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# Functional Analysis of the Murine T-Cell Receptor β Enhancer and Characteristics of Its DNA-Binding Proteins

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**The minimal T-cell receptor (TCR) β-chain (TCRβ) enhancer has been identified by transfection into lymphoid cells. The minimal enhancer was active in T cells and in some B-lineage cells. When a larger fragment containing the minimal enhancer was used, its activity was apparent only in T cells. Studies with phytohemagglutinin and 4β-phorbol-12,13-dibutyrate revealed that the enhancer activity was increased by these agents. By a combination of DNase I footprinting, gel mobility shift assay, and methylation interference analysis, seven different motifs were identified within the minimal enhancer. Furthermore, competition experiments showed that some of these elements bound identical or similar factors that are known to bind to the TCR Vβ promoter decamer or to the immunoglobulin enhancer κE2 or μEBP-E motif. These shared motifs may be important in the differential gene activity among the different lymphoid subsets.**

The antigen-specific T-cell receptor (TCR) is encoded by multiple germ line gene segments (20). The variable (V), diversity (D; in TCR β-chain [TCRβ] only), and joining (J) gene segments undergo time- and tissue-specific DNA rearrangement to assemble the complete TCR molecule (20). This process appears to be similar to that occurring in immunoglobulin genes (1, 2, 39) except for the type of lymphocytes involved. Since the fully assembled TCR gene is found only in T cells, the complete TCR message is only transcribed in such cells (20). It has been proposed that a common recombinase mediates the rearrangement of TCR and immunoglobulin genes in T and B cells, respectively (30, 42). Alt and his colleagues suggested that the control of DNA rearrangement at these loci may depend on chromosomal "accessibility" to the putative recombinase (1). The accessibility, in turn, may be linked to the transcriptional status (6, 31). If this is the case, detailed analysis of the transcriptional apparatus may shed light on the basis of tissue-specific gene rearrangement, a major developmental step during lymphocyte development.

In this article we report on the functional characteristics of the TCRβ enhancer as well as the nature of the DNA-binding proteins relevant to its function. The results reveal that factors that bind to the TCRβ enhancer are unexpectedly related to those that bind to the Vβ promoter and the immunoglobulin enhancer.

## MATERIALS AND METHODS

**Plasmid constructions.** A 695-base-pair (bp) blunted *Stu*I-*Nco*I fragment of the TCRβ enhancer element was inserted into the *Eco*RV and *Hin*cII sites of Bluescript (Stratagene). To generate the deletion mutants, plasmids linearized by digestion with either *Pst*I and *Eco*RI or *Xho*I and *Apa*I were digested with exonuclease III for various lengths of time. The ends were blunted with mung bean nuclease and ligated. After the DNA sequence of the deletion mutants was confirmed, the *Xba*I-*Sma*I fragment of the TKCAT gene from pTE2ΔSal/Nru (38) was inserted into the *Xba*I and *Sma*I sites of each Bluescript clone containing the deletion mutants. The β-galactosidase (*lacZ*)-expressing vector SFFV-lacZ was constructed from SFFVneo (9). A 3.6-kilobase (kb) *Hin*dIII-*Bam*HI fragment containing the *lacZ* gene was cut out from pMMuLV-SV-lacZ (29) and cloned into SFFVneo after removal of the neomycin resistance gene encoding aminoglycoside phosphotransferase. To allow replication of the vector, the polyomavirus origin of replication or the simian virus 40 large T antigen was cloned into the unique *Bam*HI site of the SFFV-lacZ vector.

**Cell lines.** The following cell lines were maintained in RPMI 1640 medium with 10% fetal calf serum (FCS): EL-4 and BW5147, murine T cells; Jurkat, a human T cell; PD-31, an Abelson murine leukemia virus-transformed pre-B-cell line with rearranged immunoglobulin heavy-chain (IgH) locus; DHL-9, a human B-cell line lacking surface immunoglobulin (34); M-12, a spontaneously derived murine B-cell line with low surface immunoglobulin; S194, a murine plasmacytoma; NIH 3T3, a murine fibroblast; and HeLa, a human cervical carcinoma cell line.

**Transient transfection.** Cells were transfected by the DEAE-dextran method. Briefly, $10^7$ cells were harvested, washed with RPMI 1640, and suspended in 1 ml of DEAE-dextran (1 mg/ml) in Tris-buffered saline (TBS), pH 7.5. Then, 1 ml of a DNA solution containing 30 μg of test sample and 20 μg of SFFV-lacZ control vector was mixed with cells. After incubation at room temperature for 30 min, the cells were pelleted and further washed with RPMI 1640 without FCS. Finally, the cells were suspended in RPMI 1640 with FCS and incubated at 37°C for 40 h.

**CAT assays.** The transfected cells were harvested and suspended in 200 μl of 0.25 M Tris hydrochloride (pH 7.5). After three cycles of freeze-thaw lysis, the supernatants were divided equally for chloramphenicol acetyltransferase (CAT) and β-galactosidase assays. The CAT assay was done by the method of Gorman et al. (12) with [$^{14}$C]chloramphenicol. For the normalization of transfection efficiency, β-galactosidase activities were determined by using *O*-nitrophenyl-β-D-galactopyranoside as a substrate. To determine

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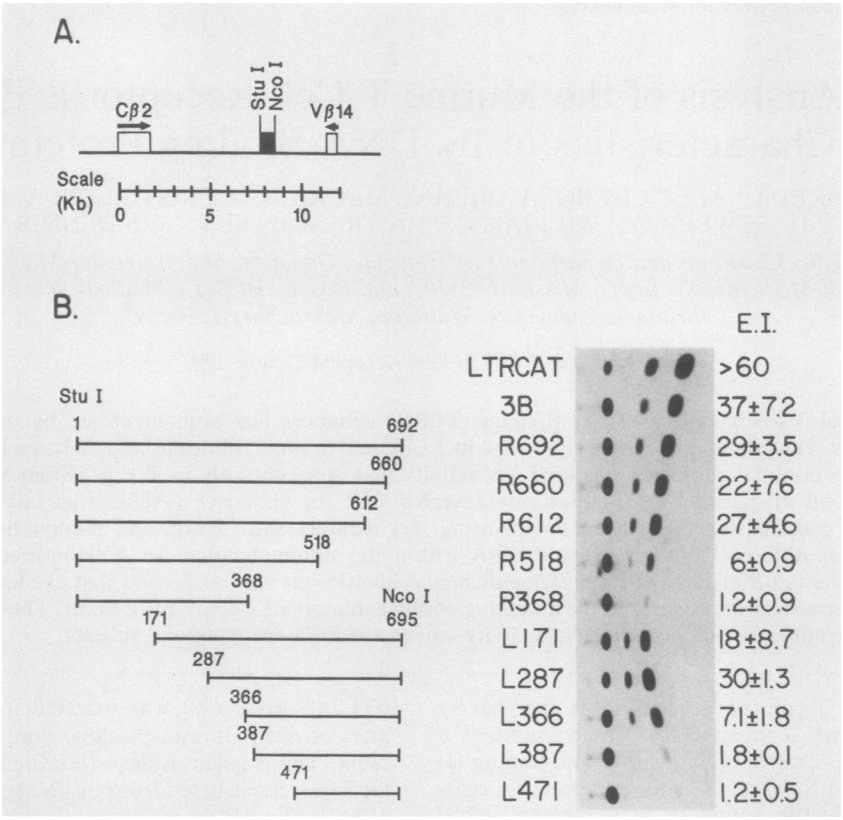


FIG. 1. Location and functional activity of the TCRβ enhancer. (A) 695-bp *Stu*I-*Nco*I TCRβ enhancer (solid box) between Cβ2 and Vβ14. Arrows indicate the transcriptional orientation. (B) Enhancement activity of each deletion mutant transfected into Jurkat cells. R and L series mutants span from the *Stu*I site to the indicated site or from the indicated site to the *Nco*I site, respectively. LTRCAT consists of the long terminal repeat of Friend spleen focus-forming virus linked to the *cat* gene. 3B is a 4-kb *Hin*dIII fragment containing the minimal TCRβ enhancer cloned 5′ to the TKCAT gene. The enhancement index (E.I.) was calculated as follows: % conversion of chloramphenicol by enhancer-containing TKCAT/% conversion of chloramphenicol by enhancerless TKCAT, normalizing the relative β-galactosidase activities of two transfectants to compare for differences in transfection efficiency.

the enhancement index, the acetylated and nonacetylated forms of chloramphenicol were excised from the thin-layer chromatograph, and the amount of radioactivity was determined by liquid scintillation counting. These results were then normalized against the β-galactosidase activity for transfection efficiency.

**DNase I footprinting.** Nuclear extracts were prepared and DNase I footprinting was performed as described previously by Anderson et al. (4). Coding-strand probe was labeled at the polylinker *Asp*-718 site 3′ to position 287 of L287; the non-coding-strand probe was labeled at the polylinker *Bam*HI site 5′ to position 612 of R612.

**GMSA and methylation interference assay.** The gel mobility shift assay (GMSA) and methylation interference assay were carried out essentially as described by Sen and Baltimore (32) with the following probes (nucleotide positions): β1 (320 to 349), β2 (338 to 370), β3 (365 to 395), β4a (384 to 413), β4 (394 to 433), β5 (454 to 487), β6 (490 to 525), β7 (552 to 584), and β8 (571 to 611). To make these probes, acrylamide electrophoresis was performed to exclude the single-stranded DNA after the annealing reactions. For GMSA, end-labeled probes (0.1 to 0.5 ng; 10,000 cpm) were incubated with 10 μg of nuclear extract in a 15 μl reaction mix [10 mM Tris (pH 7.5), 1 mM EDTA, 100 mM β-mercaptoethanol, 50 mM NaCl, 2.5 μg of poly(dI) · poly(dC), 4% glycerol] at room temperature for 20 min. Following binding, the mixtures were loaded onto a 4% acrylamide gel containing 89 mM Tris, 89 mM boric acid, and 2 mM EDTA, and electrophoresis was performed for 3 h. Gels were dried prior to autoradiography.

## RESULTS

**Deletion analysis defines the minimal TCRβ enhancer element.** The fully functional murine TCRβ enhancer was originally identified as a 695-bp *Stu*I-*Nco*I DNA fragment located between Cβ2 and Vβ14, as previously described by Krimpenfort et al. (19) and MacDougall et al. (24) (Fig. 1A). To define the minimal region necessary for the enhancer effect, further deletion clones were constructed and inserted into a TKCAT reporter gene. The TKCAT gene consists of the CAT gene linked to the herpes simplex virus thymidine kinase promoter. The normalized results, with Jurkat cells used as recipients in a transient transfection assay, showed that two fragments, spanning residues 1 to 612 (R612) and 287 and 695 (L287), maintained the full enhancer activity (Fig. 1B).

**TCRβ enhancer activity shows lymphoid cell specificity.** To ascertain the tissue specificity of the TCRβ enhancer, cell lines of different origins were transfected with R692 (692 bp) or 3B (4 kb) (Table 1). 3B is a *Hin*dIII fragment containing the TCRβ enhancer which was cloned 5′ of the TKCAT gene. Both R692 and 3B were active in T-cell lines (Jurkat and EL-4) but inactive in nonlymphoid cell lines (HeLa and

TABLE 1. TCRβ enhancer activity is lymphoid cell specific[a]

| Plasmid | Enhancement index | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | EL-4 | Jurkat | PD-31 | DHL-9 | M-12 | S194 | NIH 3T3 | HeLa |
| 3B | 24.0 | 37.0 | 7.0[b] | 0.7 | 0.1[b] | 1.3 | 1.1 | 0.3 |
| R692 | 30.0 | 29.0 | 18.0 | 10.6 | 0.3 | 1.5 | 1.1 | 0.7 |
| LTRCAT | 42.0 | 60.0 | 79.0 | 150 | ND[c] | 14.0 | 9.0 | 13.0 |

[a] All transfections were done by the DEAE-dextran method as described in Materials and Methods. The enhancement index was calculated as described in the legend to Fig. 1. LTRCAT consists of the long terminal repeat of Friend spleen focus-forming virus linked to the CAT gene.
[b] A 4-kb enhancer fragment was inserted 3′ to the TKCAT gene.
[c] ND, Not determined.

NIH 3T3). Results with 3B showed discrimination between T- and B-cell lineages, since 3B did not function in either DHL-9 or S194 cells. In contrast, R692 appeared to function (although not as efficiently as in T cells) in some B-lineage cells (PD-31 and DHL-9) but not in others (M-12 and S194). Krimpenfort et al. earlier reported that a 550-bp *Hpa*I-*Nco*I enhancer fragment functioned in 2M3, a pre-B-cell line (19). In contrast, with a 3.2-kb *Hpa*I-*Bgl*II enhancer fragment transfected into P3X63-Ag8, a plasmacytoma line, MacDougall et al. failed to see any activity (24). Together with the data reported here, it appears that the larger enhancer fragment is not active at any stage of B-cell lineage, whereas the smaller fragments show activity in some B-lineage cells.

**Minimal enhancer is inducible by PHA and PdBu.** Lindsten et al. earlier reported that transcription of both TCRα and TCRβ genes can be induced by phytohemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA) (22). For the β-chain, the induction was observed in both the 1.3-kb VDJCβ and 1.0-kb DJCβ transcripts, suggesting that the PHA- and PMA-responsive element may reside near the Cβ locus. To test whether the TCRβ minimal enhancer responds to PMA stimulation, 2 μg of PHA and 50 ng of 4β-phorbol-12,13-dibutyrate (PdBU) per ml were added to the culture medium 24 h after transfection of either L287 or TKCAT into Jurkat cells. The minimal enhancer region in L287 showed 3.9-fold inducibility by PHA plus PdBu (relative CAT activity was 1.0 for untreated TKCAT [control] and 0.8 for TKCAT treated with PHA plus PdBU; for untreated and treated L287, the values were 22.1 and 86.2 [3.9-fold induction], respectively). These results strongly hint that the induction of TCRβ transcription by PMA and PdBu might be at least partly due to the enhancer itself.

**Minimal enhancer contains at least seven distinct binding sites.** DNase I footprinting analysis was performed to detect the binding sites for nuclear proteins within the minimal enhancer. Six protected regions, denoted βE1 to βE4, βE6, and βE7, scattered throughout a 325-bp region, could be defined by using BW5147 nuclear extract (Fig. 2A). The GMSA was performed with a set of synthetic oligonucleotides containing the protected sequences (corresponding to probes β1 to β7 and β4a in Fig. 2C). As expected, the oligonucleotide probes spanning the various DNase I-protected regions formed specific DNA-nuclear protein complexes. The βE4 motif detected by DNase I footprinting was divided into two areas which were later detected by probes β4 and β4a by GMSA. One additional site, βE5, was detected by GMSA but was not detected earlier by the footprinting assay (Fig. 2B and C). As shown in Fig. 1B and 2C, deletion construct L366, which lacks the βE1 and βE2 motifs, had a reduced enhancement activity of 24% of full activity, while L387, lacking βE1 to βE3, had only basal activity. Conversely, R518, which lacks the βE7 motif, had a reduced activity of 20%. These data strongly hint that the DNA motifs identified are functionally significant, while the stepwise reduction in the activity is reminiscent of the functional redundancy of the individual motifs, as previously observed in the immunoglobulin enhancer (5, 21).

After the specificity of the complexes was established by oligonucleotide competitions, the complexes were designated bands I to VI. The nonspecific band was designated N (Fig. 2B). One striking feature of the observed GMSA patterns was the similarity of the apparent mobilities of the complexes formed. This is particularly true of band V, which appeared to be shared by β1 to β7. Bands II and III were shared by β2, β4a, and β7; band I was shared by β4a and β7; and band IV was shared by β3, β4a, and β7. β6 formed an additional complex (band VI) which was specific to this probe. Because there was no obvious DNA sequence homology among β1 to β7 to explain the presence of shared complex patterns, we performed GMSA cross-competition experiments with different probes to define the relationships among the protein factors responsible for bands I to VI.

**Cross-competition among β1 through β7.** Because band V appeared to be shared by β1 to β7, experiments were initially performed with β4 as a probe (Fig. 3A). The data clearly show that band V could be competed away by β4, β5, and β7 but not by β2 or β8. β8 is a nonspecific competitor encompassing positions 571 to 611. β1, β3, and β6 also competed away band V (data not shown). In addition, band V, with β1 and β5 as probes, was also competed with by β1 to β7 (data not shown). When 50-fold excess oligonucleotides (β3 to β7) were used for competition assays to band V, these oligonucleotides showed similar competition ability (data not shown). β2 is probably a weak competitor, since it could itself weakly bind to form band V, which could be competed away by β1, β4, β5, and β7 (data not shown). Use of β6 as a probe defined bands V and VI (Fig. 3B). As expected, band V was competed with by β3, β4, β5, and β6 and partially by β1 and β2. Band VI could be competed with only by β6 itself and thus defined a β6-specific complex. Lastly, with the β7 probe, five specific bands (I to V) were seen (Fig. 3C). Here again, band V was competed with by β3, β5, β6, and β7 and partially by β2; band IV was competed with by β3 and β7; band III was competed with by β2, β6, and β7; band II was competed with by β2 and β7; but band I was competed with by β7 itself only. Thus, the bands defined by β7 fell into three categories: one specific to β7, one shared by several other motifs, and one common to all seven probes.

These cross-competition results are summarized in Fig. 3D and indicate that the shared bands, as defined in Fig. 2A, are always cross-competed with by each other, suggesting that identical or related factors bind to the individual motifs. We conclude that the TCRβ enhancer has at least seven regions defined by footprinting and GMSA. However, because the complexes formed appeared to be shared by distinct probes, the actual number of different complexes was reduced. There were at least four non-competing complexes: band V (β1 to β7), band VI (β6), band IV (β3 and β7), and those that were defined as bands I, II, and III by β7. Although the exact nature of the protein-DNA complexes is not known at this time, these results strongly suggest that multiple complexes are formed within the enhancer by identical or similar proteins. This may partially explain the redundancy observed in the functional assays. However, none of the constructs consisting of multiple (5 to 10) copies of a single probe (β3, β5, β6, or β7) inserted downstream of TKCAT constructs could substitute for the functional activ-

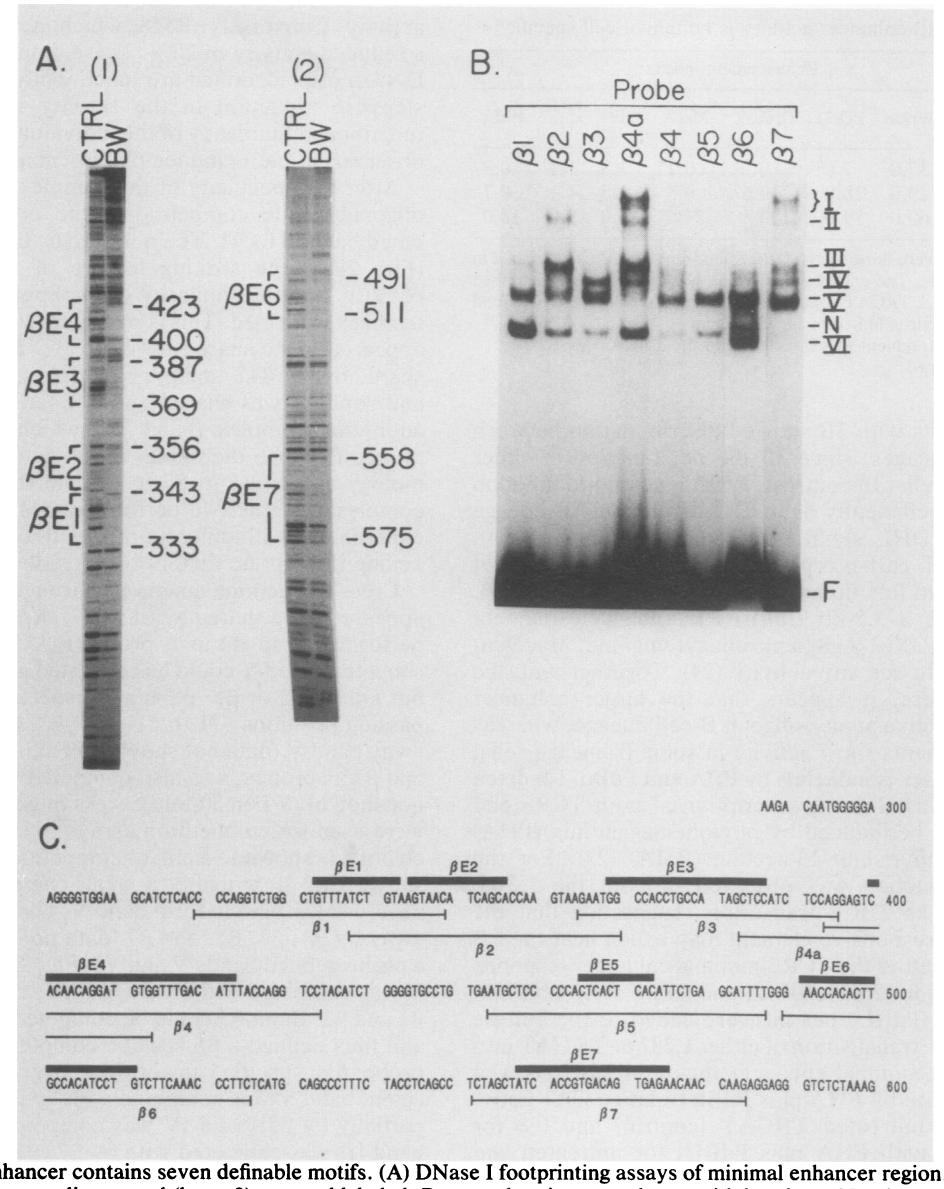


FIG. 2. Minimal enhancer contains seven definable motifs. (A) DNase I footprinting assays of minimal enhancer region. Either the coding strand (lanes 1) or the noncoding strand (lanes 2) was end labeled. Protected regions are shown with brackets. Numbers refer to the borders of protected regions. BW, DNase I-treated sample incubated with BW5147 nuclear extract. CTRL, DNase I-treated sample without nuclear extract. (B) GMSA with β1 to β7 probes. End-labeled probes were incubated with BW5147 nuclear extract. Specific complexes are designated I to VI. N designates a nonspecific complex. F indicates free DNA. (C) Sequence of the minimal enhancer spanning positions 287 to 612. Locations of βE1 to βE7 motifs are indicated by thick bars. To detect each motif, probes β1 to β7 and β4a (thin line) were synthesized.

ity of the TCRβ enhancer in the transient CAT assay systems (data not shown).

**Conserved decamer motif in Vβ promoters competes against the TCRβ enhancer motifs.** Although band V was shared by β1 to β7, there was no obvious sequence homology shared by these probes. Thus, methylation interference assays were performed to define the protein-binding sites. As shown in Fig. 4, the only suggestive common sequence shared among those forming band V was the motif TGXCA, where X is any nucleotide. As the rest of Fig. 4 shows, no other apparent homology in the contacting region could be discerned. While examining these data, we noted two striking features that related the enhancer motifs to the recently described decamer motif conserved within Vβ promoters (3, 4).

The decamer is a transcriptionally important motif whose consensus sequence can be defined as 5′-AGTGA(C/T)(A/G)TCA-3′ (4). The decamer had TGACA as its core sequence, and its 10-bp sequence differed by one base from the β2 sequence (Fig. 5A). Moreover, the methylation interference patterns of βE2 and the decamer motif were similar (Fig. 5A). Since, in the immunoglobulin heavy-chain genes, the octamer motif (ATTTAGCT) is common to the $V_H$ promoter and the IgH enhancer regions (21, 35, 36), we sought to test whether the decamer motif could compete against any of the TCRβ enhancer motifs.

By GMSA, the β2-specific band was clearly competed with by the wild-type decamer motif but not by its nonfunctional mutant analog (Fig. 5B). Similarly, most bands defined by β7, except band IV, could be competed away by the decamer (Fig. 5C). Band VI, with β6 as a probe, was also competed away by the decamer (data not shown). Therefore,

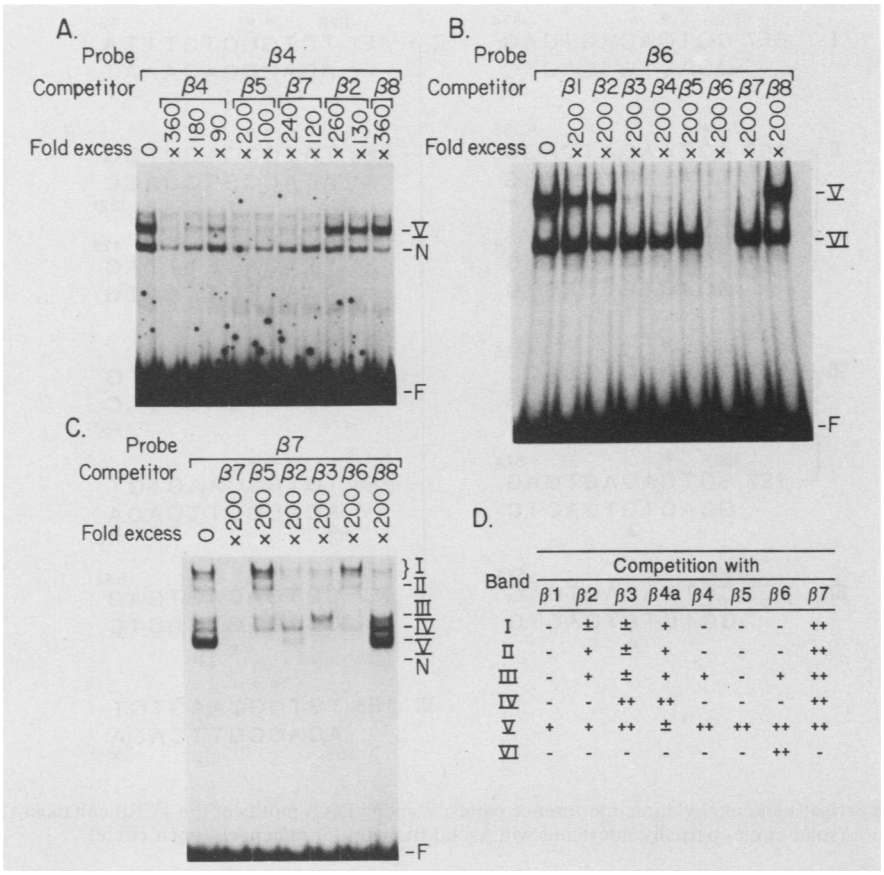


FIG. 3. GMSAs showing cross-competitions. GMSA with β4 (A), β6 (B), and β7 (C) probes were performed. End-labeled probes were incubated either with or without competitors in the presence of BW5147 (A and C) or EL-4 (B) extract. Designation of complexes is the same as in Fig. 2B. β8 is a nonspecific competitor encompassing positions 571 to 611. (D) Summary of cross-competition experiments with a 200-fold excess of competitor. Symbols: ++, good competitor; +, competitor; ±, weak competitor; −, ineffective competitor.

only band IV in the TCRβ enhancer appears to be completely independent of the decamer. As the competition pattern in Fig. 5D shows, the decamer motif seemed to bind the proteins more efficiently than the other probes used here. These data strongly suggest that there are nuclear proteins that can interact directly or indirectly with both the TCR promoter and enhancer elements. It is of particular interest that the decamer or decamerlike motifs have now been found in the enhancers (human [16] and murine [40] TCRα and CD3-δ chain [10]) and promoters (CD8 [26] and TdT [28]) of many T-cell- or lymphoid cell-specific genes.

**βE3 and βE6 are indistinguishable from the κE2 and μEBP-E motifs of immunoglobulin enhancers.** In contrast to the lack of homology described for the band V-forming sequences, βE3 was strikingly similar to κE2 in the Igκ enhancer (25). βE3 retains the perfect κE2 core sequence (GGCAGGTG). Because the TCRβ enhancer can occasionally function in B cells, we sought to clarify the relationship among the proteins that bind these homologous motifs. As shown in Fig. 6A, GMSA competition patterns between β3 and κE2 were interchangeable, and thus we conclude that βE3 is indistinguishable from κE2. When similar analysis was performed for βE6, it was found that the β6-specific band VI was identical to the band observed with μEBP-E as a probe (27) (Fig. 6B). In these assays, both κE2 and μEBP-E also competed well against band V. μEBP-E were originally reported by Peterson and Calame as being located upstream of the conventional μ enhancer motifs (27). Moreover, partially purified μEBP-E binding protein was known to be bound by murine TCRβ enhancer. Although the sequence of βE6 is similar to that of μE3 (21), the main μE3 band was not competed with by the β6 probe (data not shown). We thus conclude that βE6 is identical to or indistinguishable from μEBP-E but distinct from μE3.

## DISCUSSION

We have determined the minimal enhancer of TCRβ and have identified by DNase I footprinting and GMSA seven motifs within the minimal enhancer. The analysis of each motif allowed us to identify common elements between the TCRβ enhancer and Vβ promoter as well as the TCRβ and immunoglobulin enhancers. Most of the bands in GMSA were competed with by the Vβ decamer sequence (Fig. 5C), while βE3 and βE6 were indistinguishable from the κE2 and μEBP-E of immunoglobulin enhancers, respectively (Fig. 6A and B). The minimal enhancer was also induced by PHA and PdBu.

The developmentally regulated and tissue-specific expression of the antigen-specific receptors on T and B cells can be achieved at multiple levels. By controlling which receptor gene complex is allowed to undergo DNA rearrangement, a cell can target a particular receptor gene (TCR or immunoglobulin) for successful assembly. Although the rearrangement of $D_H$ to $J_H$ in T cells or Dβ to Jβ in B cells is occasionally observed (20), complete $VDJ_H$ rearrangements


FIG. 4. Sequence comparisons and methylation interference patterns among DNA motifs of the TCRβ enhancer. The status of G residues is shown as interfered with (solid circle) partially interfered with (solid triangle), or enhanced (open circle).

in T cells (20) or complete VDJβ rearrangements in B cells are not seen (8). Since a fully rearranged receptor gene complex is a prerequisite for functional receptor expression, tissue-specific control of receptor gene rearrangement represents a powerful regulatory mechanism.

One model of such regulated rearrangement proposes that it is the accessibility of the local chromatin structure to the putative recombinase that determines which gene complex undergoes DNA rearrangement (1, 2, 6). The second level at which antigen-specific receptor expression can be controlled is transcriptional regulation. For example, the rearranged immunoglobulin μ transgene was expressed in both B and T cells but not in nonlymphoid cells (13, 37), whereas a rearranged TCRβ transgene was preferentially expressed in T cells (19). Thus, the mere presence of a functionally rearranged TCRβ gene complex may not be sufficient for expression in B cells. These data strongly imply that the transcriptional regulation of these receptor genes is dependent not only on the *cis* elements in the promoter and enhancer regions of the TCR and immunoglobulin genes but also on the tissue-specific *trans*-activating nuclear factor(s) present in the lymphocytes.

**TCRβ enhancer is active in T cells and some B-lineage cells.** The functional analysis of the TCRβ enhancer here clearly shows its lymphoid cell-specific nature, reminiscent of the previously published transgenic mice data (19). However, the differential expression between T and B cells depended on the structure of the TCRβ enhancer being analyzed. Thus, although the enhancer activity in some B-lineage cells was clearly observed when a short enhancer (R692) was used, the activity of a larger enhancer (3B) was markedly diminished in the same cells. This is similar to the earlier observations made by Krimpenfort and colleagues for transgenic mice containing a TCR gene (19). We conclude then that the core enhancer is active in both T cells and some B-lineage cells while inactive in other B and nonlymphoid cells. Although the larger enhancer (3B) is slightly active in PD-31, a murine pre-B-cell line, it is completely inactive in DHL-9, a human B-cell tumor cell line (Table 1). On the other hand, the larger enhancer is fully active in T cells. These results suggest that the core enhancer contains a general lymphoid cell-specific element which does not discriminate between T and B cells, while one or more elements outside of the core enhancer are responsible for conferring T-cell specificity, possibly by repressing the enhancer function in B-lineage cells. One such negative element (silencer) allowing fine discrimination among lymphocyte subpopulations has been found recently adjacent to the TCRα enhancer, allowing differential gene expression between αβ and γδ T cells (41). In fact, two DNase I-hypersensitive sites downstream of Cβ2 have been reported (15). One site is identical to the TCRβ enhancer described here, while the other was mapped approximately 1 kb upstream of the enhancer. The 4-kb fragment (3B) used here contains this second hypersensitive site. While lacking enhancer function by itself, the second site becomes DNase hypersensitive in a tissue- and time-specific manner. One possible function of this site may be to act as a silencer element which regulates the fine specificity of the TCRβ enhancer.

**Motifs in the TCRβ enhancer are related to motifs in the Vβ promoter and the immunoglobulin enhancer.** To characterize the *cis*- and *trans*-acting elements that constitute the TCRβ core enhancer, deletional analysis, followed by DNase I footprinting and GMSA, was performed. The functional activity of the TCRβ enhancer was reduced stepwise, as previously observed for the immunoglobulin enhancer, con-

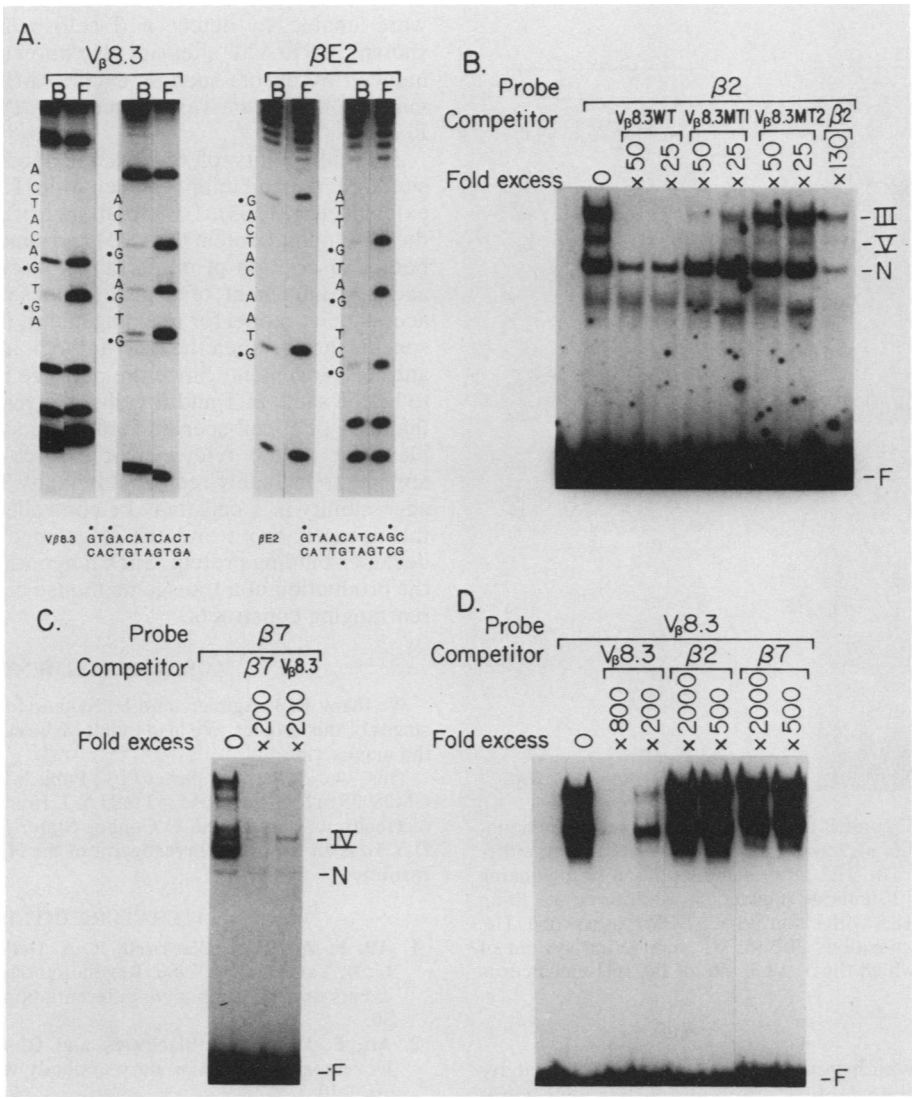


FIG. 5. Decamer of the Vβ8.3 promoter competes against βE1 through βE7 enhancer motifs. (A) Sequence and methylation interference pattern of βE2 and Vβ8.3 decamer. Preparative binding reactions were carried out with partially methylated end-labeled probes. The bound (B) and free (F) fragment bands were eluted from the acrylamide gel, treated with piperidine, and analyzed with a 15% sequencing gel. Affected G residues are indicated by solid circles. (B) GMSA with β2 as a probe. Wild-type Vβ8.3 promoter (30-mer) containing the decamer motif (Vβ8.3 WT) and mutants (67-mer) which are mutated either within the 10-bp of the decamer (Vβ8.3 MT2) or within the flanking sequence just 3′ of the decamer (Vβ8.3 MT1) were used. Both Vβ8.3 MT1 and Vβ8.3 MT2 are known to affect decamer binding (4). (C) GMSA with β7 as a probe. (D) GMSA with the Vβ8.3 decamer as a probe.

sistent with the presence of some redundant elements within the core enhancer (Fig. 1C). Seven regions could be identified that bound nucleus-binding factors. Surprisingly, initial analysis of these factors revealed that a given factor could bind to several of the seven motifs identified, providing a possible explanation for the observed functional redundancy.

Detailed comparison of the TCRβ core enhancer motifs described here revealed a surprising relationship between the TCRβ enhancer and the immunoglobulin enhancer and between the TCRβ enhancer and a conserved Vβ promoter element. Specifically, TCRβ enhancer elements βE3 and βE6 were indistinguishable from κE2 in the Igκ enhancer (21, 25) and μEBP-E in the IgH enhancer (27), respectively. Thus, the three lymphoid-specific enhancers have elements in common that are crucial for their function. For the minimal TCRβ enhancer, no lymphoid-specific binding protein has been identified by GMSA (data not shown).

Since the immunoglobulin $V_L$ and $V_H$ promoters and IgH enhancer share a conserved octamer motif, we sought to see whether any of the TCRβ core enhancer motifs was shared by the TCR promoter. Previously, we described a decamer motif shared among the Vβ promoters which is functionally indispensable (3, 4). Although the DNA sequence of the βE2 was highly similar to the decamer, it showed only a weak binding to the nucleus-binding factor that binds to the decamer motif in the Vβ promoter. However, the Vβ promoter decamer could effectively compete against most GMSA bands (I, II, III, V, and VI) in the minimal enhancer. These data strongly suggest that common nuclear factors bind to both the TCRβ promoter and enhancer. It is of note that both the murine and the human TCRα enhancers also contain a decamer motif (16, 40). Thus, the decamer motif is found in the TCR Vβ promoter and α and β enhancers. Since the TCRβ gene complex rearranges before the TCRα gene complex, it is possible that the location of the decamer

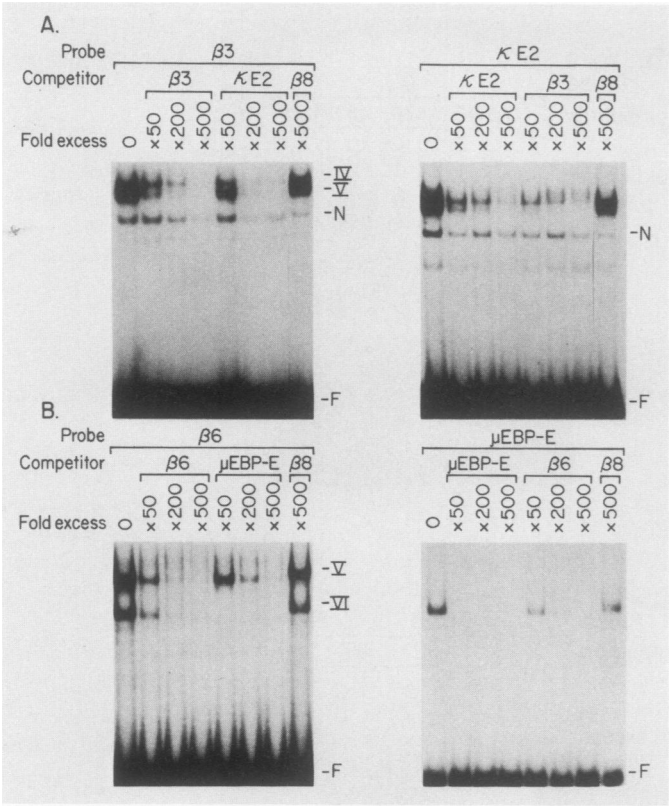


FIG. 6. βE3 and βE6 motifs are indistinguishable from immunoglobulin enhancer motifs κE2 and μEBP-E. (A) GMSA with either β3 or κE2 as a probe. The κE2 probe includes 27 bp of 5′-flanking sequence and 13 bp of 3′-flanking sequence in addition to the 10-bp core sequence. (B) GMSA with either β6 or μEBP-E as a probe. The μEBP-E probe spans positions 307 to 351 (numbering system of Ephrussi et al. [7] in which the 5′ *Xba*I site of the IgH enhancer is position 1).

motifs determines which gene complex rearranges first by binding the appropriate factors, allowing earlier accessibility.

In fact, the location of the octamer sequence among the immunoglobulin genes is consistent with this notion. The octamer is found in both the promoter and the enhancer regions of the earlier-rearranging heavy-chain gene system, while the light-chain enhancer lacks the octamer sequence. Furthermore, since the decamer or its analog is also found in many T-cell-specific genes, including murine CD3-δ enhancer (10), murine CD8 promoter (26), and human TdT promoter (28), we propose that the presence of the decamer-binding protein may be important in the coordinated expression of these genes in T cells. Furthermore, we had previously reported that the Vβ decamer sequence and the cyclic AMP response element-activating transcriptional factor (CRE/ATF) sequence are functionally interchangeable for expression in T cells (4), suggesting that identical or closely related factors mediate a function through the decamer or CRE/ATF sequence. These facts suggest that the decamer or CRE/ATF sequence may play an important role in coordinate gene expression in T-cell development. Although many CRE/ATF-binding factors have been cloned from nonlymphoid cells (11, 14, 17, 23), a T-cell-specific decamer- or CRE/ATF-binding factor has not yet been reported.

We have performed GMSA with a series of nuclear extracts that bind to the decamer and related sequences but were unable to detect a T-cell-specific band (data not shown). CRE/ATF sites are also known to be regulated and induced by agents such as cyclic AMP and E1A (18). The minimal enhancer was induced by PHA and PdBu (see Results).

The inducibility of the Igκ enhancer, especially the κB site, by PMA and lipopolysaccharide (LPS) has been studied extensively (31, 33). LPS treatment of pre-B cells activates the κB binding protein (NF-κB) and simultaneously activates both transcription of unrearranged κ constant-region genes and rearrangement of κ genes. These results support the accessibility model for rearrangement, in which T- or B-cell-specific factors open the relevant loci, allowing transcription and rearrangement. Since the putative recombinase is likely to be the same in T and B cells, it is reasonable to postulate that a T- or B-cell-specific factor(s) may induce the accessible state in the relevant loci. Since the decamer motif appears to be highly recurrent in many T-cell-specific genes, accessibility in T cells may be controlled by the presence of the decamer motif and the stage-specific induction of the decamer-binding protein. Such a hypothesis can be tested by the production of a transgenic mouse containing appropriate rearranging constructs.

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