Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 3560–3564, May 1988
Immunology

Identification of murine nuclear proteins that bind to the conserved octamer sequence of the immunoglobulin promoter region

(DNA-binding proteins/purification/renaturation)

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Communicated by Edwin D. Kilbourne, January 26, 1988 (received for review October 30, 1987)

ABSTRACT **Sequence-specific DNA-affinity chromatography was used to purify a nuclear protein from the B-cell leukemia cell line BCL_1 that specifically binds to the octamer sequence ATTTGCAT, previously shown to be important in the regulation of immunoglobulin genes. This protein has a molecular mass of approximately 70 kDa and is responsible for the protein–DNA interaction specific to lymphoid cells. Other proteins of molecular mass 80–90 kDa and 50–55 kDa that specifically bind to the octamer sequence were also identified. These results demonstrate that the octamer is recognized by several biochemically distinct nuclear proteins, perhaps to differentially regulate the expression of immunoglobulin genes.**

Recent studies indicate that gene transcription may be modulated by DNA sequence both within the transcription unit and in flanking sequences (1–3). Promoters and enhancers represent two classes of DNA elements that appear to cooperate in the control of efficient transcription. Enhancer elements can potentiate transcription from an appropriate promoter region in a tissue-specific manner, somewhat independent of their orientation or distance from the promoter. The promoter region contains sequences upstream of the transcription initiation site that are involved in the tissue-specific activation or repression of transcription. Although it is not clear how these DNA elements function, it has been demonstrated that they specifically bind nuclear proteins that may be involved in the control of gene expression (1–4).

The octameric sequence ATTTGCAT represents an important regulatory element in the promoter region of immunoglobulin genes (5–13). This conserved sequence is found 70–90 nucleotides 5′ to the site of transcription initiation of both heavy and light chain variable-region genes. Deletion of DNA sequences that encompass the octamer abolishes immunoglobulin promoter activity (5–8, 13). Moreover, several studies have shown that the octamer is sufficient to impart B-cell specificity to variable-region promoters (7, 8, 10–12). Interestingly, this sequence also appears to be a transcriptional control element in the promoters of genes that are expressed in other tissues (14–22). Thus, this sequence may represent a flexible control element that is acted upon by various cell-type-specific transfactors to regulate transcription.

Employing the electrophoretic migration inhibition assay and DNase I protection studies, several groups have demonstrated that nuclear proteins from B cells, T cells, and nonlymphoid cells bind directly to the octameric sequence, giving rise to several protein–DNA complexes (23–29). We previously identified four complexes (arbitrarily numbered 1–4) in nuclear extracts from the B-cell leukemia cell line BCL_1 (23, 24). Comparison of this pattern to the patterns obtained with nuclear extracts from other cell lines revealed that species 1 and 2 were expressed in both lymphoid and nonlymphoid cell lines (23). In contrast, species 3 was specific to lymphoid cells that transcribe immunoglobulin genes suggesting that this protein–DNA interaction may be involved in the selective expression of immunoglobulin genes in B cells. Subsequent experiments performed in our laboratory have demonstrated that species 4 represents a protein–DNA interaction specific to some, but not all, B-cell lines that express immunoglobulin transcripts (ref. 23 and unpublished data). In this paper, both conventional and DNA affinity chromatography were used to identify a 70-kDa protein responsible for the lymphoid-specific protein–DNA interaction from the BCL_1 cell line. We also describe the involvement of at least two other proteins with molecular masses of approximately 80–90 kDa and 50–60 kDa that specifically bind to the octamer sequence, giving rise to species 1, 2, and 4. Together these results suggest that distinct nuclear proteins bind to a common transcription regulatory element, perhaps to differentially regulate immunoglobulin expression.

MATERIALS AND METHODS

Gel Migration Inhibition Assay. The gel migration inhibition assay was performed as previously described (23, 24), using either a 3′-end-labeled (ref. 30, pp. 115–116) 322-base-pair (bp) *Bam*HI/*Nco* I restriction fragment or a 5′-end-labeled (ref. 30, p. 122) 188-bp *Hae* III/*Nco* I restriction fragment. Both fragments were derived from the plasmid PMK3 (23, 24, 31), which contains the heavy chain variable region gene expressed in the BCL_1 cell line. In some cases, 1 μg of heparin sodium salt (Sigma) was substituted for sheared salmon sperm DNA. For affinity-purified fractions, the binding assay was performed in the absence of competitor.

Purification of the Lymphoid-Specific Octamer-Binding Factor from Nuclear Extracts of BCL_1 Cells. The nuclear extracts were prepared from cell lines grown either *in vitro* or *in vivo* as described (32). The *in vivo* BCL_1 cells were teased from the spleens of BALB/c mice injected at least 4 weeks beforehand in the peritoneum with BCL_1 cells. The BCL_1 tumor cells represented greater than 85% of the cells recovered from the spleens of these mice. In a typical purification, approximately 150 mg of nuclear extract derived from the BCL_1 cells grown *in vivo* was precipitated by 50% ammonium sulfate and centrifuged at 12,000 × *g* for 30 min and the pellet was resuspended in buffer 1 [20 mM Hepes, pH 7.9/20% (vol/vol) glycerol/0.1 M KCl/0.2 mM EDTA/0.5 mM phenylmethylsulfonyl fluoride/1 mM dithiothreitol]. The solubilized pellet was applied to a (3 cm × 70 cm) Sephacryl S-300 column (Pharmacia) equilibrated with buffer 1 and fractions containing octamer-binding activity were pooled and applied to a 10-ml DEAE Bio-Gel agarose column (Bio-Rad) equilibrated with buffer 1. The octamer-binding activity flowed through the column under these conditions and was subsequently applied to a 5-ml column containing heparin-agarose (Bethesda Research Laboratories) equilibrated with buffer 1. The column was washed with buffer 1

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and the bound octamer-binding proteins were eluted with 2 column volumes of buffer 1 containing 0.4 M KCl. The sequence-specific DNA-affinity column was generated by first synthesizing a 44-base oligonucleotide containing the octamer sequence in the middle and an aminoethyl phosphate group at the 5′ end by using an Applied Biosystems model 380A DNA synthesizer and Aminolink-1 (Applied Biosystems, Foster City, CA). The 5′-amino-oligonucleotide was then gel purified, annealed with a complementary oligonucleotide lacking the 5′ amino group, and attached to Affi-Gel 10 (Bio-Rad) activated support in 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (pH 4.8). The coupling reaction was allowed to proceed overnight at 4°C, after which the resin was thoroughly washed and stored in buffer containing 10 mM Tris·HCl (pH 7.6), 0.3 M NaCl, 1 mM EDTA, and 0.2% sodium azide. DNA-affinity chromatography was performed essentially as described with modifications (4). Briefly, the heparin-agarose eluate was equilibrated to 0.1 M KCl in buffer 1 and heparin was added to a concentration of 0.4 mg/ml as a competitor for nonspecific DNA-binding proteins. The mixture was allowed to stand for 10 min at 4°C and then was added to a 3 ml DNA-affinity column. The bound proteins were washed with at least 5 column volumes of buffer 1 and the octamer-binding activity was eluted by sequential additions of buffer 1 containing 1 M KCl and buffer 1 containing 0.5 M KCl. The 1 M and 0.5 M KCl eluates were pooled and equilibrated to 0.1 M KCl for a second pass over a 1-ml affinity column in the presence of heparin at 0.2 mg/ml.

NaDodSO$_4$/PAGE. Nuclear extracts were analyzed by NaDodSO$_4$/PAGE at various stages of purification by mixing the extracts with an equal volume of nonreducing NaDodSO$_4$/solubilizing buffer [0.0625 M Tris·HCl, pH 6.8/10% (vol/vol) glycerol/3% NaDodSO$_4$] and boiling the mixture for 2 min. The samples were then loaded onto a slab gel containing a 4% stacking gel and a NaDodSO$_4$/10% polyacrylamide separating gel (33) and subjected to electrophoresis at a constant current of 25 mA. Samples for elution and renaturation were similarly treated. Silver staining was performed as described (34).

Elution and Renaturation of Octamer-Binding Proteins from NaDodSO$_4$/Polyacrylamide Gels. Nuclear proteins separated by NaDodSO$_4$/PAGE were recovered as described (35) with modifications. Between 0.1 and 0.3 mg of unfractionated nuclear extract was separated on a (14 × 16 cm) 10% separating gel, the appropriate lane was cut into sections with a razor blade, and the proteins were eluted by crushing the gel pieces with a glass rod and soaking them for 2 hr at room temperature in 0.5 ml of 0.1% NaDodSO$_4$/0.05 M Tris·HCl, pH 7.5/0.1 mM EDTA/1 mM dithiothreitol/0.1 M KCl, containing fraction V bovine serum albumin (Boehringer Mannheim) at 0.1 mg/ml as a carrier protein. The gel pieces were pelleted by centrifugation and the proteins were freed of NaDodSO$_4$ by precipitation with a least 3 vol of acetone for 30 min in a dry ice bath. The proteins were pelleted by centrifugation and the protein pellets were rinsed once with 80% acetone to remove residual NaDodSO$_4$ and then dried under reduced pressure. The pellets were resuspended in 20 μl of boiling hot 6 M guanidine hydrochloride and allowed to incubate at room temperature for 15 min prior to the addition of 1 ml of buffer 1. This mixture was incubated at room temperature for 2–12 hr to renature the proteins. After this, the protein mixture was diluted further with 1 ml of buffer 1 and concentrated in Centricons (Amicon) prior to assay in the gel migration inhibition assay. Two times affinity-purified nuclear extracts (10-fold concentrated) were similarly treated, except apomyoglobin (Beckman) was used in place of bovine serum albumin as the carrier protein.

DNase I "Footprinting." The DNase I protection (footprint) analysis was performed as described (24), using the 188-bp *Hae* III/*Nco* I restriction fragment labeled at the 5′ end (ref. 30, p. 122).

RESULTS

Purification of the Protein Responsible for the Lymphoid-Specific Protein–DNA Interaction. To obtain large numbers of cells for purification of the octamer-binding proteins, we used nuclear extracts derived from BCL$_1$ cells grown *in vivo* because as many as 1 × 10^9 cells could be obtained from each mouse spleen. Examination of the protein–DNA interactions

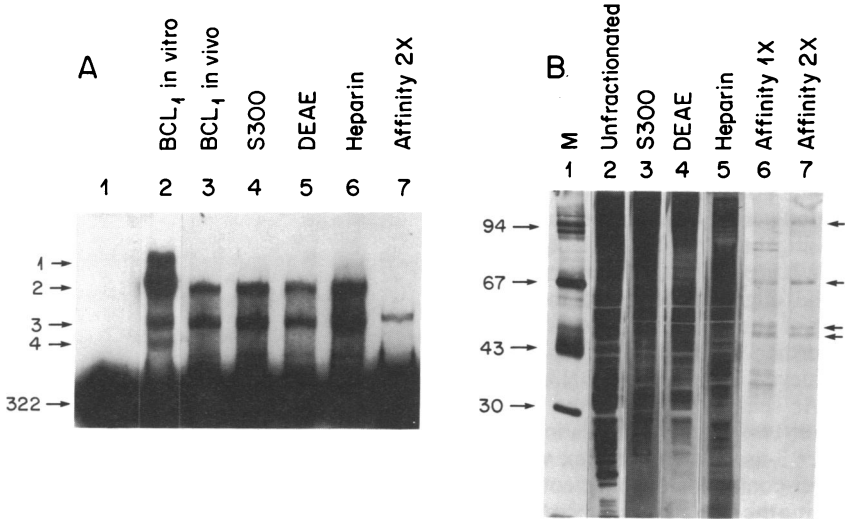

FIG. 1. Purification of octamer-binding proteins by column chromatography. (*A*) The gel migration inhibition assay was used to assay for the octamer-binding proteins after each chromatography step. Protein–DNA complexes (species) 1–4 are indicated by arrows along the left margin. The first lane shows the migration of the free 322-bp fragment in the absence of nuclear extract. (*B*) NaDodSO$_4$/PAGE was used to examine the proteins contained in unfractionated nuclear extract from BCL$_1$ cells grown *in vivo* (lane 2), the S-300 pool subsequently applied to the DEAE column (lane 3), the DEAE column flow-through (lane 4), the heparin-agarose column 0.4 M KCl eluate (lane 5), and the eluates from one (lane 6) and two (lane 7) sequence-specific DNA-affinity column chromatography steps. The molecular mass (M) markers phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), and carbonic anhydrase (30 kDa) in lane 1 are indicated along the left margin. Predominant proteins visualized by silver staining in lane 7 are indicated with small arrows on the right margin.

formed when nuclear extracts derived from these cells were used revealed two protein–DNA complexes (Fig. 1*A*, lane 3). These two protein–DNA complexes comigrated with species 2 and 3 of the *in vitro* grown BCL_1 pattern (Fig. 1*A*, compare lanes 2 and 3). This pattern is similar to the pattern we previously obtained with nuclear extracts derived from *in vitro* BCL_1 cells and normal B cells stimulated with lipopolysaccharide (23, 24), suggesting that the cells grown *in vivo* may be activated.

A combination of conventional and sequence-specific DNA-affinity chromatography was employed to purify the proteins that specifically bound to the octamer sequence (Fig. 1). Nuclear extracts derived from BCL_1 cells grown *in vivo* were adjusted to 50% ammonium sulfate, and the precipitated proteins were separated on an S-300 gel filtration column. A peak of octamer-binding activity involving both species 2 and 3 reproducibly eluted off the column immediately after the major protein peak at an elution/void volume of 1.3–1.4 (data not shown). Thus, in a manner similar to that described for transcription factor Sp 1 (4), the proteins responsible for species 2 and 3 migrated as native complexes of molecular mass greater than 200 kDa. Samples containing species 2 and 3 were pooled and applied to a DEAE column, and the DEAE flow-through was subsequently bound to a column containing heparin-agarose that had been equilibrated with buffer containing 0.1 M KCl. The octamer-binding proteins responsible for species 2 and 3 were recovered from the heparin-agarose column by using buffer containing 0.4 M KCl. Fig. 1*A* shows an analysis, using the gel migration inhibition assay, of the octamer-binding proteins contained in the S-300 pool (lane 4), DEAE flow-through (lane 5), and heparin-agarose eluate (lane 6). Although it was not possible to quantitate the level of activity of these proteins accurately after each column chromatography step, we estimate a 9- to 10-fold purification of the species 3 octamer-binding proteins, with a 50–60% recovery of the total activity (data not shown). These estimates were obtained by titrating each purified fraction in the gel migration inhibition assay and comparing the amount of radioactivity shifted into species 3 by each column fraction to the radioactivity shifted into species 3 by unfractionated extract.

$NaDodSO_4$/PAGE analysis of the active fractions after each chromatography step revealed a complex mixture of proteins (Fig. 1*B*). To obtain further purification of the octamer-binding proteins, we employed sequence-specific DNA-affinity chromatography using 5′-amino-oligonucleotides coupled to Affi-gel 10. Interestingly, species 3 was the predominant protein–DNA complex observed in material eluted after one DNA-affinity chromatography step, while activity comigrating with species 2 remained predominantly in the flow-through fraction (data not shown). This was surprising, since species 2-associated activity was formed in the gel migration inhibition assay using the same oligonucleotides that were attached to the column. It is possible that steric restraints imposed by conjugating the oligonucleotides to the beads prevented efficient binding of the species 2-associated protein(s) to the column. Further purification of species 3-associated protein was achieved by a second DNA-affinity column purification step. As seen in Fig. 1*A* (lane 7), only species 3 was observed after two affinity purification steps. This affinity-purified species 3-associated complex was specifically inhibited by octamer-containing oligonucleotides but not by oligonucleotides lacking the octamer sequence (data not shown). The two-times purified fraction represents a purification of approximately 15,000–20,000-fold, with a cumulative yield of 10–30% of the total activity as determined by estimates of the binding activity as described above.

$NaDodSO_4$/PAGE examination of the proteins eluted after one DNA-affinity chromatography step (Fig. 1*B*) revealed a marked reduction in the number of the proteins visualized by silver staining (compare lanes 2–5 with lane 6). Moreover, two passes over the DNA-affinity column reduced the protein pattern to several predominant bands (Fig. 1*B*, lane 7). Two proteins are seen close together between 50 and 55 kDa (lower two arrows). Other protein bands are visible at approximately 70 kDa and 105 kDa. Thus, using a combination of conventional and sequence-specific DNA-affinity chromatography, we were able to purify several biochemically distinct proteins, one or more of which appeared to specifically bind to the octamer sequence in a lymphoid-specific manner.

A 70-kDa Protein Binds to the Octamer Sequence to Form the Lymphoid-Specific Protein–DNA Complex. Using sequence-specific DNA-affinity chromatography, we isolated proteins in the molecular mass range of 50–55 kDa, 70 kDa, and 105 kDa. To establish which of these proteins were responsible for the formation of the lymphoid-specific protein–DNA complex, we separated the nuclear proteins contained in affinity-purified nuclear extracts by $NaDodSO_4$/PAGE, cut the appropriate lanes into slices, eluted the proteins, renatured their activity, and tested their ability to bind the octamer sequence in the gel migration inhibition assay. The experiment shown in Fig. 2*A* was performed with an extract that was affinity purified two times, as shown in Fig. 1*B*, lane 7. Species 3 eluted from a gel slice corresponding in molecular mass to 60–70 kDa (Fig. 2*A*, lane 6). When the eluted protein tested in lane 6 (species 3) was reanalyzed by $NaDodSO_4$/PAGE under nonreducing conditions (Fig. 2*B*, middle lane), a single major protein band (indicated by the small arrow) was seen at 70 kDa by using silver staining. Under reducing conditions the migration of this protein did not change (data not shown), suggesting that the lymphoid-

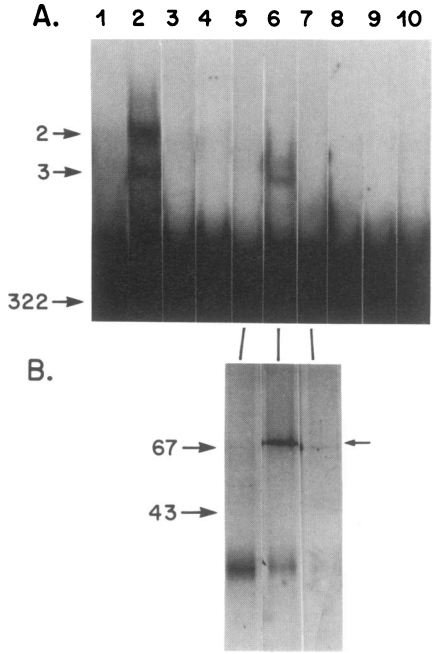

FIG. 2. Elution and renaturation of affinity-purified proteins from $NaDodSO_4$/polyacrylamide gels. (*A*) Twice-affinity-purified BCL_1 extract (as shown in Fig. 1*B*, lane 7) was separated on a $NaDodSO_4$/10% polyacrylamide gel and the appropriate lane was cut into 0.5-cm slices. The protein in each slice (lanes 3–10) was recovered by using apomyoglobin (Beckman) as the carrier protein and was tested by using the gel migration inhibition assay. Lanes 1 and 2 contain no extract and unfractionated extract derived from BCL_1 cells grown *in vivo*, respectively. Species 2 and 3 are indicated along the left margin. (*B*) The proteins tested in lanes 5, 6, and 7 in *A* were analyzed on a $NaDodSO_4$/10% polyacrylamide gel under nonreducing conditions and were visualized by silver staining. The small arrow points to the 70-kDa protein in the middle lane. The positions of the molecular mass markers (kDa) are indicated with the larger arrows along the left margin of *B*.

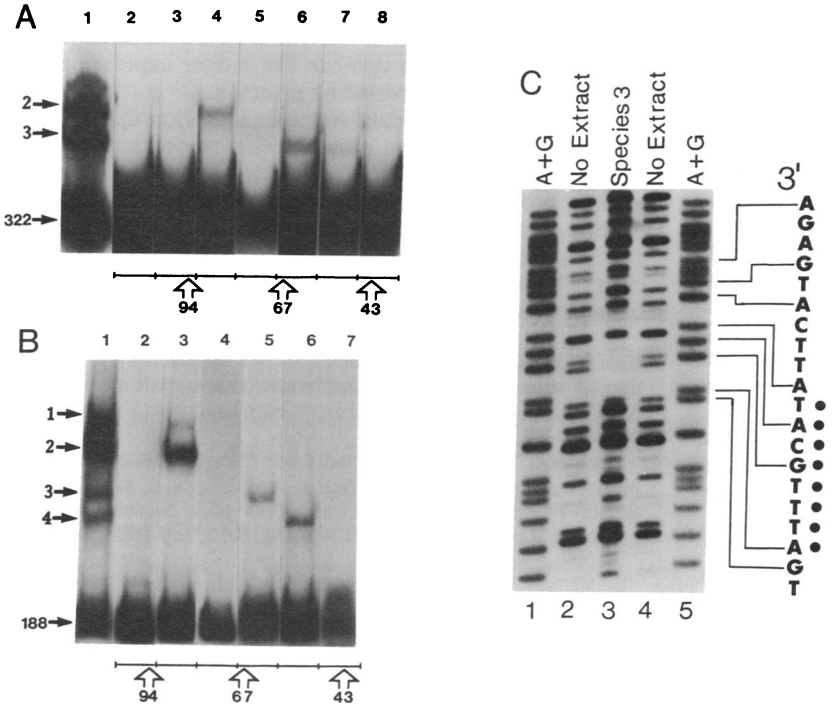

FIG. 3. Elution and renaturation of octamer-binding proteins from $NaDodSO_4$/polyacrylamide gels. (*A*) Unfractionated nuclear proteins derived from BCL_1 cells grown *in vivo* were separated on a $NaDodSO_4$/10% polyacrylamide gel and the appropriate lane was cut into 1-cm sections prior to eluting the protein, renaturing its activity, and testing it in the gel migration inhibition assay. Species 2 and 3 and the unbound 322-bp fragment are indicated along the left margin. (*B*) $NaDodSO_4$/PAGE-separated nuclear proteins derived from BCL_1 cells grown *in vitro* were similarly analyzed with the 188-bp restriction fragment. Species 1–4 are indicated along the left margin. The positions of the molecular mass markers (kDa) relative to each gel slice are indicated below *A* and *B*. (*C*) Autoradiogram of DNase I protection analysis of the species 3 complex isolated by $NaDodSO_4$/PAGE. Lane 3 shows the DNase I cleavage pattern of the 188-bp fragment extracted from the gel migration-retarded species 3 complex shown in lane 5 of *B*. Lanes 1 and 5 represent the products of the A+G-specific modification/cleavage reaction of the 188-bp DNA fragment. Lanes 2 and 4 show the pattern of DNase I cleavage of the unbound DNA fragment (digested in the presence of the material assayed in lane 5 of *B*). The octamer sequence ATTTGCAT is indicated by the dark circles to the right of the figure.

specific protein–DNA complex species 3 is formed through the interaction of a single protein with the octamer sequence. It should be noted, however, that the migration of the 67- and 43-kDa protein standards was altered upon reduction such that assignment of the molecular mass of the lymphoid-specific octamer-binding protein changed from 70 to 60 kDa.

At Least Three Proteins of Different Molecular Mass Bind to the Octamer to Give Rise to Four Distinct Protein–DNA Complexes. To identify the molecular mass of the proteins responsible for other protein–DNA interactions involving the octamer, unfractionated nuclear extract from BCL_1 cells grown *in vivo* was separated by $NaDodSO_4$/PAGE and the appropriate lane was cut into 1-cm slices. The protein in each gel slice was eluted, renatured, and tested in a gel migration inhibition assay (Fig. 3*A*). The protein eluted from a gel slice in the molecular mass range of 60–70 kDa (Fig. 3*A*, lane 6) formed a complex that comigrated with species 3, corresponding to the 70-kDa protein purified by DNA-affinity chromatography (Fig. 1*B*, lane 7). In contrast, activity associated with species 2 (Fig. 3*A*, lane 4) repeatedly eluted from gel slices in the molecular mass range 80–90 kDa. In Fig. 3*B*, a similar experiment was performed with a nuclear extract derived from BCL_1 cells grown *in vitro* to examine the molecular mass of the proteins that give rise to all four gel migration inhibition species. Once again, the molecular mass for protein giving rise to species 3 (lane 5) was in the range of 60–70 kDa while the protein giving rise to species 2 (lane 3) corresponded to 80–90 kDa. Interestingly, species 1-associated activity consistently eluted with species 2 (lane 3), suggesting that species 1 involved proteins or protein complexes of molecular mass similar to that of species 2. Species 4 (lane 6) corresponded to a molecular mass between 50 and 60 kDa.

Finally, we wished to determine whether the species 2- and 3-associated proteins maintained specificity for the octamer sequence after being isolated by $NaDodSO_4$/PAGE and then denatured prior to renaturation. The region protected (footprint) within the octamer-containing DNA fragment by the isolated species 3 interaction (shown in Fig. 3*B*, lane 5) included only the octamer sequence, as shown in Fig. 3*C*, lane 3. An identical pattern of protection was obtained by using the species 2 complex isolated in Fig. 3*B*, lane 3 (data not shown). Thus, the species 2- and 3-associated nuclear proteins could independently recognize the octamer sequence in the absence of the other octamer-binding proteins after being denatured and subsequently renatured.

DISCUSSION

We used sequence-specific DNA-affinity chromatography and renaturation of proteins eluted from $NaDodSO_4$/polyacrylamide gels to purify the protein responsible for the lymphoid-specific protein–DNA interaction as well as determine the molecular mass of other nuclear proteins that specifically bind to the octamer regulatory element of immunoglobulin heavy chain variable region genes. Protein in the molecular mass range of 80–90 kDa was isolated that formed gel migration retarded complexes (species 1 and 2) comigrating with protein–DNA complexes observed in nuclear extracts derived from both lymphoid and nonlymphoid cells. In contrast, proteins of approximately 70 kDa (species 3) and 50–60 kDa (species 4) interacted with the octamer sequence to form protein–DNA complexes restricted to lymphoid cells and thus may be involved in the lymphoid-specific expression of immunoglobulin genes. Thus, octamer–protein interactions common to both lymphoid and nonlymphoid cells appear to involve nuclear proteins distinct from the proteins involved in octamer–protein complexes specific to lymphoid cells. These differential interactions may serve to control the lymphoid-specific expression of immunoglobulin genes in B cells. Furthermore, while previous studies have demonstrated a lymphoid-specific octamer–protein interaction that may be involved in lymphoid-specific expression of immunoglobulin genes, it has not been clear whether this complex involved one or more proteins with the octamer sequence (23–25). Here we demonstrate that his lymphoid-specific interaction may be formed through the interaction of a single protein with the octamer sequence.

The DNA-affinity columns used in these studies selectively enriched for a 70-kDa octamer-binding protein responsible for species 3. However, proteins of 50–55 kDa and 105 kDa were also present in the affinity-purified extracts but had no apparant octamer-binding activity in the gel migration inhibition assay. Two-dimensional $NaDodSO_4$/PAGE and amino acid sequence analyses revealed that actin is present in the

50- to 55-kDa range of the affinity-purified extracts (data not shown). Moreover, variations in the purification scheme before affinity chromatography (data not shown) and elution of proteins from $NaDodSO_4$/PAGE gels demonstrated that only the 70-kDa protein was required for the species 3-associated activity. Thus, the 50- to 55-kDa and 105-kDa proteins most likely represent contaminants that either bind nonspecifically to the column or copurify with the 70-kDa protein through protein–protein interactions.

The inability of the affinity columns to select species 1 and 2 did not allow precise determination of the molecular mass of the proteins responsible for the formation of these complexes. However, we were able to recover activity associated with both species from $NaDodSO_4$ gels by using extracts from BCL_1 cells. Interestingly, species 1 and 2 activity consistently eluted together from the same gel slices, suggesting that they both involved either proteins or protein complexes of similar molecular mass. However, DNase I protection assays (24) and methidiumpropyl-EDTA-iron(II) footprinting (data not shown) have established that species 1 from both BCL_1 and HeLa extracts involves the interaction of one or more proteins with not only the octamer sequence but also 13 nucleotides 3′ to the octamer on the noncoding strand and 13 nucleotides 5′ to the octamer on the coding strand (24). This suggests that the recognition specificity of this molecule(s) is different from that of the other octamer-binding proteins. Mutation studies have shown that sequences upstream of the conserved octamer sequence may also be important in the regulation of immunoglobulin gene transcription (8, 13). Thus, specificity of species 1-associated nuclear protein(s) for this region may be of functional significance. In addition, the distinct recognition specificity of species 1 suggests it is composed of either an entirely unique protein(s) or a protein–protein complex involving species 2 that contains a distinct DNA-binding site.

DNase I protection studies using unfractionated nuclear extracts revealed that species 2, 3, and 4 each protect identical nucleotides of the octamer sequence in the heavy chain gene of BCL_1 (24). Identical results were obtained with species 2- and 3-associated proteins that were first separated from each other by $NaDodSO_4$/PAGE, indicating that these octamer-binding proteins contain either similar or identical recognition sites for the octamer sequence. This suggests that each protein may be encoded by either a family of related genes or a single gene that is differentially spliced at the RNA level. Alternatively, one or more protein precursors may be specifically cleaved to give rise to octamer-binding proteins of lower molecular mass. However, differences in the expression of species 2 and 3 between lymphoid and nonlymphoid cells (23), as well as the selective enhancement of species 3 in mitogen-activated B cell (24), suggest that the species 3-related protein is regulated independently of the species 1- and 2-associated protein(s). Using an immunoglobulin κ-chain promoter DNA fragment, Staudt *et al.* (29) demonstrated that an increase in the formation of a lymphoid-specific complex (NF-A2) in mitogen-stimulated B cells was sensitive to cycloheximide, suggesting that this increase required new protein synthesis. If regulation of the species 3-associated protein similarly requires new synthesis, then activation of an immunoglobulin structural gene may require a series of sequential activation events involving multiple genes encoding regulatory proteins. In contrast, Sen and Baltimore (36) reported that protein(s) that bind to immunoglobulin κ-chain gene enhancer sequences (NF-kB) may be selectively activated in mature B cells through posttranslational mechanisms. If such posttranslational mechanisms alone serve to control the binding of the octamer-specific proteins, they must be able to modulate the binding of the 70-kDa protein (species 3) and the 50- to 55-kDa protein(s) (species 4) separately from the species 1- and 2-associated protein(s) found in both lymphoid and nonlymphoid cell types. However, it is just as likely that both mechanisms may operate coordinately to regulate the proper expression and function of the octamer-binding proteins.

Taken together, our data support the notion that a single DNA regulatory element may function to control immunoglobulin gene expression by interacting with more than one nuclear protein. Elucidation of the structure of these sequence-specific DNA-binding proteins and the mechanisms governing their expression and activation should allow a clearer understanding of the role of promoter-specific proteins in transcriptional control of gene expression.

Note Added in Proof. During review of this manuscript the purification of what appears to be the human counterpart of species 2 (OTF-1) and species 3 (OTF-2) have been reported (37, 38).

We thank Teresa Eversole and Leon Carayannopoulas for technical assistance. J.H.H. and N.F.L. are fellows of the Leukemia Society of America. This work was supported by National Institutes of Health Research Grants AI18499, AI18016, and GM31689.

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