin heavy-chain switch recombination.

Immunoglobulin M (IgM^+) B cells can undergo an immunoglobulin class switch, a process by which they switch to the expression of a different heavy-chain (H-chain) constant (C) region while maintaining the same antibody specificity (13). The expressed immunoglobulin C_H region is generally switched at the genomic level by recombination between switch (S) sequences, which consist of tandem direct repeats of short sequences located 5′ to each C_H gene except C_δ (Fig. 1A) (7, 18, 42). The S sequences differ between C_H genes, although they do have homologous elements (26). Recombination between Sμ and a downstream S sequence results in deletion of DNA located between the two sites of recombination (including the previously expressed C_μ gene) and association of the C_H gene downstream of the recombination site with the expressed H-chain variable-region gene (6, 44). Different H-chain classes possess various effector functions, so the result of such a C_H switch is to associate a single antibody-binding specificity with alternative effector functions.

The molecular mechanisms that control antibody class switching are not understood. Evidence to date indicates that transcription of unrearranged C_H genes precedes switching to the same gene, suggesting that accessibility of the C_H gene determines the frequency of switching to that gene (22, 37, 39, 43). However, the mechanism by which a C_H gene is selected for transcription and how this directs the subsequent switch recombination is unknown.

The normal cellular DNA recombinase presumably present in all dividing cells does not appear to be sufficient for effecting switch recombination. This can be concluded from experiments in which S sequences delivered into cells by retroviral vectors undergo switch recombination much more frequently in cells whose endogenous genes are undergoing switch recombination than in other cells (28). It is possible that there is a special switch recombinase or that the normal cellular DNA recombinase involved in the process but that additional factors are required to direct the recombinase to the S sequences.

We have begun a search for B-cell-specific DNA-binding factors that may be involved in directing or regulating switch recombination. We report here the identification of a nuclear protein which binds to two different sites in the region 5′ to the Sα tandem repeats and which is present in nuclear extracts from pre-B and B-cell lines and in spleen cells but not in extracts from plasmacytomas or B-cell hybridomas or from a variety of non-B cells.

MATERIALS AND METHODS

Preparation of nuclear extracts. Nuclear extracts were prepared as described by Dignam et al. (8), in some cases after isolation of nuclei (3); 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 10 μg of leupeptin per ml were added to solutions just before use. Protein concentrations were determined by the Bradford assay (supplied by Bio-Rad Laboratories, Richmond, Calif.).

Band shift assays. Band shift assays were carried out as described previously (34) except that 6% (bisacrylamide/acrylamide ratio of 1:29) gels were used in some experiments, and all gels were prepared and run in 0.5× TBE (pH 8.3; 50 mM Tris) running buffer. All DNA fragments were ^{32}P 3′ end labeled with Klenow enzyme (Boehringer-Mannheim Biochemicals, Indianapolis, Ind.) and α-^{32}P-labeled deoxynucleoside triphosphates (3,000 Ci/mmol; Dupont, NEN Research Products, Billerica, Mass., or Amersham Corp., Chicago, Ill.) to 2×10^7 to 5×10^7 cpm/μg; 2,000 to 5,000 cpm was loaded per lane.

Methylation footprinting experiments. DNA fragments ^{32}P labeled at one end only were treated with dimethyl sulfate for 10 s either before (methylation interference) or after (methylation protection) incubation with nuclear extract in a 10× reaction mixture (100 μg of extract protein and 1 to 2 ng of ^{32}P-end-labeled DNA in a total volume of 100 μl). In both cases, the methylation reaction was quenched by addition of 2 μl of β-mercaptoethanol. If quenching was delayed for 1 to 5 min, no protection was seen. The reaction mixture was

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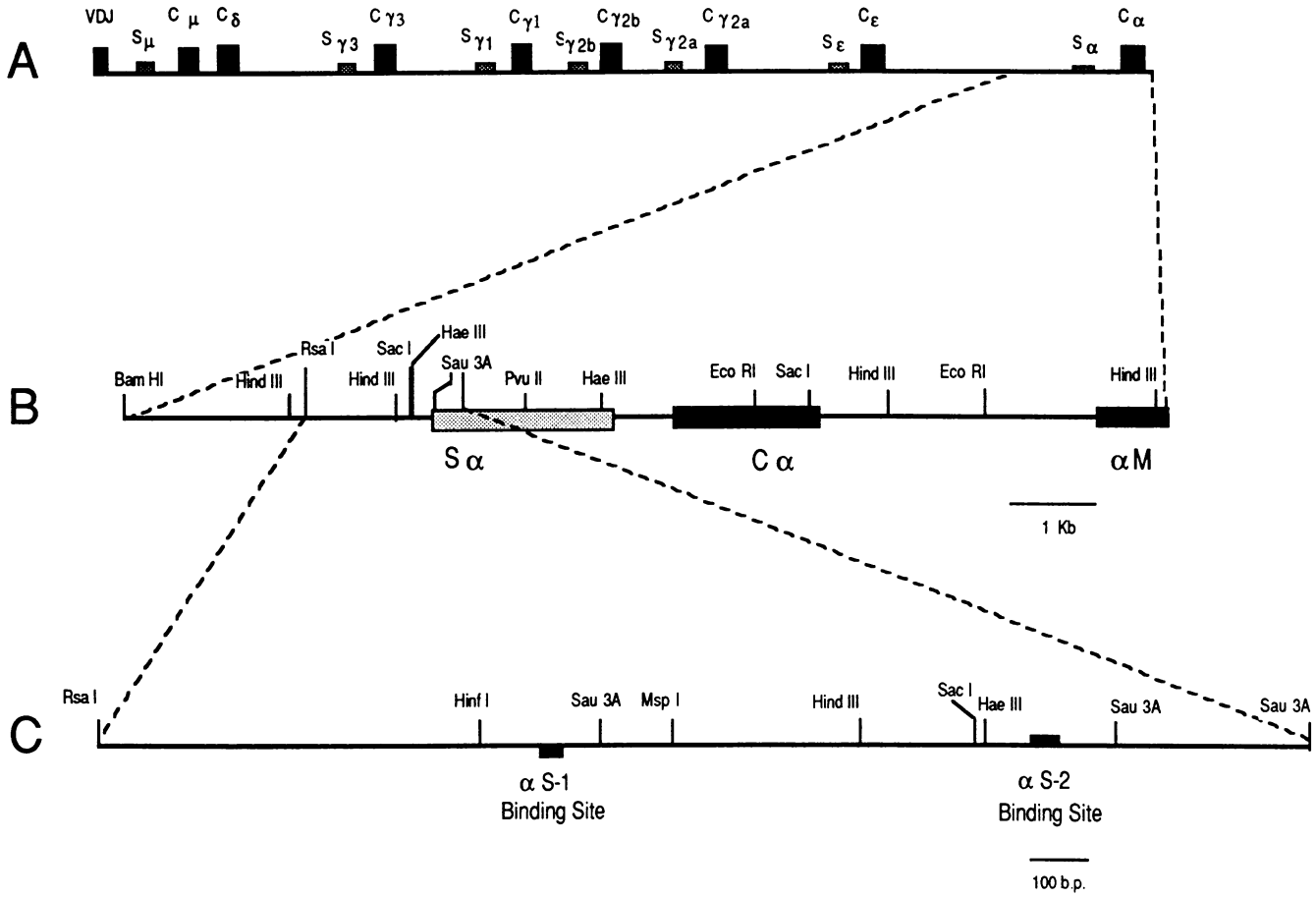

FIG. 1. Map of the immunoglobulin heavy-chain locus and germ line C_α gene, with locations of the binding sites αS-1 and αS-2 indicated. (A) Arrangement of immunoglobulin H-chain genes in a B cell expressing IgM (not to scale). (B) Restriction enzyme map of the germ line Sα-C_α-containing DNA segment in the I/St strain of mice. (C) Expanded restriction enzyme map of the Sα region indicating the two binding sites for the B-cell-specific nuclear factor. Fragments tested in the band shift assay included all those shown in line C except the *Sac*I-*Hae*III fragment. The Sα *Sau*3A-*Pvu*II and *Pvu*II-*Hae*III fragments (line B) were also tested.

loaded directly onto a polyacrylamide band shift gel for methylation protection; for methylation interference, the DNA was precipitated, ethanol washed, and dried by lyophilization before use in the 10× band shift assay. After electrophoresis, the bound and free bands were located by autoradiography of the wet gel and excised. The DNAs were eluted, concentrated by passage through a NACS ion-exchange column (Bethesda Research Laboratories, Inc., Bethesda, Md.), and precipitated. To cleave the DNA at the methylated guanine bases, samples were resuspended in 50 μl of 1 M piperidine and heated at 90°C for 30 min. Piperidine was removed by three cycles of lyophilization before the samples were loaded on 6% sequencing gels (24). The same end-labeled DNA was cleaved with the Maxam-Gilbert G- and G+A-specific chemical reactions to generate G and G+A marker lanes.

Competition experiments. ^{32}P-end-labeled DNA fragments (0.1 ng; 2,000 cpm per lane) were added to nuclear extracts with competitor DNAs and incubated for 15 to 30 min before electrophoresis through the band shift gel. The results obtained were the same irrespective of the order of addition to the reaction of labeled and competitor DNAs.

Subcloning and isolation of *Alu*I fragments containing binding sites αS-1 and αS-2. *Alu*I fragments of 39 and 47 base pairs (bp) containing binding sites αS-1 and αS-2, respectively, were ligated into the *Sma*I site of the plasmids Bluescribe and Bluescript (Stratagene, La Jolla, Calif.); 55- and 65-bp αS-1- and αS-2-containing fragments were excised by *Bam*HI-*Asp*718 and *Eco*RI-*Bam*HI restriction enzyme digestions, respectively. These digests released the subcloned *Alu*I fragments with a minimum of linker sequence. Fragments were separated on acrylamide gels and eluted from crushed gel pieces into 0.5 M ammonium acetate–1 mM EDTA. The eluate was separated from the acrylamide by centrifugation in microfuge tubes, filtered through 0.45-μm-pore-size membrane filters (Millipore Corp., Bedford, Mass.), and concentrated by butanol extraction. DNA was ethanol precipitated and suspended in Tris-EDTA (10 mM Tris hydrochloride [pH 7.5], 1 mM EDTA) for ^{32}P end labeling.

Other S-sequence plasmids. Sμ sequences were excised on a 1.8-kilobase-pair (kb) *Hin*dIII fragment of pM2-20 (23); *Eco*RI digestion of pBR1. 4 gave a 1.4-kb Sγ2b-containing fragment (21); Sγ1 fragments and subfragments were obtained from pγ1EH10 (25), and the latter were subcloned into Bluescribe (Stratagene).

RESULTS

Detection of nuclear B-cell-specific DNA-binding factors. We used the electrophoretic mobility shift assay (11, 12) to search for nuclear factors that may be involved in switch

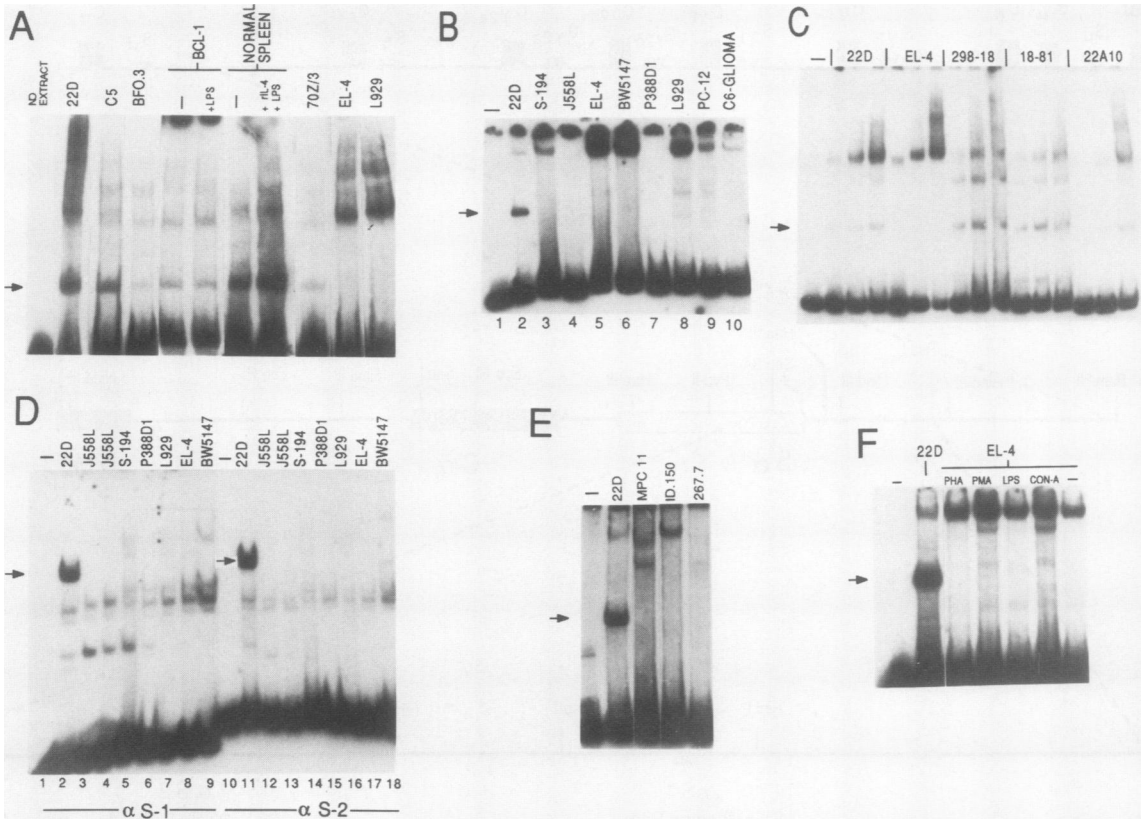

FIG. 2. Mobility shift assays to test cell type distribution of nuclear factors binding to fragments containing sites αS-1 and αS-2. Arrows indicate B-cell-specific bound bands. Nuclear extracts (5 or 10 μg of protein unless otherwise indicated) from the cell types indicated above the lanes were incubated with the following fragments: (A) *Hin*fI-*Msp*I (332 bp) fragment containing binding site αS-1 electrophoresed on a 4% acrylamide gel; (B) *Hin*fI-*Sau*3A (210 bp) αS-1 fragment (6% gel); (C) *Hae*III-*Sau*3A αS-2 fragment (248 bp; 5, 10, and 15 μg of nuclear extract protein used with each set of three lanes; 4% gel); (D) subcloned 39- and 47-bp *Alu*I fragments containing binding sites αS-1 and αS-2, respectively (6% gel); (E) *Hin*fI-*Sau*3A (αS-1) fragment incubated with nuclear extracts from 22D (10 μg), MPC11 (20 μg), ID.150 (20 μg), and 267.7 (15 μg) cells; (F) *Hin*fI-*Msp*I (αS-1) fragment incubated with nuclear extracts from EL-4 cells that had been treated for 3 days with phytohemagglutinin, phorbol myristate acetate, lipopolysaccharide, and concanavalin A (6% gel) (the same result was obtained with the αS-2 fragment [data not shown]).

recombination, testing restriction enzyme fragments from the region 5′ to and within the Sα segment extending from the 5′ *Hin*dIII site on the map shown in Fig. 1B to the *Hae*III site near the 3′ end of the Sα sequence. The legend to Fig. 1 indicates the fragments tested. All but two fragments gave identical binding patterns with nuclear extracts from B- and non-B-cell lines (data not shown). Two of the fragments (*Hin*fI-*Msp*I and *Hae*III-*Sau*3A; Fig. 1C) contained binding sites for factors present in nuclear extracts from B-lineage cells. Figures 2A and C display autoradiographs of band shift assays of the 332-bp *Hin*fI-*Msp*I and the 248-bp *Hae*III-*Sau*3A fragments, respectively, incubated with nuclear extracts from a variety of B- and non-B-cell lines; Fig. 2B and E show results for the 210-bp *Hin*fI-*Sau*3A fragment. Table 1 lists the cell lines used. The arrows in Fig. 2 indicate a retarded band observed after incubation of end-labeled DNA fragments with nuclear extracts from cell lines 22D (IgM^+), 22A10 (IgM^+), C5 (IgE^+), and BFO.3 (IgA^+), all of which were derived from the I.29 B-cell lymphoma. IgM^+ cells from this lymphoma can be induced to undergo H-chain switching in culture by treatment with lipopolysaccharide (38). Retarded bands of the same size were also observed after incubation with extracts from the B-cell leukemia BCL_1 (IgM^+), pre-B-cell lines 18–81, 298–18, and 70Z/3, and extracts from normal spleen cells. Bound bands of the same mobility were not detected with nuclear extracts from the T-cell lymphomas EL-4 and BW5147, with extracts from the macrophage-monocyte cell line $P388D_1$, or with extracts from the nonlymphoid cell line PC-12 (neuronal progenitor cells), C6 (glioma cells), or L929 (fibroblasts). The lack of binding activity in the EL-4 extract did not appear to be due to the presence of an inhibitory factor, since mixing the EL-4 and 22D extracts did not reduce the binding activity present in the 22D extracts (data not shown). Furthermore, this factor (or factors) could not be induced in EL-4 T cells after treatment with reagents that activate T cells (phorbol myristate acetate, phytohemmaglutinin, or concanavalin A) or with lipopolysaccharide (which does not activate T cells) (Fig. 2F).

This band was not detected after incubation of the *Hin*fI-*Sau*3A fragment with nuclear extracts from the plasmacytoma cell line S194 or J558L (Fig. 2B, lanes 3 and 4) or MPC11 (Fig. 2E) or from the B-cell hybridomas 267.7 and ID.150 (Fig. 2E). The hybridoma ID.150 was derived by fusion of an IgA^+ cell from the I.29 lymphoma with a plasmacytoma cell (40), and 267.7 was derived by fusion of an antigen-specific normal B cell with a plasmacytoma cell (19). Therefore, this binding activity is present in pre-B- and B-cell lines expressing a variety of isotypes and also in normal spleen cells but appears to be lost or inactivated

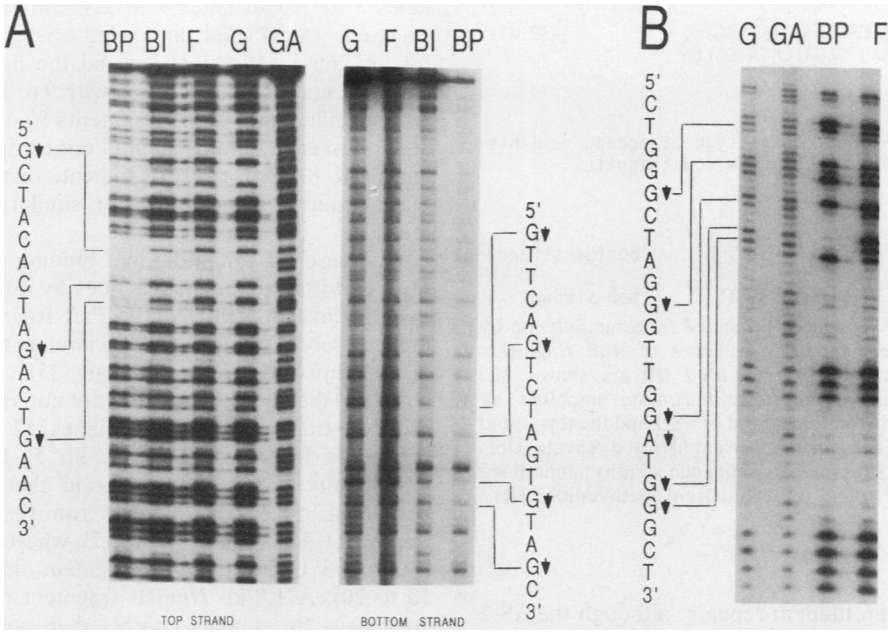

FIG. 3. Methylation footprints of the B-cell-specific factor bound to the αS-1 and αS-2 sites. (A) Footprint on a 3'-end-labeled fragment containing binding site αS-1. Lanes: BP, methylation protection ladder; BI, methylation interference ladder; F, free (unbound) DNA methylation ladder; G, methylation ladder; GA, acid depurination ladder. Arrows next to guanine nucleotides in sequences indicate methylation protection. (B) Footprint on a 3'-end-labeled *Alu*I fragment containing binding site αS-2 by methylation protection. The footprint on the top strand is shown. No methylation protection of the single G on the bottom strand was seen.

when mature B cells differentiate to secretion of immunoglobulin. This binding activity was not further induced by treatment of pre-B-cell (70Z/3) and B-cell (22D, 22A10, and BCL-1) lines and normal spleen cells with lipopolysaccharide, interleukin-4, or both (Fig. 2A and data not shown).

By testing subfragments of the *Hin*fI-*Sau*3A and *Hae*III-*Sau*3A fragments in the band shift assay, we localized their binding sites to small *Alu*I fragments and designated these sites αS-1 (the 5' site) and αS-2, respectively (Fig. 1C). By using these fragments, a specific retarded band was detected after incubation with nuclear extracts from B and pre-B cells but not after incubation with extracts from plasmacytomas, T-cell lymphomas, or a macrophage line or from the nonlymphoid lines tested (Fig. 2D and data not shown).

TABLE 1. Cell lines

Designation	Description	Reference or ATCC no.
298-18	Abelson leukemia virus-transformed pre-B-cell line	2
18-81	Abelson leukemia virus-transformed pre-B-cell line	4, 32
70Z/3	Pre-B-cell line	29
22D	IgM^+ line from I.29 B-cell lymphoma	1
22A10	IgM^+ line from I.29 B-cell lymphoma	1
C5	IgE^+ line from I.29 B-cell lymphoma	39
BFO.3	IgA^+ line from I.29 B-cell lymphoma	35, 36
BCL-1	IgM^+ B-cell leukemia	20, 31
S194	Plasmacytoma	16
J558L	Plasmacytoma	27
MPC11	Plasmacytoma	10
267.7	B-cell hybridoma	19
ID.150	B-cell hybridoma	40
EL-4	T-cell lymphoma	TIB 39
BW5147	AKR thymoma	17
$P388D_1$	Macrophage-monocyte cell line	TIB 63
L929	Fibroblast cell line	Flow Laboratories, Inc., McLean, Va.
C6 glioma	Glioma cell line	CCL 107
PC-12	Pheochromocytoma cell line	15

Methylation footprints of the B-cell-specific factor(s) bound to sites αS-1 and αS-2. To precisely locate the DNA sequences that bind this factor (or factors), we performed methylation interference (33) and methylation protection (41) experiments. End-labeled fragments containing the two B-cell-specific binding sites were partially methylated with dimethyl sulfate either before (methylation interference) or after (methylation protection) incubation with nuclear extracts. The bound fragments were separated from the free fragments by electrophoresis through a band shift gel, and the bound and free fragments were visualized by autoradiography of the wet gel. Bound and free DNA was eluted from the gel and subjected to piperidine cleavage at the methylated guanine bases. Figure 3 shows the piperidine cleavage ladders of free DNA and of DNA where bound protein interfered with or protected DNA from methylation alongside chemical G and G+A sequence ladders (24). Protected guanine residues extended over 15 (αS-2) to 17 (αS-1) nucleotides. No methylation protection of the single G on the bottom strand of site αS-2 was seen (data not shown). Footprints were not detected at either αS-1 or αS-2 in methylation interference or DNase protection experiments, possibly because the binding factor dissociates readily from the DNA.

The sequences of the *Alu*I fragments containing the αS-1 and αS-2 sites (Fig. 4A and B) differ from each other, although if they are arranged as inverted repeats and then compared, 50% of their nucleotides are identical (Fig. 4C). Neither of these fragments is located within the region

A
ctcaactcatgccagccaaGTTCAGTCTAGTGTAGCaag -39 nts.
gagttgagtacggtcggttCAAGTCAGATCACATCGttc

B
ctggactggGCTAGGGTTGGATGGgctcaataactgggctaatccaag -48 nts.
gtggtgaccCGATCCCAACCTACCcgagttattgacccgattaggttc

C
αS-1 5' GCTACACTAGACTGAAC 3' (bottom strand)
αS-2 5' GCTAGGGTTGGATGG 3' (top strand)

FIG. 4. Alignment of sequences protected from methylation by the B-cell-specific nuclear factor. Sequences of *Alu*I fragments containing binding sites αS-1 (A) and αS-2 (B) are shown. (C) Binding sequences aligned to illustrate maximum homology by writing the sequence of the bottom strand of αS-1 and the top strand of αS-2 so that the sequences form imperfect inverted repeats. Dots between aligned binding sites indicate sequence identity; underlined and overlined bases are those protected from methylation. Nts., Nucleotides.

containing the Sα 80-bp tandem repeats, although the αS-2 site is located only 120 bp 5′ to a 320-bp *Sau*3A fragment that consists of four Sα 80-bp consensus repeats (9) (Fig. 1).

Binding sites αS-1 and αS-2 compete with each other and with other S sequences for binding of the B-cell-specific nuclear factor. Because the cell specificity of the binding activities for these two sites appeared identical, and because the bound DNA fragments had similar mobilities in the band shift experiments (Fig. 2D, lanes 2 and 11), we decided to determine whether these two different DNA sequences were actually binding the same factor. We examined this by competition band shift experiments and found that the unlabeled αS-2 *Alu*I fragment competed with a labeled αS-1 *Hin*fI-*Sau*3A fragment for binding to the nuclear factor as well as did the homologous fragment (Fig. 5A; compare lanes 3 to 5 with lanes 9 to 11). Similarly, when the αS-2 fragment was labeled, unlabeled αS-1 appeared to compete for binding as effectively as did the homologous fragment (Fig. 5B; compare lanes 3 to 5 with 9 to 11). The competition was specific, since DNA fragments from the region immediately 3′ to each binding site did not compete (Fig. 5A and B, lanes 6 to 8). These results indicate that these two different DNA sequences both bind with similar affinities to a single factor.

We searched for additional binding sites for this factor near or within other S sequences by additional competition experiments. A 2-kb *Hin*dIII-*Pst*I fragment that is located immediately 5′ to the Sγ1 49-bp tandem repeats, but does not include any of the tandem repeats (25), competed for binding with the labeled αS-1 fragment but not as well as did the αS-1 or αS-2 fragment (Fig. 5A, lanes 12 to 14). When two fragments from within this 2-kb 5′ Sγ1 fragment were individually tested, it was found that only one of them competed, a 0.55-kb fragment from the middle of the 2-kb fragment (Fig. 5A, lanes 15 to 17), whereas a 0.7-kb fragment from the 3′ end of the 2-kb fragment did not compete (lanes 18 to 20). A 1.8-kb *Hin*dIII fragment consisting mostly of consensus Sμ repeats competed in some experiments for binding to αS-2 but usually not as well as in the experiment shown in Fig. 5B (lanes 12 and 13); this Sμ fragment competed less well with αS-1 (Fig. 5A, lanes 24 to 26). The site (or sites) within the 1.8-kb Sμ fragment probably does not consist of consensus Sμ sequences, since an 80-bp Sμ fragment containing Sμ tandem repeats that was subcloned from the 1.8-kb *Hin*dIII fragment did not compete for the B-cell-specific factor (data not shown). Similarly, a 320-bp *Sau*3A fragment consisting of Sα consensus repeats (Fig. 1C) competed poorly (data not shown). A 0.8-kb *Eco*RI-*Hin*dIII fragment located 5′ to the 1.8-kb *Hin*dIII fragment did not compete (data not shown). A 1.4-kb fragment containing the 3′ portion of the Sγ2b repeats and the adjacent 3′

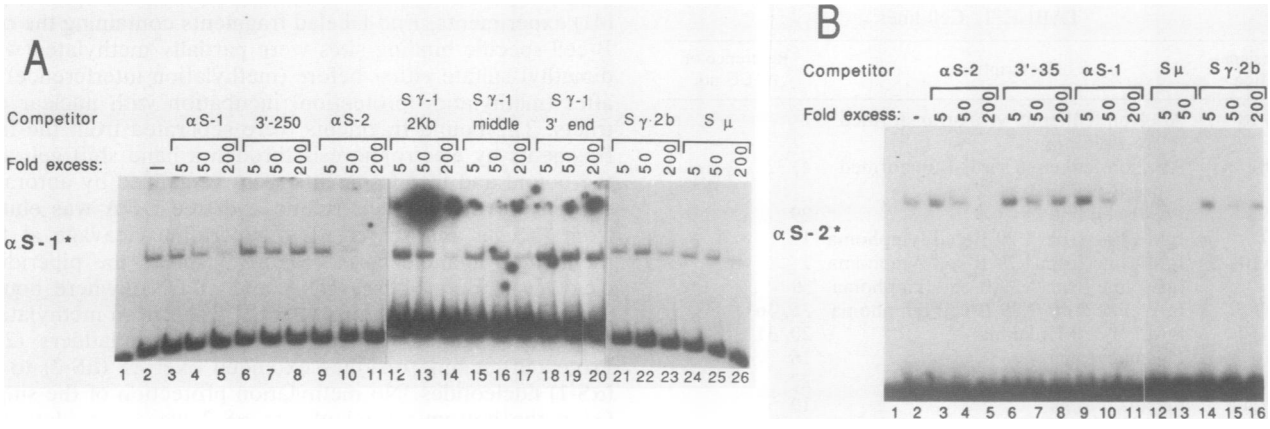

FIG. 5. Mobility shift competition experiments. (A) The ^{32}P-end-labeled 210-bp *Hin*fI-*Sau*3A fragment (from the *Hin*fI-*Msp*I fragment [Fig. 1C]) containing the αS-1 binding site incubated with 10 μg of 22D nuclear extract protein and with the indicated unlabeled competitor DNA fragments at 5-, 50-, and 200-fold molar excess. (B) The ^{32}P-end-labeled *Alu*I αS-2 fragment incubated with 4 μg of 22A10 nuclear extract protein with or without competitor DNA. Competitor fragments: αS-1, subcloned *Hin*fI-*Sau*3A fragment (210 bp) (A) and subcloned *Alu*I fragment (55 bp) (B) containing the αS-1 binding site; αS-2, subcloned *Alu*I fragment (65 bp) containing the αS-2 binding site; 3′-250, the 250-bp *Msp*I-*Hin*dIII fragment located 3′ to the αS-1 binding site; Sγ1 2Kb, a *Hin*dIII-*Pst*I fragment subcloned from the 5′ end of pγ1EH10 (25) (p5′γ1HP-2); Sγ1 middle, a 0.8-kb *Bam*HI-*Kpn*I fragment from p5′γ1HP-2; Sγ1 3′ end, a 0.7 kb *Kpn*I-*Pst*I fragment from p5′γ1HP-2; Sγ2b, a 1.4-kb *Eco*RI fragment from pBR1.4 containing a portion of the Sγ2b sequence (21); Sμ, a 1.8-kb *Hin*dIII fragment subcloned (pM2-20) from the 5′ portion of Sμ (23); 3′-35, a subcloned 35-bp *Alu*I fragment located 3′ to the αS-2 binding site (from the 250-bp *Hae*III-*Sau*3A fragment).

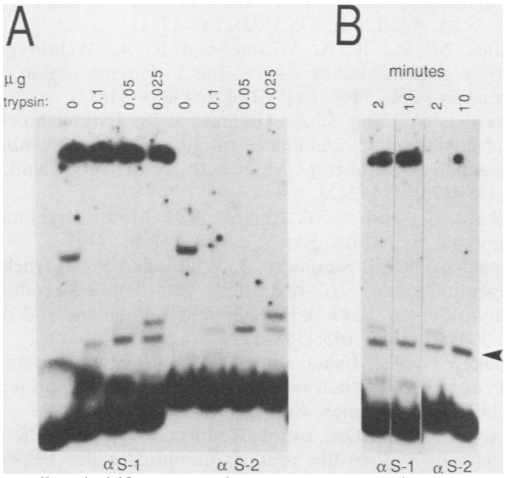

FIG. 6. Band shift assay using 22D extracts after partial proteolysis with trypsin. (A) 22D nuclear extracts (5 μg of protein) treated for 10 min with 0.1, 0.05, or 0.025 μg of trypsin were incubated with ^{32}P-end-labeled *Alu*I fragments containing binding site αS-1 or αS-2 (as indicated). (B) 22D nuclear extracts (5 μg) were incubated for 2 or 10 min with 0.05 of μg trypsin and used in the band shift assay. The arrowhead indicates the band corresponding to DNA bound to the smallest tryptic fragment of Sα-BP that retained binding activity.

sequences competed poorly (Fig. 5A, lanes 21 to 23; Fig. 5B, lanes 14 to 16).

Although the mass excess of nonspecific competitor DNA associated with the introduction of a 200-fold molar excess of the 1.8-kb Sμ competitor fragment is large, it is less than 10% of the poly(dI-dC) · poly(dI-dC) nonspecific competitor DNA routinely included in the band shift assay. Although some of the competition seen at this molar excess may be due to the large mass excess of nonspecific competitor, the data suggest that there may be additional binding sites for this B-cell factor near other S regions.

Partial proteolysis of Sα-BP indicates that the same-size domain binds to sites αS-1 and αS-2. Competition between αS-1 and αS-2 for the B-cell-specific binding factor indicated that these sites bound the same factor despite their distinct sequences. To provide further evidence that a single factor is involved, we compared the αS-1 and αS-2 *Alu*I fragments side by side in a band shift assay using partially proteolyzed nuclear extracts from 22D cells. Figure 6 shows the results of one such band shift experiment using nuclear extracts digested for various times or with various amounts of trypsin. The identical patterns of bound bands observed with digested extracts indicated that the two proteolytic fragments retaining the DNA-binding domain for αS-1 and αS-2 were indistinguishable in size. These results further support the conclusion that sites αS-1 and αS-2 bind the same protein. We have termed this protein Sα-BP.

DISCUSSION

The band shift assay was used to identify a nuclear B-cell-specific DNA binding protein, Sα-BP, with two binding sites 5′ to Sα. Sα-BP is present in nuclear extracts from all pre-B- and B-cell lines tested as well as in extracts from normal spleen cells. Sα-BP was not detected in extracts from three plasmacytomas or from two B-cell hybridomas, nor was it detected in extracts from any of the non-B-cell lines tested, including cells of the lymphoid lineage, T-lymphomas BW5147 and EL-4, and the macrophage-monocyte cell line P388D$_1$. To the extent that transformed cells are representative or normal B-cell stages, these results indicate that Sα-BP is regulated during differentiation of the B lineage, being present in pre-B and B cells but absent from plasma cells that are mature B cells terminally differentiated for secretion of immunoglobulin. The presence of Sα-BP cannot be sufficient to allow a cell to undergo H-chain switching, since it is present in cells that do not switch, e.g., pre-B cells and BCL-1 cells, and also in the postswitch cell lines C5 (IgE$^+$) and BFO.3 (IgA$^+$) derived from the I.29 B cell line. It is interesting that the plasmacytoma and hybridoma cell lines we tested consistently lacked Sα-BP, because both the MPC11 plasmacytoma and 267.7 hybridoma cell lines have been shown to undergo H-chain switching in culture, but the sites of recombination do not involve the tandemly repeated switch sequences (10, 19). Consequently, it is possible that Sα-BP is a factor necessary, but by itself not sufficient, for normal switch recombination using S sequences.

The nucleotide sequences of binding sites αS-1 and αS-2 were quite distinct, with a maximum of 50% identity. However, competition experiments revealed that the binding sites αS-1 and αS-2 compete for binding to Sα-BP, indicating that Sα-BP possesses a binding site capable of binding these two distinct sequences. Although it is formally possible that Sα-BP has different binding sites for αS-1 and αS-2, occupancy of one of which causes a conformational change in Sα-BP that prevents binding to the other, this is unlikely given the identical pattern of bound bands seen when nuclear extracts subjected to partial digestion with trypsin were incubated with DNA fragments containing the two different binding sites. There are precedents for a single DNA-binding protein recognizing different binding sites. The yeast HAP1 transcription activator binds distinct sequences upstream of the *CYC1* and *CYC7* genes (30), and the HeLa cell protein TEF-1 binds two different simian virus 40 enhancer motifs (5).

We do not know the function of Sα-BP. Attempts to show *cis*-acting enhancer activity by Sα-BP binding sites αS-1 and αS-2 in transient transfection assays with the binding sites inserted in a *fos* promoter-driven chloramphenicol acetyltransferase expression vector (14) as monomers, as homo- and heterodimers, and as homotrimers have yielded negative results (data not shown). These results argue against involvement of Sα-BP in the induction of transcription that appears to precede switch recombination (22, 37). The binding sites for Sα-BP are located 1.4 and 2.3 kb 3′ to the initiation site for the major RNA species transcribed from the unrearranged C_α gene in IgM$^+$ cells of the I.29 lymphoma (37; G. Radcliffe, Y. C. Lin, M. Julius, K. Marcu, and J. Stavnezer, submitted for publication). Possible functions of the protein in immunoglobulin H-chain switch recombination include aligning S-sequence DNAs in order to promote recombination, association with switch recombination proteins in a recombination complex, or attaching S sequences to nuclear matrix components within a putative recombination compartment of the nucleus. A recent examination of postswitch Sμ-Sα sequences has revealed nucleotide substitutions, duplications, and deletions of portions of Sα at recombination sites (9). To account for this observation, a model has been proposed in which DNA polymerase strand switching mediates switch recombination, resulting in mutations and sequence duplications in the products on resolution of the recombination intermediates. In this model, binding sites αS-1 and -2 could function as replication initiation sequences. The competition seen with heterologous S sequences suggests that specific sites that bind Sα-BP

may be associated with other S sequences; these sites were not composed of the switch region tandem direct repeats.

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ADDENDUM ADDED IN PROOF

We have now localized the binding site for Sα-BP in the 5′ $S_{\gamma 1}$ fragment. Sα-BP binds at a site located about 300 bp 5′ to the initiation sites of RNAs transcribed from the unrearranged $C_{\gamma 1}$ gene in spleen cells treated with interleukin 4.

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