Table 3. The effect of trypsin and antiserum treatment on adhesion of cells to TSP and fibronectin. In experiment 1, B16-F10 cells were treated with cycloheximide (20 μ*M*) for 60 minutes before being washed and then harvested with EDTA (1 m*M*) or EDTA (1 m*M*) plus trypsin (1 mg/ml). After 15 minutes at 37°C, soybean trypsin inhibitor (1 mg/ml) was added. Cells were resuspended in serum-free DMEM containing cycloheximide (20 μ*M*), and adhesion assays were performed on TSP- and fibronectin-coated slides (see Table 1). In experiment 2, cells harvested with EDTA in the absence of cycloheximide were treated with a 1:40 dilution of antiserum to GP140 or preimmune serum for 10 minutes at 37°C and plated on either TSP- or fibronectin-coated slides. The apparent potentiation of cell adhesion to TSP in the presence of antiserum to GP140 was a consistent finding and is not understood at this time.

Treatment of cells	Adherent cells per square millimeter	
	TSP	Fibronectin
Experiment 1		
EDTA	489	285
Trypsin/EDTA	3	111
Experiment 2		
Preimmune serum	505	923
Antiserum to GP140	1070	5

receptor. Cells were treated with trypsin in the presence of cycloheximide to prevent protein synthesis and then tested for their ability to adhere to TSP and fibronectin. A similar procedure was used by Tarone *et al.* (*38*) to characterize the cell fibronectin receptor. Trypsin treatment totally abolished cell adhesion to TSP, whereas it inhibited adhesion to fibronectin to a lesser extent (Table 3). These results suggest that the TSP receptor is protein and is more sensitive to proteolysis than the fibronectin receptor. We also treated cells with a polyclonal antiserum (antiserum to GP140) to cell surface adhesion–related glycoproteins of 140,000 molecular weight (*39*). This antiserum is a potent inhibitor of fibronectin-mediated cell adhesion. Cells treated with antiserum to GP140 adhered to TSP but not to fibronectin (Table 3). These data suggest that the molecular mechanism involved in TSP-mediated cell-substratum adhesion is distinct from that involved in fibronectin-mediated adhesion.

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Protein-Binding Sites in Ig Gene Enhancers Determine Transcriptional Activity and Inducibility

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Individual protein-binding sites within the mouse immunoglobulin heavy chain and kappa light chain gene enhancers were altered, making it possible to examine the functional role of the sites during transcription. The E motifs, which bind factors that are present in many if not all cells, mostly behave as transcriptional activating sites. The only known heavy chain enhancer site that binds a lymphocyte-specific factor, the "octamer" site, plays a critical role in transcription but only in a truncated form of the enhancer. In the full enhancer, no one site is crucial because of an apparent functional redundancy. The site in the kappa enhancer that binds a factor specific to mature B cells, κB, was crucial to the constitutive activity of the enhancer in B cells. This factor is also inducible in pre-B cells, and the site was necessary for inducibility of the kappa enhancer. Thus, the sites defined by protein binding are important for the functional activity of immunoglobulin enhancers, with the sites that bind proteins restricted in their cellular distribution playing the most important roles.

MATURATION OF B LYMPHOCYTES in adult bone marrow is a paradigm for differentiation of a cell lineage (*1*). At the pre-B cell stage, rearrangement and transcription of the immunoglobulin (Ig) heavy chain gene takes place (*2*). In more mature cells, light chain genes are rearranged and transcriptionally activated, and Ig tetramers are then expressed on the cell surface (*3*). Agents such as bacterial lipopolysaccharide, cycloheximide, and phorbol esters can induce kappa gene expression at earlier stages (*4*). In both the Ig heavy chain and kappa light chain genes, the large intron between the variable and constant regions contains a transcriptional enhancer element (*5*). These cis-acting sequences function independently of position and orientation and are virtually restricted to activity in cells of the B-lymphoid lineage although a portion of the heavy chain en-

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hancer is active in non-B cells (*5*). To determine if these enhancer sequences contain the crucial DNA elements for differentially activating the transcription of Ig genes, we have been dissecting the enhancers into individual, short, sequence motifs and studying the role of each element (*6, 7*).

Several studies have identified putative trans-acting factors that interact with defined motifs within the Ig enhancers (*7–15*). In vivo dimethyl sulfate protection experiments (*14, 15*) first identified sites we have called E motifs that are variants of an apparent consensus sequence 5′-CAGGTGGC-3′. There are four examples in the heavy chain enhancer and three examples in the kappa enhancer (Fig. 1A). In vitro, distinct proteins, NF-μE1 and NF-μE3, bind to the E1 motif in the heavy chain enhancer and to the E3 motifs in the heavy chain and kappa enhancers (*7, 9*). Also, a conserved octanucleotide motif, 5′-ATTTGCAT-3′, that binds two factors, occurs in the heavy chain enhancer (Fig. 1A, OCTA) as well as in Ig promoters. The NF-A1 factor is found in all cell types, and the NF-A2 factor is restricted mainly to cells of the B lineage (*10, 11*). A site present in the kappa enhancer but not in the heavy chain enhancer (Fig. 1A, the B site) binds yet another factor, NF-κB, that is constitutively present in mature B cells and whose binding can be posttranslationally induced in earlier B cells as well as in non-B cells (*8*).

To define the functional role of these protein-binding sites, we made clustered mutations in each site using synthetic oligonucleotides (*16*) (Fig. 1A). We measured activity of wild-type and mutant enhancers in plasmids containing a truncated c-*fos* promoter driving transcription of the bacterial chloramphenicol acetyltransferase (CAT) gene (Fig. 1B) (*17, 18*). It was essential to test the enhancers in the context of heterologous promoters and protein-coding sequences because these portions of Ig genes are themselves tissue-specifically regulated (*6*). In preliminary studies, we obtained similar results testing the activity of various mutant enhancers by CAT production or by directly measuring messenger RNA (mRNA) accumulation by S1 nuclease assay. Because the body of the mRNA is not altered in the mutant constructs, the level of CAT activity should reflect the influence of enhancer mutations on the rate of transcription. Transient transfections of the truncated promoters alone gave minimal CAT activity (*19*). Transcription in S194 myeloma cells was increased at least 330-fold with the full 700-bp heavy chain enhancer (called μ700) cloned immediately upstream or 130-fold when cloned 2.5-kb downstream of the c-*fos* promoter (the Xho I site in Fig. 1B) in either orientation.

Mutations in any one E motif or in the OCTA motif within μ700 reduced enhancement by no more than a factor of 2 when tested in the 5′ position. This suggested that if these motifs were important for μ700 enhancer function, loss of any one could be compensated by other sites. We therefore tested combinations of E motif and OCTA mutations. Introduction of mutations in both E3 and E4 caused only a slight reduction in enhancer activity (Fig. 2, line 2). The combination of $E1^-$, $E3^-$, and $E4^-$ or the combination of $E2^-$ and $E3^-$, however, decreased activity to 1.4 and 34% of wild-type activity (Fig. 2, lines 3 and 4). These data were reproducible in three experiments, two measurements in each. Thus, the E motifs may be functionally redundant, but the E1 and E2 motifs can play important roles in the enhancer effect.

In previous studies, the 300 bp at the 3′ end of the heavy chain enhancer (μ300) provided 30 to 50% of the enhancement provided by the 700-bp fragment (*20*). The μ300 portion contains the E3, E4, and OCTA motifs and was used to assess their transcriptional roles without the influence of other upstream motifs (legend to Fig. 1). When transfected into J558L myeloma cells, we found it gave 70% of the level of μ700. The μ300 enhancer altered in either E3 or E4 displayed 40 to 60% of its wild-type activity. These data are shown in Fig. 3A where we have set the wild-type levels for both orientations at 100% and for which the level of activity with no enhancer (ENH^-) is given as a relative value to the wild type in each orientation. In contrast to the E3 and E4 results, altering the OCTA motif caused a reduction in the activity by a factor of 10 when tested in either orientation (Fig. 3A, O^-). Combining $E4^-$ and $OCTA^-$ mutations had little added effect, but the further mutation of the E3 site (Fig. 3A, $E3^-$, $E4^-$, and O^-) caused another decrease to one-fifth the activity of the $OCTA^-$ mutation (Fig. 3A, O^-). This combination represents one-fiftieth the activity of the wild type. Truncation of the μ300 enhancer to 165 bp also emphasized the importance of the OCTA site because in this construct altering

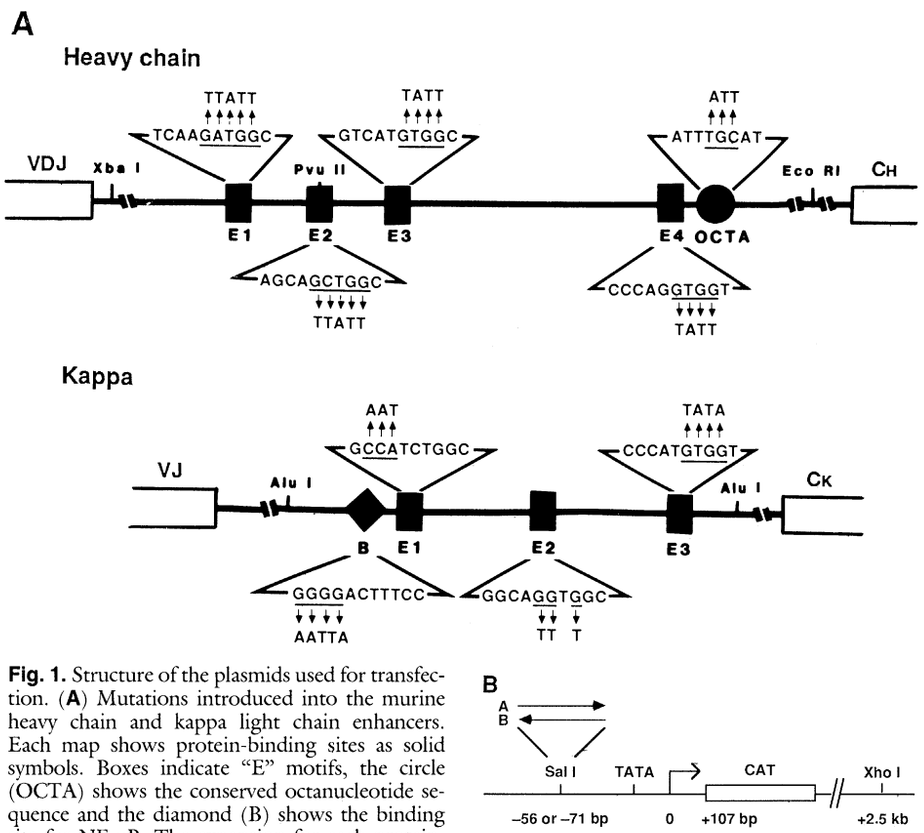

Fig. 1. Structure of the plasmids used for transfection. (**A**) Mutations introduced into the murine heavy chain and kappa light chain enhancers. Each map shows protein-binding sites as solid symbols. Boxes indicate "E" motifs, the circle (OCTA) shows the conserved octanucleotide sequence and the diamond (B) shows the binding site for NF-κB. The expansion for each protein-binding site gives its wild-type sequence and the nucleotides replaced by mutations are underlined. E motifs are shown as 10-bp sequences to correspond to Church *et al.* (*15*). Arrows indicate clusters of nucleotides introduced by mutations. Open boxes are exons for the variable and constant regions and the thin line indicates intron sequences. The heavy chain enhancer referred to as μ700 extends between the Xba I and Eco RI sites and the segment referred to as μ300 extends between the Pvu II and Eco RI sites. The portion of the kappa enhancer between the Alu I sites is 473 bp. (**B**) Insertion sites for enhancer fragments into a c-*fos*-CAT fusion plasmid (*17, 18*) either 56 or 71 bp upstream (Sal I) or 2.5 kb downstream (Xho I) of the mRNA cap site mapped by S1 nuclease assay (*18*). The A orientation is the natural orientation as given for each enhancer in (A).

OCTA left little residual activity (Fig. 2, lines 5 and 6). Results closely corresponding to these were obtained in two experiments. Similar findings were obtained in S194 and MPC-11 myeloma cells as well as in PD31 pre-B cells. Thus the OCTA and E3 motifs have significant roles in enhancer activity while E4 may have a lesser role.

Although the μ700 enhancer was inactive in nonlymphoid cells, we obtained a sixfold enhancer effect in 3T3 fibroblast cells with the B orientation of the μ300 fragment (Fig. 3B). Alterations in E3 decreased this activity by a factor of 3, but changes in E4 and OCTA had no effect (Fig. 3B); there was no stimulation by the A orientation of wild type (compare WT, A orientation to ENH^-, A orientation), but an $E3^-$ alteration decreased transcription below the basal level seen with either wild type or no enhancer, which were essentially equivalent. This implies that the lack of an enhancer effect in the A orientation may be caused by a balance between stimulatory and inhibitory sequences. Further experiments showed that the inhibitory sequence, which lies close to the Eco RI site in the 3′ end of the fragment and decreases transcription only from closely situated promoters, appears to be that described in studies in vitro (*13*). Therefore, the $E3^-$ mutation in the heavy chain had an equal effect in lymphoid and nonlymphoid cells, but the $OCTA^-$ mutation only affected lymphoid transcription.

A similar mutational analysis was carried out for the kappa enhancer. We transfected S194 myeloma cells with constructs bearing the 473-bp kappa enhancer and showed stimulation of transcription ninefold in the A orientation and 18-fold in the B orientation. (Fig. 3C; WT values are both set at 100% and the ENH^- bars give relative differences between enhancer-containing constructs and that with no enhancer.) Equivalent levels were observed in either the upstream or downstream position. Unlike the heavy chain enhancer, single mutations in the whole enhancer significantly decreased activity. Mutation of the B motif, which binds a protein, NF-κB, that is constitutively present only in mature B cells (*7*), had the most dramatic effect by reducing activity essentially to unenhanced levels. The $E2^-$ mutation caused a reduction in enhancement by a factor of 10. Mutations in either the E1 or E3 sites caused reductions by approximately a factor of 3, except that the $E3^-$ mutation had no effect in the B orientation where the site was placed far from the promoter. Neither the wild-type nor mutant enhancers were active in 3T3 cells. Therefore, all motifs contribute to activity in myeloma cells.

Because compounds, such as bacterial lipopolysaccharide (LPS), cycloheximide (CYC), and phorbol esters, that induce kappa gene expression in pre-B cells also activate the binding of NF-κB (*4, 8*), we investigated the role of the B motif in the induction process. After PD31 pre-B cells were transfected with a plasmid bearing the wild-type kappa enhancer, exposure of the cells to LPS for various lengths of time resulted in increases in transcriptional activity that correlated positively with length of treatment (Fig. 3D, solid bars). The inducibility of the kappa enhancer, however, was abrogated by mutation of the B motif (Fig. 3D, open bars). The B^- mutation also decreased enhancer activity before induction, probably because uninduced PD31 cells contain a small, but detectable amount of NF-κB (*7*). No E motif alteration impaired inducibility, suggesting that the effect was specific to the B site. Thus, the B site is responsible for inducibility of the kappa enhancer. Similar results were obtained with phorbol ester

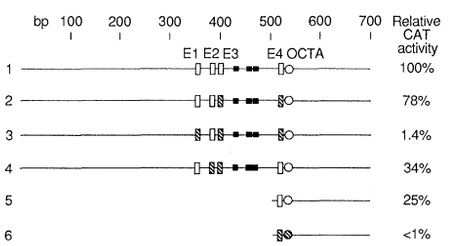

Fig. 2. Activity of heavy chain enhancer mutations in S194 myeloma cells. Enhancer fragments were inserted into the Sal I site in the B orientation. Open symbols give the position of E and OCTA motifs and solid boxes are sequences homologous to the SV40 core motifs (*7, 14, 15*). Hatched boxes indicate mutated sites and lost portions of the lines show deletions. The deleted enhancers were made by inserting an Xho I linker at a Dde I site in μ700. The 56-bp promoter was used for line 4 and the 71-bp promoter (which has a basal activity five times as great as that of the 56-bp promoter) was used for lines 1 to 3 and 5 and 6. Each mutant is compared to the wild type in the corresponding promoter construct. CAT values are averages of two independent transfections. These were obtained from three separate experiments.

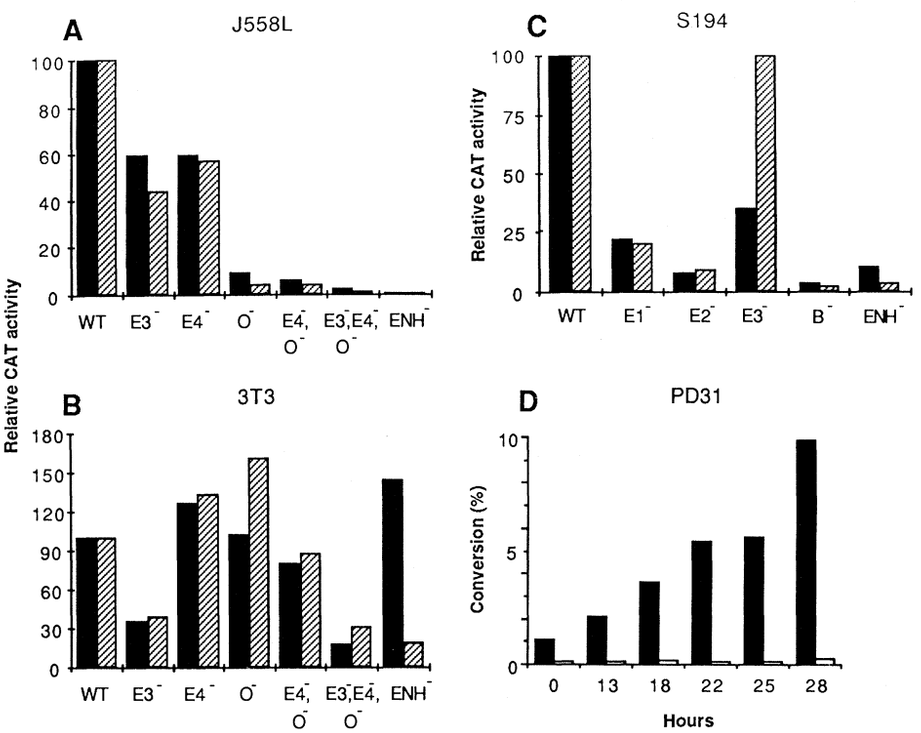

Fig. 3. Effect of mutations on Ig enhancer transcription rates and inducibility. (**A**) The μ300 heavy chain enhancers were tested in J558L myeloma cells using the 56 bp promoter. The pair of bars for each enhancer gives the A orientation (solid) and the B orientation (hatched). In each experiment, each value is the average of two independent transfections; repeated experiments (n = 2 to 4) gave similar results in which the standard deviations were C.V. < 25% compared to the values given. Absolute percent conversions of chloramphenicol to acetylated forms are: wild-type, A orientation = 3.4%; wild-type, B orientation = 20.6%; and no enhancer = 0.02%. (**B**) Transfection of μ300 heavy chain enhancers into BALB/c 3T3 cells as in (A). Absolute percent conversions of chloramphenicol to acetylated forms are: wild-type, A orientation = 2.2%; wild-type, B orientation, 17.9%; and no enhancer, 3.2%. (**C**) The kappa enhancer mutants were tested in S194 cells with the 71-bp promoter as in (A). The absolute percent conversion of chloramphenicol to acetylated forms is: wild-type, A orientation, 8.8%; wild-type, B orientation, 17.6%; and no enhancer, 1.1%. (**D**) PD31 cells were transfected with either the wild-type (left, solid bar) or B^- (right, open bar) kappa enhancers. All samples were harvested 48 hours after transfection, but bacterial lipopolysaccharide (10 μg/ml) was added for the number of hours indicated before harvest. Activity is given as absolute percent conversion of [^{14}C]chloramphenicol to acetylated forms. The vector without enhancer gave less than 0.1% conversion and was not inducible.

induction and with the enhancer cloned into the Xho I site 3′ of the CAT gene. Kappa enhancer induction dependent on NF-κB binding was also seen in two very early pre-B cell tumor cell lines, HAFTL-1 and 38B9. Before induction these lines had no detectable NF-κB and essentially no enhancer activity, suggesting the kappa enhancer is inactive at early stages of B-cell differentiation because of the lack of NF-κB.

To be certain that the transcriptional effects of the various mutations correlated with reduced protein binding, we tested the mutant motifs in an electrophoretic mobility shift assay (*21*). For the heavy chain enhancer, we found by competition with mutant or wild-type DNA fragments (Fig. 4A, lanes 1 to 3 for μE1) or by direct binding (Fig. 4A, lanes 7 to 12 for μE3 and OCTA), that the mutations eliminated formation of the NF-μE1, NF-μE3, NF-A1, and NF-A2 nucleoprotein complexes. For the kappa enhancer, the E3$^-$ and B$^-$ mutations eliminated the previously described NF-μE3 and NF-κB nucleoprotein complexes (Fig. 4B, lanes 1 to 4 and 7, 8). We also detected binding of a distinct nuclear protein to the heavy chain E2 motif (termed NF-μE2) and to the kappa chain E2 motif (termed NF-κE2). These do not cross-compete for binding with either the E1 or E3 sites and are distributed in many cell types. Competition analysis showed that the μE2$^-$ mutation excluded NF-μE2 binding (Fig. 4A, lanes 4 to 6) and direct binding analysis showed the κE2$^-$ excluded NF-κE2 binding (Fig. 4B, lanes 5 and 6). No binding was detected to the μE4 or to the κE1 motifs despite a significant transcriptional effect of the latter.

Functional analyses of mutant Ig heavy chain and kappa light chain enhancers have shown that the previously observed protein-binding sites have important roles in enhancer function. In these experiments, positioning the enhancer close to the truncated promoters may allow it to function as part of the promoter. We observed, however, activation of the truncated promoters by wild-type or selected mutant enhancers in the 3′ position to similar degrees as in the 5′ position, suggesting the effects do not depend on distance. Also, for the heavy chain enhancer, the effect of readthrough from the sterile μ promoter has been eliminated by the use of the B orientation of μ700 or either orientation of μ300 in which direct tests have shown that no readthrough occurs (*22*).

The E sites in both the heavy chain and kappa enhancers bind several distinct proteins but may function similarly and, at least in the heavy chain, appear to be redundant. Their effects may be specific to lymphoid cells because, in vivo, binding to E3 and other E motifs in the endogenous heavy chain enhancer occurred only in B lymphoid cells (*14, 15*). The in vitro experiments here and previously reported (*7*), however, indicate that the nuclear proteins binding to E motifs are distributed in many tissue types. Our results show that the E3$^-$ mutation decreased enhancer activity in both lymphoid and nonlymphoid cells. Thus, the E motifs probably do not directly determine the lymphoid-specificity of Ig enhancers. A specific chromatin conformation or other factors may regulate binding of E motif proteins to chromosomal enhancer sequences.

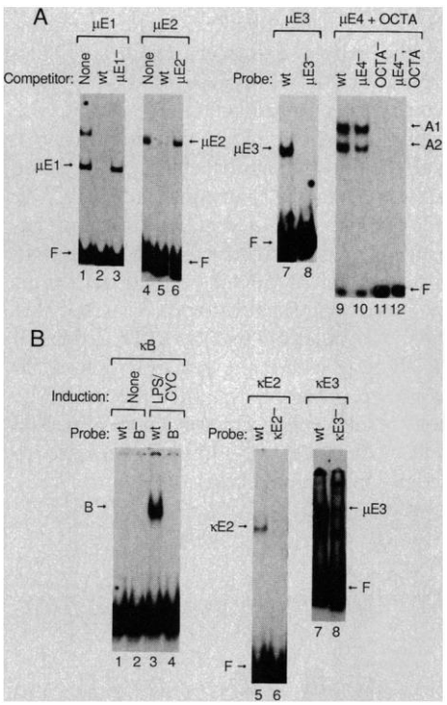

Fig. 4. Effect of mutations on the binding of nuclear factors. (**A**) Electrophoretic mobility shift assay of fragments bearing heavy chain enhancer motifs (*13*). For binding reactions we used the following labeled fragments and nuclear extracts. (Lanes 1 to 3) A 45-bp Hinf I–Pst I fragment containing the μE1 motif analogous to that used by Weinberger *et al.* (*9*) in an extract from EW, a human B lymphoma; also included was 25 ng of identical unlabeled competitor fragment that had a wild-type (lane 2) or mutant μE1 (lane 3); (lanes 4 to 6) a 45-bp Sau 3A to Nco I fragment containing the μE2 motif (generated by inserting an Nco I site into the μE3 motif by site-directed mutagenesis) in an extract from the murine T cell BW5147; also included was 4 ng of unlabeled competitor fragment that had a wild-type (lane 5) or mutant (lane 6) μE2 motif; (lanes 7 and 8) a 70-bp Alu I to Alu I fragment [denoted μ70 in (*7*)] containing the μE3 motif in WEHI 231, murine B-cell lymphoma extract; (lanes 9 to 12) a 50-bp Dde I to Hinf I fragment [denoted μ50 in (*7*)] containing the μE4 and OCTA motifs in WEHI 231 extract. (**B**) Binding reactions were done with the following labeled kappa enhancer fragments and nuclear extracts. (Lanes 1 to 4) A 70-bp Dde I–Hae III fragment [denoted κ3 in (*7*)] containing either the wild-type (lanes 1 and 3) or mutant (lanes 2 and 4) B motif. Nuclear extracts were from 70Z/3 cells that were untreated (lanes 1 and 2) or treated for 4 hours with bacterial lipopolysaccharide (LPS) at 10 μg/ml and cycloheximide (CYC) at 10 μg/ml (lanes 3 and 4) to induce NF-κB binding. (Lanes 5 and 6) A 52-bp Sau 3A–Hinc II fragment containing either the wild-type or mutant κE2 motif was incubated in 38B9 extract. (Lanes 7 and 8) An 85-bp Hae III to Dde I fragment [denoted κ2 in (*7*)] was used in an extract from uninduced 70Z/3 cells.

Our results provide evidence that proteins binding to non-E motifs may determine the tissue restriction of heavy chain enhancer activity to lymphoid cells and kappa enhancer activity to the mature B-cell stage. The evidence is clearest in the kappa enhancer where the B motif, binding the NF-κB protein, seemed the key site because mutation of it left little or no residual activity and abolished inducibility. Because the B$^-$ mutant had no basal activity, it is difficult to prove whether the B site is responsible for induction of the enhancer. The fact that NF-κB binding activity is inducible, however, suggests that it plays a causative role. In addition, most of the induction of kappa gene expression in pre-B cells may result from enhancer activation because the kappa promoter works approximately equally at all stages of B-cell development (*23*). Our results extend observations associating levels of NF-κB with kappa gene expression and enhancer activity (*8*). Because induction of NF-κB can be correlated to in vivo function of the kappa enhancer, this provides a forceful argument for a crucial role of the enhancer in situ.

In the μ300 enhancer, the OCTA$^-$ mutation had the greatest effect but only in lymphoid cells. Thus, it would appear to interact with a lymphoid-specific factor. The octanucleotide-binding protein NF-A2 has been found in cells at all stages of B lymphoid differentiation, but not in nonlymphoid cells (*10, 11*). The OCTA motif, however, also interacts with a ubiquitous factor. Nevertheless, the binding of NF-A2 likely determines the activity of the OCTA element since the motif functions at different B-cell stages when NF-A2 is present and not in nonlymphoid cells. The OCTA motif is also crucial to Ig promoter activity—it can confer lymphoid specificity on genes that are otherwise not specific to these cells (*24*). We did find that the effects of OCTA mutation depended on the version of the enhancer tested. Mutation of the OCTA motif in μ700 had little effect on its activity in lymphoid cells. This suggests that there may be one or more other tissue-specific factors that can bind to the heavy chain enhancer and can substitute for the OCTA factor.

Our studies show that most of the individual protein-binding sites have roles in

enhancer activity, and certain of them may determine B-cell specificity. Studies of purified factors will be needed to reveal the mechanisms by which protein binding confers the enhancement effect.

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16. Mutagenesis in bacteriophage M13 was carried out exactly as described by M. Zoller and M. Smith [*DNA* **3**, 479 (1984)]. Alternatively, 0.4 μg of oligonucleotide was annealed to 1 μg of heteroduplex plasmid carrying a single-stranded gap over the mutation site. Annealed products were repaired for 1 hour at 14°C with Klenow DNA polymerase and T4 DNA ligase in 50 m*M* tris-Cl, *p*H 7.6, 10 m*M* $MgCl_2$, 10 m*M* dithiothreitol, 1 m*M* spermidine, 1 m*M* ATP (adenosine triphosphate) and BSA (bovine serum albumin) at 0.1 mg/ml, and the repair products were transformed into bacteria (P. Sarnow and D. Baltimore, unpublished results). Mutants were selected by colony filter hybridization with the mutagenic oligonucleotide. Mutations were confirmed by DNA sequencing [A. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980)].
17. The *fos*-CAT fusion plasmids, described by Gilman *et al.* (*18*), were modified by placing a Xho I linker 2.5 kb downstream from the mRNA cap site (at a unique Nde I site). Sal I or Xho I–linkered enhancer fragments were cloned into the Sal I or Xho I sites in both orientations. The μ300 or smaller heavy chain enhancers were prepared by inserting Xho I linkers into the Pvu II site or Dde I sites in μ700.
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19. About 2×10^7 lymphoid cells were transiently transfected with 10 μg of plasmid DNA in the DEAE-dextran procedure [R. Grosschedl and D. Baltimore (*6*)]. Transfections of 0.3×10^7 3T3 cells with calcium phosphate coprecipitation and cell lysates and CAT assays were carried out according to M. Gilman *et al.* (*18*). Assays were standardized to either 50 or 100 μg of protein for each sample for 1 hour with 0.1 μCi of [^{14}C]chloramphenicol. 3T3 transfections were normalized by cotransfection of a plasmid bearing the RSV promoter driving the β-galactosidase gene. Lymphoid transfections were normalized by repeated transfection ($n = 4$ to 6) and relating values to transfection of pSV2 CAT in each case.
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21. Mobility shift electrophoresis and preparation of nuclear extracts was performed according to (*7*) and (*11*). Reactions used 0.25 to 0.5 ng of end-labeled probe fragment (10,000 cpm), 10 μg of nuclear extract and 2.5 μg poly-d(IC) (Pharmacia) in 10 m*M* tris (*p*H 7.5), 5 m*M* NaCl, 1 m*M* dithiothreitol, 1 m*M* EDTA, and 5% glycerol in 15 μl. After incubation at 25°C for 15 minutes, samples were loaded onto 4% (30:1) polyacrylamide gels buffered with 6.7 m*M* tris-Cl (*p*H 7.5), 3.3 m*M* sodium acetate, and 1 m*M* EDTA.
22. M. Lenardo and D. Baltimore, unpublished observations.
23. J. Pierce and D. Baltimore, unpublished observations.
24. T. Wirth, L. Staudt, D. Baltimore, unpublished results.

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