Nuclear factors binding to the human immunoglobulin heavy-chain gene enhancer

---

Hiroaki Maeda, Kazuo Araki, Daisuke Kitamura, Jiyang Wang and Takeshi Watanabe*

Department of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University, Higashi-ku, Fukuoka 812, Japan

---

Received January 14, 1987; Revised and Accepted March 3, 1987

ABSTRACT

The human immunoglobulin heavy chain (IgH) gene contains at least two tissue-specific regulatory regions, which are similar to the mouse IgH gene. One is the J-C enhancer and another is located in the 5' promoter region. Using an electrophoretic mobility shift assay and DNase I footprint, we have examined the interaction of factors in B cell nuclear extracts with the two regulatory regions of the human IgH gene. We have identified a nuclear factor in mouse B cell nuclear extracts which bound to specific sequence in the human IgH enhancer. This factor is apparently not present in mouse fibroblast nuclear extracts. We also found factor(s) which bound to the highly conserved octanucleotide sequence within the human IgH enhancer and 5' promoter regions.

INTRODUCTION

The immunoglobulin heavy-chain (IgH) gene is specifically expressed in cells of the B lymphocyte lineages. It has been reported that at least two regulatory regions in the intron of the IgH gene are involved in such a B cell-specific gene transcription. One is located in the intron between the joining (J) and constant (C) region of the IgH gene and it acts as a transcriptional enhancer (1-3). The IgH enhancer appears to function most efficiently in B cell lineages but to be inactive in non-lymphoid cells. In the B lymphoid cells, it enhances the transcription rate of the gene started from the promoter nearby. Another regulatory element is located 5' of the transcription start site in the immunoglobulin (Ig) heavy and κ -light chain variable region (4-9). This element directs B lymphoid-specific transcription of Ig gene even in the presence of nonspecific viral enhancers. Such a B-cell specific activity of Ig gene regulatory elements suggested that their functions may be positively and/or negatively regulated by cellular trans-acting factors (10-14).

Mouse and human Ig light chain promoters possess a highly conserved octanucleotide sequence, ATTTGCAT, at approximately 70 base pairs (bp)

upstream of the transcription start site (15,16). Mouse and human IgH promoters contain the identical sequence in inverted orientation, ATGCAAAT, at the similar position. In the mouse IgH enhancer, the same octamer sequence is also present (16). On the other hand, the human IgH enhancer contains two similar octanucleotides which deviate from the consensus motif by substituting a guanine for an adenine residue at the seventh position (ATTTGCGT) and a thymidine for a guanine residue at the fifth position (ATTTTCAT) (17,18). Recently, a nuclear factor has been identified which binds to the octamer sequence in the mouse heavy and κ -light chain promoters and also to the octamer in the mouse heavy-chain enhancer (19,20). Hromas and Ness observed two patterns of specific binding of nuclear factors to the κ -light chain enhancer element. The binding pattern of nuclear factor(s) of cell lines which actively transcribe κ -light chain genes was different from that of cell lines which do not express Ig genes (20). Using dimethyl sulphate (DMS) protection experiments, Ephrussi et al. have demonstrated that alterations in the pattern of guanine reactivity to DMS are consistent with the tissue-specific binding of protein molecules to the mouse IgH enhancer and they proposed four different binding sites (21,22). Utilizing DNase I footprinting in vitro, Augereau and Chambon (23), and Schlokot et al. (24) have also demonstrated the cellular factors interacting in trans with the mouse IgH enhancer sequences.

Mercola et al. showed that positive factor(s) acting on the mouse IgH enhancer are present in lymphoid cells but not in fibroblasts (25). We have previously reported that microinjection of nuclear factor(s) prepared from mouse myeloma cells induced the expression of the human γ_1 heavy chain (HIG1) gene integrated into the L cell transformants and demonstrated that cell-type specific trans-acting factor(s) is involved in the activation of the human IgH enhancer (26). In order to examine whether such factor(s) in mouse myeloma cell nuclear extracts bind to regulatory elements of the HIG1 enhancer, we have used an electrophoretic mobility shift assay which is based on the altered mobility of DNA-protein complexes during a low ionic strength polyacrylamide gel electrophoresis. In the present study, we describe nuclear protein(s) prepared from mouse myeloma cells which specifically bind to the unique sequences in the HIG1 enhancer, and also nuclear protein(s) which bind to the octamer sequences present in both the 5' promoter and enhancer region of the HIG1 gene.

MATERIALS AND METHODS

DNA and plasmids

All of the probes used in this study were derived from human γ_1 heavy chain (HIG1) gene (27) and mouse γ_{2a} heavy chain (NL-1H) gene (28,29). The restriction cleavage site maps are shown in Fig. 1. HIG1 gene was cloned from a human plasma cell leukemia line, ARH77 (27). The 5' promoter region of HIG1 gene was subdivided into the HP1 (118 bp, HaeIII-AluI) and HP2 (72 bp, HaeIII-

(A) HIG1(human)

(B) NL1-H chain(mouse)

Figure 1 DNA fragments used in the experiments. (A) DNA fragments containing 5' promoter and J-C intron enhancer regions of the human immunoglobulin γ_1 heavy chain (HIG1) gene. The HP1, HE3 and HE5 fragments contained the octamer sequences ATGCAAT, ATTTGCGT and ATTTTCAT, respectively (see Figs. 3,4). The putative viral enhancer core sequence, GTGGTTTIG, is present in the HE2 fragment (see Fig. 8). (B) DNA fragments containing 5' flanking octamer sequence and viral enhancer core sequence of the mouse immunoglobulin γ_{2a} heavy chain (NL-1H) gene. The N1 fragment contained the octamer sequence ATGCAAT (28) and the N2 fragment contained three viral enhancer core sequences, namely AAAACCAC, GTGGTTTGAA and GTGGTTTIGAA (Fig. 8). All fragments were prepared as described in the Materials and Methods. Black boxes represent exons of HIG1 and NL-1H genes. L, leader sequence; V, variable region; D, diversity segment; J, joining region.

HaeIII) elements (Fig. 1A, Fig. 3B). The J-C enhancer region was also subdivided into five parts, which were the HE1 (160 bp, AluI-HpaII), HE2 (120 bp, HpaII-BglII), HE3 (123 bp, BglII-AluI), HE4 (133 bp, AluI-AluI) and HE5 (97 bp, AluI-HpaI) elements (Fig. 1A, Fig. 4). The HP1 contained the octamer sequence (ATGCAAAT). The octamer-like sequences in the HE3 and HE5 elements deviated from the consensus motif, ATTTGCAT, by substituting a guanine for an adenine residue in the seventh position (ATTTGCGT) and a thymidine for a guanine residue in the fifth position (ATTTICAT), respectively (Fig. 4). The HE2 element has the putative "viral enhancer core sequence", GTGGTTTGTG, which is exactly conserved in the human and mouse IgH gene enhancer and polyoma virus enhancer (1,30) (Fig. 8). NL-1H gene was cloned from a mouse hybridoma cell line, NL-1, which produced a monoclonal antibody against the common acute lymphocytic leukemia (cALL) antigen (28). The element containing the 5'promoter octamer sequence or viral enhancer core sequence was prepared from NL-1H gene as the N1 (130 bp, SphI-Sau3AI) or the N2 (137 bp, PvuII-DdeI) element (Fig. 1B). All probes were end-labeled with [γ -³²P]ATP using T4 polynucleotide kinase.

The various recombinant plasmids were prepared for competition assay. The AluI site of the HP1 (HaeIII-AluI) fragment was converted into the EcoRI site. The BglII site of the HE3 (BglII-AluI) fragment was converted into the BamHI site and the HpaI site of the HE5 (AluI-HpaI) fragment into the BamHI site. The HaeIII-EcoRI (pHP1), BamHI-AluI (pHE3) or AluI-BamHI (pHE5) fragment was cloned into the HincII and BamHI (EcoRI) sites of pUC19. pHE2 was prepared by ligating the HE2 (HpaII-BglII) fragment into the HincII site of pUC19 at blunt-end after treatment with DNA polymerase I.

Nuclear extracts

Nuclear extracts were prepared from the cells of mouse myeloma cell line, NS1, or mouse fibroblast, L cells, according to the method of Dignam et al. (31) and Maeda et al. (26). The soluble nuclear extracts in 0.15 M NaCl solution were applied to a Sephacryl S-300 column (2 x 90 cm) equilibrated with buffer containing 20 mM HEPES (pH 8.0), 20% (v/v) glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM PMSF and 0.5 mM DTT. The fractionated samples were divided into six groups (S1 to S6) and concentrated to one tenth of original volume. Each sample was aliquoted and stored at -80°C.

Electrophoretic mobility shift assay

A few nanograms of end-labeled DNA fragments were incubated at room temperature for 30 min. with 50 μ g of nuclear extracts, 10 μ g of poly(dI-dC)·poly(dI-dC)(Pharmacia), 10 mM Tris-HCl (pH 7.6), 50 mM NaCl, 1 mM DTT, 1

mM EDTA and 5% (v/v) glycerol in a total volume of 30 μ l. Samples were then electrophoresed on a 5% polyacrylamide gel (acrylamide:bisacrylamide weight ratio of 30:1) containing 6.7 mM Tris-HCl (pH 7.5), 3.3 mM Na-acetate and 1 mM Na-EDTA. Electrophoresis was done for 120-180 min. at 11 V/cm at 4°C with buffer-recirculation. The gels were then dried and autoradiographed.

DNase I footprinting

The HE2 probes for DNase I footprinting were prepared from the 120 bp HpaII-BglII fragment by end-labeling with T4 polynucleotide kinase and [γ -³²P]ATP, and digesting with HaeIII, which yielded the 116 bp end-labeled fragment. The HE3 and HE5 probes for DNase I footprinting were prepared from the 353 bp BglII-HpaI fragment by end-labeling with T4 polynucleotide kinase and [γ -³²P]ATP, and digesting with AluI. The end-labeled 116 bp fragment of HE2, 123 bp fragment of HE3 and 97 bp fragment of HE5 were purified by polyacrylamide gel electrophoresis. DNase I Footprinting was performed according to the method of Carthew et al. (32). The labeled DNAs were incubated with nuclear extracts and poly(dI-dC)·poly(dI-dC) in a 100 μ l volume at room temperature for 30 min. Samples were then treated with DNase I (Bethesda Research Laboratories) at a final concentration of between 0.5 and 10 μ g/ml for 60 sec. at room temperature. DNase I digestion was stopped by the addition of 3 μ l of 0.5 M EDTA, and electrophoresis of complexes was performed at 11 V/cm at 4°C for 120-180 min. as described above. The position of complexes were localized by autoradiography of the wet gels at 4°C. Radioactive complexes were excised from the gel and shaken overnight at 37°C in 10 mM Tris-HCl (pH 7.8), 1 mM EDTA, 0.2% SDS, 0.3 M NaCl and 1 μ g/ml tRNA. The labeled DNAs were extracted with phenol/chloroform/isoamylalcohol and chloroform/isoamylalcohol and precipitated with ethanol, electrophoresed in an 8% polyacrylamide-8 M urea sequencing gel and visualized autoradiographically.

RESULTS

Nuclear proteins binding to the fragment of human IgH gene containing the octamer sequence or viral enhancer sequence

In order to study the interaction of nuclear factors with regulatory regions of HIG1 gene, we have utilized an electrophoretic mobility shift assay which detects the slower migration of DNA-protein complexes with respect to free DNA on low ionic strength polyacrylamide gels. At first, the three probes, HE2, HE3, and HP1 were tested. The HE2 probe (120 bp, HpaII-BglII) contained viral enhancer core sequence, GTGGTTTG (Fig. 8). The HE3 (123 bp, BglII-AluI) and HP1 (118 bp, HaeIII-AluI) probes contained the octamer

Figure 2 Binding of nuclear extracts to the probes containing the octamer sequences or viral enhancer core sequence. (A) Binding pattern of probes from HIG1 gene. Lanes 1 and 2, the HP1 probe; lanes 3 and 4, the HE2 probe; lanes 5 and 6, the HE3 probe. (B) Binding pattern of probes from NL-1H gene. Lanes 1 and 2, the N1 probe; lanes 3 and 4, the N2 probe. Arrows denote the major mobility shift complexes.

sequences which are derived from the HIG1 enhancer and promoter, respectively. The octamer sequence (ATTGCGT) in the HE3 probe had a single base variation of the consensus octamer motif (ATTGCAT) (Fig. 4). Radiolabeled fragments were incubated with nuclear extracts from the NS1 myeloma cells or the L cells (fibroblast cells) in the presence of poly(dI-dC)·poly(dI-dC) as a non-specific competitor. The sample were then electrophoresed through a low ionic strength polyacrylamide gel and the protein-DNA complex was visualized by autoradiography. As shown in Fig. 2A, the HP1 and HE3 probes which contained the octamer sequence yielded major discrete complexes of similar large mobility in both nuclear extracts of NS1 cells and L cells (Fig. 2A, Lanes 1,2,5,6). When the HE2 probe was incubated with the NS1 nuclear extracts, a distinct band with small mobility shift was detected but it was not observed with the L cell nuclear extracts (Fig. 2A, lanes 3,4). Thus, this small mobility complex appeared to be B lymphoid cell specific.

We next examined whether there are similar factors binding to fragments of the mouse IgH gene promoter or enhancer. The N1 (130 bp, SphI-Sau3AI) and

N2 (137 bp, PvuII-DdeI) probes (Fig. 1B) of NL-1H gene which correspond to the HP1 and HE2 probes of HIG1 gene, respectively, were incubated with the same nuclear extracts. The similar large mobility shift pattern to that of the HP1 probe was seen in the N1 probe with the nuclear extracts of NS1 cells or L cells (Fig. 2B, lanes 1,2). The band with larger mobility shift was octamer-sequence specific. When the N2 probe was incubated with NS1 or L cell nuclear extracts, the large and small mobility shift components were demonstrated (Fig. 2B, lanes 3,4). The small mobility shift component (indicated by arrow) was competed off by the N2 fragment but the band with large mobility was not competed off (data not shown). As in the case of the human IgH enhancer, the component with small mobility shift was formed specifically with the NS1 nuclear extracts but not with the L cell nuclear extracts. Thus, nuclear extracts of mouse myeloma cells appeared to contain the factor(s) which specifically bind to the DNA fragments containing the viral enhancer core sequence.

Fractionation of nuclear extracts by gel filtration

The nuclear extracts prepared from mouse myeloma NS1 cells were fractionated with Sephacryl S-300 gel filtration (Fig. 3A). The fractions were pooled and divided into six groups, S1 to S6 and concentrated to one tenth of the original volume. In the region between -4 and -194 bp from the transcription start site, the HP2 (72 bp, HaeIII-HaeII) probe showed no appreciable binding (Fig. 3B, lanes 1-6). The HP1 (118 bp, HaeIII-AluI) probe yielded major large mobility bands with the S4 and S5 fractions (Fig. 3B, lanes 9-11). A fragment of plasmid pBR322 (HaeIII-HaeIII, 123 bp long) did not show any binding (Fig. 3B, lanes 13-18). Assuming that DNA-binding protein is a globular protein, molecular weights of proteins in the S4 and S5 fractions will be between 25 and 440 kDa. When the NS1 extracts were applied on HPLC with TSK-3000 gel filtration column (TOYO SODA), the factor(s) binding to the HP1 probe migrated as a native species of between 53 and 120 kDa (data not shown). An enhancer region in J-C intron was divided into five parts, that is, HE1 to HE5 (Fig. 1A, Fig. 4). The HE3 probe displayed the same pattern of retarded bands as those of the HE5 probe with the proteins of the S4 and S5 fractions (Fig. 4, lanes 15-17, 27-29). It is noteworthy that essentially the same pattern was seen when the HP1 probe was incubated with the NS1 nuclear proteins of the S4 and S5 fractions. As the HE3, HE5 and HP1 probes contained the octamer sequence, it was suggested that there might be specific factor(s) binding to the octamer sequence in the NS1 nuclear extracts. This was confirmed by DNase I footprinting analysis as described below. In contrast to

Figure 3 Gel filtration of the NS1 nuclear extracts and interactions of the fractions with the 5' promoter region of HIG1 gene. (A) Fractionation of the NS1 nuclear extracts by Sephacryl S-300 gel filtration. The fractions were divided into six groups, S1-S6. The estimated molecular weight of each fraction is shown in the lower portion of the figure. (B) Interactions of the 5' promoter fragments of HIG1 gene with the fractionated NS1 nuclear extracts. Lanes 1-6, the HP2 probe; lanes 7-12, the HP1 probe; lanes 13-18, the pBR322 probe as a negative control. Arrow denotes the major mobility shift complexes. CACAT is capping site of the HIG1 gene. Open circle indicates the octamer sequence.

Figure 4 Electrophoretic mobility shift assay of J-C intron enhancer fragments of HIG1 gene with the fractionated NS1 nuclear extracts. Lanes 1-6, the HE1 probe; lanes 7-12, the HE2 probe; lanes 13-18, the HE3 probe; lanes 19-24, the HE4 probe; lanes 25-30, the HE5 probe. Arrows denote the major mobility shift complexes. Thick arrow shows the viral enhancer core sequence and open circles are the octamer sequences.

the HE3 and HE5 probes, the HE2 probe produced the bands of small mobility complex in the S4 and S5 fractions (Fig. 4, lanes 10,11). Although a faint band was seen in the HE1 probe after long exposure, the intensity of the band was much lower than those of the HE2, HE3 or HE5 probes. No band was found with the HE4 probe.

The specificity of mobility shift components

To examine the specificity of the binding of nuclear proteins to the DNA, competition experiments were carried out. At first, the specificity of large mobility bands of the HE3 probe containing the octamer sequence were examined. As shown in Fig. 5, addition of 70 ng of unlabeled pUC19 plasmid did not affect the binding of the nuclear protein(s) in the S4 fraction to the end-labeled HE3 probe (lane 2). Equivalent amounts of the pHE3 or pHPI plasmid containing the octamer sequence effectively competed for the binding to the HE3 probe (lanes 3,4). Similarly, the binding to the HE5 probe was competed with either the pHE3 or pHPI plasmid (data not shown). The same results were obtained, when the nuclear protein(s) in the S5 fraction was used. These results suggested the presence of the nuclear protein(s) in the S4 and S5 fractions, which specifically bind to the octamer sequence.

Figure 5 Competition experiments showing the octamer sequence-specific binding. The end-labeled HE3 fragment (0.1 ng) was incubated with 1 μ g of poly(dI-dC)·poly(dI-dC), 70 ng of various kinds of unlabeled plasmids as competitors and 5 μ g of the S4 fraction. Lane 1, no competitor; lanes 2-4, pUC19, pHE3 and pHP1, respectively, as competitors. Arrow denotes the complex with the octamer binding protein.

Then, the binding specificity of the nuclear protein(s) in the S4 and S5 fractions to the HE2 probe containing the viral enhancer core sequence was examined by the competition assay. The competition was performed by incubating the S4 fraction with the end-labeled HE2 probe and 70 ng of unlabeled plasmids (Fig. 6, lanes 1-6). The plasmids, pUC19, pHP1 and pHE5 failed to compete for the binding to the HE2 probe (Fig. 6, lanes 2,4,5). The pHE2 plasmid completely inhibited the formation of small mobility complex with the HE2 probe, and the pHE3 plasmid partially reduced the binding to the HE2 probe (Fig. 6, lanes 3,6). There is a partially homologous sequence (TATTTAG) between the HE2 and HE3 fragments as shown in Fig. 8, which might have caused the partial reduction of the binding to the HE2 probe by pHE3 plasmid. Similar results were obtained with the N2 probe which contained the mouse IgH enhancer core sequence (Fig. 6, lanes 7-12). The binding to the N2

Figure 6 Competition experiments showing the specificity of the HE2 and N2 fragment-binding proteins. The labeled fragment (0.1 ng) was incubated with 1 μ g of poly(dI-dC)·poly(dI-dC), 70 ng of various kinds of unlabeled plasmids as competitors and the S4 fractions (5 μ g). pUC19 (lanes 2 and 8), pHE2 (lanes 3 and 9), pHP1 (lanes 4 and 10), pHE5 (lanes 5 and 11), and pHE3 (lanes 6 and 12) were used as competitors. Lanes 1 and 7 have no competitor. Lanes 1-6, the HE2 probe; lanes 7-12, the N2 probe. Arrows denote the major mobility shift complexes.

probe was efficiently competed for by the plasmid pHE2, and partially by the plasmid pHE3 (Fig. 6, lanes 9,12) while the binding was not affected by the plasmid pUC19, pHP1 or pHE5 (Fig. 6, lanes 8,10,11). We obtained the same results by using the nuclear protein(s) of the S5 fraction (data not shown). These results imply that the nuclear protein(s) responsible for the formation of small mobility complex may recognize a common sequence between the human HE2 and mouse N2 probes, and may possess low affinity to the sequence in the HE3 probe.

DNase I footprinting analysis

DNase I footprint analysis was carried out to delineate more precisely the binding domain(s) of protein(s) present in complexes formed by the HE2, HE3 or HE5 probes. The DNA-protein complexes were briefly digested with DNase I, and the mixtures were then subjected to a low ionic strength polyacrylamide gel electrophoresis. DNA bound to protein(s) was eluted from the respective retarded bands, denatured, and examined by an 8% polyacrylamide-8 M urea sequencing gel electrophoresis.

The large mobility complex of the HE5 probe incubated with the S4 fraction showed a distinct region of complete protection (Fig. 7A). Protein(s) in the HE5 complexes protected a 21-nucleotide region of the non-coding strand which included the octamer (ATTTTCAT) possessing single base variation of the consensus octamer motif (Fig. 7A). The region protected by the incubation of the HE3 probe with the S4 fraction was a 31-nucleotide

Figure 7 DNase I footprinting analysis. DNase I footprinting was performed as described in the Materials and Methods. Strand cleavage at A and G was prepared according to the procedure of Maxam and Gilbert (44). (A) Footprints induced by the S4 fraction on the non-coding strand of the HE5 fragment. The octamer sequence is boxed. (B) Footprints induced by the S4 fraction on the coding strand of the HE3 fragment. The octamer sequence is boxed and the underline indicates the fourth binding site (E4) proposed in mouse IgH enhancer by Ephrussi et al. (21). (C) Footprints induced by the S4 and S5 fractions on the coding strand of the HE2 fragment. Underline indicates the third binding site (E3) proposed in mouse IgH enhancer by Ephrussi et al. (21). Open circles show the sequence similar to the sequences in lymphotropic papovavirus (LPV) enhancer (42).

region on the coding strand which spanned from 214 to 244 bp (Fig. 7B). This region also contained the octamer (ATTTGCGT) which was one base variation of the octamer motif. These results indicated that even if the octamer sequence varies by one nucleotide, a specific DNA binding by nuclear protein(s) can

Figure 8 Summary of the results of the DNase I footprinting. Sequences of the human (HIG1) and mouse (1) immunoglobulin heavy chain enhancers are compared [according to the numbering of Hayday et al. (30)]. The residues which match between human and mouse sequences are indicated by asterisks. Dashes indicate manually inserted spaces necessary to maintain homologies. Arrows indicate the putative viral enhancer core sequences and the octamer sequences are boxed. Protected regions are indicated by brackets and the regions of partial protection are shown by dashed brackets. The thick bar (LC1-LN2) shows the region which Augereau and Chambon reported as a lymphocyte-specific protection site. Closed triangles show the homologous sequences found in the HE2 and HE3 fragments. LC1-LN1, LC1-LN2, LN3, LC2-LN4 and LC3-LC4-LN5 are the regions protected from DNase I digestion which was shown in vitro in B lymphoid cell nuclear extracts by Augereau and Chambon (23). The open and filled circles denote the positions of the protected and hypermethylated G residues, respectively, which were observed in vivo in B lymphoid cells by Ephrussi et al. (21). NF-Eu1 and NF-Eu3 are the binding sites for the nuclear proteins, NF-Eu1 and NF-Eu3 which were reported by Weinberger et al. (33,34). IgNF-A is the binding site for the octamer (ATGCAAT) binding protein, IgNF-A which was reported by Singh et al. (19).

still occur. Furthermore, the protected region in the HE3 probe contained the protein-binding site, E4, which Ephrussi et al. proposed from the results of the DMS-protection experiments (Fig. 7B, Fig 8).

The small mobility complex which was formed by the HE2 probe with the S4 and S5 fractions appeared to be B lymphoid cell specific, since the nuclear extracts of L cells did not form this complex. The 5' and 3' boundaries of the protected region map to positions 119 and 138 bp, respectively, on the coding strand (Fig. 7C). The sequence of major protected region is TTTTAGGAAGCAAAAACA. This region contained a part of the third binding site (E3) proposed by Ephrussi et al. (Fig. 7C, Fig. 8) and also contained the sequence, AGGAAGC, which is homologous to the sequence found in lymphotropic papova virus (LPV) enhancer (Fig. 7C). Any binding protein to the viral core sequence, GTGGTTTTG, in the HE2 probe was not detected under the present conditions (data not shown).

DISCUSSION

In the present study, we have shown the nuclear proteins which bound to specific DNA sequences within the HE2, HE3 and HE5 probes of enhancer region or the HP1 probe of promoter region of the human IgH gene by using an electrophoretic mobility shift assay and DNase I footprinting. The protein(s) in the both nuclear extracts of NS1 myeloma cells and L cell fibroblasts showed the similar discrete large mobility complexes with the HE3, HE5 and HP1 probes containing the octamer sequence. Besides, the NS1 nuclear extracts yielded a distinct small mobility complex with the HE2 fragment containing the viral enhancer core sequence (Fig. 2,4). The gel filtration with Sephacryl S-300 or TSK-3000 column showed that the molecular weight of the NS1 nuclear factors which exhibited the binding activities to above probes was between 53 and 120 kDa (Fig. 3,4). DNase I footprinting was carried out to clarify the binding sites of the nuclear proteins (Fig. 7). The results are summarized in Fig. 8. The protein binding sites of the mouse IgH enhancer are also shown in Fig. 8, which have been recently reported (19-21,23,33,34).

A highly conserved octanucleotide has been identified within mouse Ig heavy and light chain promoter regions and mouse IgH enhancer region (15,16). Recently, the presence of the protein(s) binding to the octamer was reported from several laboratories (19,20,23,35). We have demonstrated that the similar octamer sequences with one nucleotide change are present in the human IgH enhancer region and that the protein(s) binding to the octamer sequence in promoter region also appeared to bind to those octamer-like sequences in the human IgH enhancer. However, single base variation of the human IgH enhancer octamer sequences may cause the reduced affinity for the octamer binding proteins(s). Staudt et al. have shown that mutations of each single base in the octamer motif interfered with binding of octamer-specific proteins (41). In fact, although the binding to the HE3 and HE5 probes completely competed with plasmids containing octamer-like sequence (Fig. 5), the binding to the HP1 probe containing the complete octamer motif was only partially competed with plasmids containing the HE3 or HE5 fragments (Wang et al., in preparation).

The octamer binding protein(s) are not restricted to B cells but they have been detected also in L cells (Fig. 2) or HeLa cells (19,20,35). Moreover, the octamer sequence has been found in several genes such as Ig heavy and light chain promoters (15,16), IgH enhancer (16), histone H2B gene promoter (35), human U2 small nuclear RNA (snRNA) gene promoter (36),

amphibian U1 (37,38) and U2 (39) snRNA gene promoters and the simian virus 40 (SV40) enhancer (16), suggesting that the octamer binding protein(s) may be ubiquitous protein(s) present in various kinds of cells. However, the promoter regions of Ig genes containing this octamer sequence appeared to direct a tissue-specific transcription of the Ig gene. It cannot function in non-lymphoid cells even in the presence of viral enhancer (5-9). A deletion of the octamer destroyed a tissue-specific promoter activity of Ig genes in B cells (4,8,9,16). Thus, the octamer sequence in Ig gene is certainly associated with positive or negative regulation of Ig gene transcription. There are several possible explanations for the fact that the octamer-binding protein(s) are involved in the tissue-specific transcription of Ig genes. First, the octamer-binding protein(s) for Ig gene may belong to a family of proteins recognizing the octamer motif but having distinct functional domain(s) involved in the specific transcription of Ig gene. This possibility is supported by the evidence that unique octamer-binding protein(s) may exist in B-lymphoid cells but not in other cells (40,41). Second, the octamer-binding proteins may contribute to the transcriptional regulation through interaction with other molecules with specific function for Ig gene expression. Third, the octamer-binding protein(s) may belong to the repressor molecule(s) or non-functional molecule(s) and may acquire the positive function after modification with other molecule(s) present in B-lymphoid cells.

The binding protein(s) to the HE3 fragment also covered the E4 region which Ephrussi et al. (21) proposed as a protein binding site from the in vivo DMS-protection experiments in B cells (Fig. 8). Moreover, the HE3 region protected from DNase I digestion in the present study was highly conserved between mouse and human IgH genes. These data suggest that the HE3 region may be an important region in the regulation of Ig gene expression.

As shown in Fig. 2 and 8, myeloma cell nuclear extracts contained the protein(s) which bind to the specific sequence in the HE2 fragment, but they were apparently not present in fibroblast nuclear extracts. Sequence analysis showed that the region protected from DNase I digestion was TTTTAGGAAGCAAAAACA. Augereau and Chambon demonstrated the similar protected region (LC1-LN2) in the mouse IgH enhancer and suggested the presence of lymphoid-specific protein(s) binding to this region (32). The sequence, AGGAAGC, is similar to that found in lymphotropic papovavirus (LPV) enhancer (42). The region protected by HE2-binding protein(s) contained a part of the E3 site which Ephrussi et al. proposed in mouse IgH enhancer (21). It is

notable that the restricted region of the mouse IgH enhancer, which corresponds to the HE2 region in the human IgH enhancer, is known to be important for IgH enhancer activity in lymphoid cells (14,43). These data suggested that the HE2 region may interact with DNA-binding protein(s) specifically present in B cells and be involved in the regulatory control of the IgH enhancer activity.

The HE2 fragment contains a putative viral enhancer core sequence. Under the present conditions, any binding protein to this sequence was not detected. Augereau and Chambon also demonstrated only the weak binding (LN3) to two out of three core sequences in mouse IgH enhancer (23). Thus, the viral enhancer core sequence in IgH gene enhancer may not play a crucial role in the regulatory control of IgH gene expression. Mouse IgH enhancer has been shown to contain the binding site (E1) for nuclear protein, NF-Eu1 (33). Under the present experimental conditions, the binding site corresponding to the mouse E1 was not detected in human IgH enhancer (Fig. 4).

Recently, applying in vivo functional assay (26), we have found that the fractions of nuclear proteins from mouse myeloma cells with molecular weights of between 53 to 120 kDa contained the transcription-enhancing factors for the human IgH enhancer, in which the binding proteins to the HE2 as well as HE3 probes were also present (Araki et al., manuscript in preparation). They might have an important regulatory role in a tissue-specific expression of IgH gene. But, further purification is required to clarify the nature of the binding protein(s) for the human IgH enhancer element. In the present study, we have investigated on the interaction of mouse myeloma cell nuclear proteins with the human IgH enhancer since the active transacting factor(s) for the human IgH enhancer were found in mouse nuclear protein. Characterization of human lymphocyte nuclear factor(s) acting on the human IgH enhancer have been also in progress in our laboratory and will be reported elsewhere.

ACKNOWLEDGMENT

We thank Miss Y. Tsurusaki for help in preparing the manuscript. This work was supported in part by a grant from the Ministry of Education, Science and Culture in Japan.

*To whom correspondence should be addressed

REFERENCES

1. Gittes, S.D., Morrison, S.L., Oi, V.T. and Tonegawa, S. (1983) Cell 33, 717-728.

2. Banerji, J., Olson, L. and Schaffner, W. (1983) *Cell* 33, 729-740.
3. Neuberger, M.S. (1983) *EMBO J.* 2, 1373-1378.
4. Bergman, Y., Rice, D., Grosschedl, R. and Baltimore, D. (1984) *Proc. Natl. Acad. Sci. USA* 81, 7041-7045.
5. Foster, J., Stafford, J. and Queen, C. (1985) *Nature* 315, 423-425.
6. Picard, D. and Schaffner, W. (1985) *EMBO J.* 4, 2831-2838.
7. Gopal, T.V., Shimada, T., Baur, A.W. and Nienhuis, A.W. (1985) *Science* 229, 1102-1104.
8. Mason, J.O., Williams, G.T. and Neuberger, M.S. (1985) *Cell* 41, 479-487.
9. Grosschedl, R. and Baltimore, D. (1985) *Cell* 41, 885-897.
10. Ishihara, T., Kudo, A. and Watanabe, T. (1984) *J. Exp. Med.* 160, 1937-1942.
11. Schöler, H. and Gruss, P. (1985) *EMBO J.* 4, 3005-3013.
12. Borrelli, E., Hen, R., Wasylyk, C., Wasylyk, B. and Chambon, P. (1986) *Proc. Natl. Acad. Sci. USA* 83, 2846-2849.
13. Sassone-Corsi, P., Wildeman, A. and Chambon, P. (1985) *Nature* 313, 458-463.
14. Wasylyk, C. and Wasylyk, B. (1986) *EMBO J.* 5, 553-560.
15. Parslow, T.G., Blair, D., Murphy, W.J. and Granner, D.K. (1984) *Proc. Natl. Acad. Sci. USA* 81, 2650-2654.
16. Falkner, F.G. and Zachau, H.G. (1984) *Nature* 310, 71-74.
17. Rabbitts, T.H., Forster, A., Baer, R. and Hamlyn, P.H. (1983) *Nature* 306, 806-809.
18. Mills, F.C., Fisher, L.M., Kuroda, R., Ford, A.M. and Gould, H.J. (1983) *Nature* 306, 809-812.
19. Singh, H., Sen, R., Baltimore, D. and Sharp, P.A. (1986) *Nature* 319, 154-158.
20. Hromas, R. and Ness, B.V. (1986) *Nuc. Acids Res.* 14, 4837-4848.
21. Ephrussi, A., Church, G.M., Tonegawa, S. and Gilbert, W. (1985) *Science* 227, 134-140.
22. Church, G.M., Ephrussi, A., Gilbert, W. and Tonegawa, S. (1985) *Nature* 313, 798-801.
23. Augereau, P. and Chambon, P. (1986) *EMBO J.* 5, 1791-1797.
24. Schlokot, U., Bohmann, D., Schöler, H. and Gruss, P. (1986) *EMBO J.* 5, 3251-3258.
25. Mercola, M., Gorman, J., Mirell, C. and Calame, K. (1985) *Science* 227, 266-270.
26. Maeda, H., Kitamura, D., Kudo, A., Araki, K. and Watanabe, T. (1986) *Cell* 45, 25-33.
27. Kudo, A., Ishihara, T., Nishimura, Y. and Watanabe, T. (1985) *Gene* 33, 181-189.
28. Kudo, A., Nishimura, Y. and Watanabe, T. (1985) *J. Immunol.* 135, 642-645.
29. Nishimura, Y., Yokoyama, M., Araki, K., Kudo, A. and Watanabe, T. (1987) *Cancer Res.* in press.
30. Hayday, A.C., Gillies, S.D., Saito, H., Wood, C., Wiman, K., Hayward, W.S. and Tonegawa, S. (1984) *Nature* 307, 334-340.
31. Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) *Nuc. Acids Res.* 11, 1475-1489.
32. Carthew, R.W., Chodosh, L.A. and Sharp, P.A. (1985) *Cell* 43, 439-448.
33. Weinberger, J., Baltimore, D. and Sharp, P.A. (1986) *Nature* 322, 846-848.
34. Sen, R. and Baltimore, D. (1986) *Cell* 46, 705-716.
35. Sive, H.L. and Roeder, R.G. (1986) *Proc. Natl. Acad. Sci. USA* 83, 6382-6386.
36. Mangin, M., Ares, M. and Weiner, A.M. (1986) *EMBO J.* 5, 987-995.

37. Ciliberto, G., Buckland, R., Cortese, R. and Philipson, L. (1985) EMBO J. 4, 1537-1543.
38. Krol, A., Lund, E. and Dahlberg, J.E. (1985) EMBO J. 4, 1529-1535.
39. Mattaj, I.W., Lienhard, S., Jiricny, J. and DeRobertis, E.M. (1985) Nature 316, 163-167.
40. Landolfi, N.F., Capra, J.D. and Tucker, P.W. (1986) Nature 323, 548-551.
41. Staudt, L.M., Singh, H., Sen, R., Wirth, T., Sharp, P.A. and Baltimore, D. (1986) Nature 323, 640-643.
42. Mosthaf, L., Pawlita, M. and Gruss, P. (1985) Nature 315, 597-600.
43. Kadesch, T., Zervos, P. and Ruezinsky, D. (1986) Nuc. Acids Res. 14, 8209-8221.
44. Maxam, A.M. and Gilbert, W. (1980) Methods Enzymol. 65, 490-560.