Characterization of Human TCR Vβ Gene Promoter

ROLE OF THE DODECAMER MOTIF IN PROMOTER ACTIVITY*

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During T-lymphocyte development, the T-cell antigen receptor (TCR) gene expression is controlled by its promoter and enhancer elements and regulated in tissue- and development stage-specific manner. To uncover the promoter function and to define positive and negative regulatory elements in TCR gene promoters, the promoter activities from 13 human TCR Vβ genes were determined by the transient transfection system and luciferase reporter assay. Although most of the TCR Vβ gene promoters that we tested are inactive by themselves, some promoters were found to be constitutively strong. Among them, Vβ6.7 is the strongest. 5′-Deletion and fragmentation experiments have narrowed the full promoter activity of Vβ6.7 to a fragment of 147 base pairs immediately 5′ to the transcription initiation site. A decanucleotide motif with the consensus sequence AGTGATGTCACT has been found to be conserved in most TCR Vβ gene promoters. There are three such decamer motifs in the promoter region of Vβ6.7, and the contribution of each such motif to the promoter activity has been examined. Further site-directed mutagenesis analyses showed that: 1) when two Ts in the decamer were mutated, the promoter activity was totally abolished; 2) when two additional nucleotides 3′ to the end of decamer were mutated, the promoter activity was decreased to two-thirds of the full level; and 3) when the element with the sequence AGTGATGTCACT was inserted into other promoters, the original weak promoters become very strong. Taken together, our data suggest that the positive regulatory element in Vβ6.7 should be considered a dodecamer rather than a decamer and that it confers strong basal transcriptional activity on TCR Vβ genes.

T-lymphocyte development is similar to that of B-cell maturation in that the germ line variable (V), diversity (D), and joining (J) gene segments of the T-cell antigen receptor (TCR) are somatically recombined to form a V-D-J (for β-chain) or V-J (for α-chain) exon encoding the variable domain portion of the receptor. Studies in B-cells support the idea that to achieve the recombination, the rearranging Ig gene segments must be transcriptionally activated (1–4). If this prerequisite also applies to TCR genes, then the promoter elements that control transcriptional activity of TCR V genes will play a pivotal role in rearrangement. Specific transcriptional factors may bind to distinct DNA sequence motifs in the promoter region and control transcription. These factors can also affect the accessibility of germ line loci to the recombinational machinery, thus regulating gene expression in a tissue- or developmental stage-specific way.

A 10-base pair decamer sequence motif, AGTGAYRTCA, was found to be conserved in the promoter regions of most murine and human TCR Vβ genes (5–7). Elimination of this decamer motif in the murine Vβ3.3 promoter reduced transcriptional activity (8). Binding of thymic factors to this decamer motif was found to be developmentally regulated, and no decamer binding activity was detected in nuclear extracts prepared from thymuses of severe combined immunodeficiency mice, suggesting that the decamer motif plays an important role in the connection between murine TCR V gene transcription and rearrangement (9).

The most highly conserved portion in the decamer motif is an inverted repeat with the sequence TGA–TCA. This palindromic feature links the decamer to other regulatory elements, such as the c-AMP response element, TGACGTCA, and the AP-1 binding site, TGAAGCA (10, 11). We have observed some discrete differences in the location and the consensus sequence of the decamer motif between different human TCR Vβ subfamilies (7). Little is known regarding the role of the decamer motif in promoter function or whether differences in the decamer motif may affect promoter activity.

In this study, we have characterized 14 promoters of human TCR Vβ genes, with a specific emphasis on Vβ6.7. By using a transient transfection system, we have shown that human TCR Vβ promoters vary in terms of their strength. Whereas most promoters tested are weak, the promoters of the Vβ8 subfamily, particularly Vβ6.7, are constitutively strong. Sequence analyses have revealed three decamer-like motifs in the promoter region of Vβ6.7, and each of them contributes differently to promoter activity. The one located most 5′ to the transcription initiation site has essentially no effect on promoter activity. In contrast, the one located proximal to the initiation site forms the major contribution to promoter strength. A 5′ deletion study has shown that a fragment of 147 bp immediately 5′ upstream of the transcription initiation site can constitutively drive the reporter luciferase gene expression. Further shortening of this fragment results in a decrease in reporter gene expression. Therefore, this fragment represents the minimal promoter element of Vβ6.7. Inside this fragment there are a CACCC motif, a TATA box, and two decamer-like motifs. Further site-directed mutagenesis analyses have shown that a 12-nucleotide sequence, AGTGATGTCACT, is responsible for the high promoter activity. Therefore, the regulatory element in the promoter of Vβ6.7 should be considered a dodecamer rather than a decamer. The possible contributions to Vβ6.7

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The abbreviations used are: V, variable; D, diversity; J, joining; bp, base pair(s); TCR, T-cell antigen receptor; CTP, cytidine triphosphate; RLU, relative light unit.
promoter activity from additional transcription factor binding sites 3’ behind the dodecamer are also discussed.

MATERIALS AND METHODS

Cell Culture and Total Cellular RNA Isolation—Human peripheral blood T-cells were cultured in RPMI 1640 medium with 10% fetal calf serum. CDS+ T-cells were depleted following treatment with an anti-CD8 monoclonal antibody (OKT 8) and anti-mouse Ig-coated magnetic beads (Dynal, Great Neck, NY). CD4+CD8+ T-cells were activated with anti-Vβ6.7-specific monoclonal antibody OT145 (12). After a second refreezing, more than 80% of T-cells were Vβ6.7-positive. Cells were washed once with phosphate-buffered saline, and the pellet was spun down. Total cellular RNA was isolated using an acidified guanidinium/phenol/chloroform isolation kit (RNazol, TEL-TEST, Friendswood, TX) and used to identify the transcription initiation sites by RNase protection and primer extension methods.

RNase Protection and Primer Extension—To prepare an antisense RNA probe, a double-stranded DNA fragment spanning the first and the second exons plus the 5’-untranslated portion of Vβ6.7 was subcloned into a T/A vector that contains a T7 promoter. The template plasmid DNA was digested upstream at an unique XhoI site. The antisense RNA probe was synthesized. The primer was end-labeled with [γ-32P]ATP, and polynucleotide kinase (Promega, Madison, WI) in the presence of T7 RNA polymerase and [γ-32P]CTP. The labeled RNA probe was hybridized with total cellular RNA isolated from Vβ6.7+ T-cells. The protected products were purified once with phenol/chloroform extraction, denatured, and loaded on a 6% polyacrylamide, 7% urea sequencing gel. The dideoxyl-terminated sequencing reaction product from a plasmid DNA with a known sequence was loaded alongside as a size marker. After electrophoresis, the gel was dried and exposed to x-ray film at −70 °C for 12–48 h.

In primer extension experiments, an antisense oligonucleotide primer with the sequence 5’-TGGATCTGCCCCCAGAGA-3’ was synthesized. The primer was end-labeled with [γ-32P]ATP, and polynucleotide kinase following the protocol in TaqTrack sequencing system (Promega). The end-labeled primer was annealed to 5 µg of total cellular RNA isolated from Vβ6.7+ cells at 65 °C for 10 min and then extended at 56 °C for 1 h in the presence of reverse transcriptase, dNTPs, and RNase inhibitor (cDNA Cycle Kit, Invitrogen, San Diego, CA). The cellular RNA isolated from a mouse thymoma cell line BW5147, which contains no human TCR Vβ genes, was used in a parallel extension reaction as a negative control. The extension products were denatured and loaded on sequencing gel with a size marker as described in the RNase protection assay.

Preparation of Constructs and Site-directed Mutagenesis—The 5’-flanking sequences were PCR-amplified from either genomic DNA or λ phage DNA clones. The sequences of 5’ sense and 3’ antisense oligonucleotide primers used for 14 human TCR Vβ genes were listed in Table I. These PCR amplified fragments were 500 bp and were inserted 5’ to the reporter luciferase gene segment in pGL-2/Basic vector (Promega). The sequences and orientations of these fragments inside pGL-2 were confirmed by sequencing. The plasmid DNAs used for transfection were purified by cesium chloride banding. The site-directed mutagenesis was achieved using the PCR-overlapping method (13).

Transient Transfection—Human Jurkat T-cell line cells were grown to confluence in RPMI 1640 medium plus 10% fetal bovine serum and other supplements. Cells were transfected with plasmid DNA contain-

ing promoter-reporter constructs (described above) by a modified DEAE-dextran electroporation method. Briefly, cells were washed twice with serum-free RPMI 1640 medium and resuspended in the same medium at a concentration of 17 × 10^6/ml. 600 µl of cell suspension was placed in a 0.45-cm electroporation cuvette (Gene Pulser, Bio-Rad) followed by adding 180 µl of DEAE-dextran (100 µg/ml) and 10 µg of promoter-reporter plasmid DNA. To evaluate the transfection efficiency, 5 µg of plasmid DNA containing a β-galactosidase gene segment was co-transfected into the cells. The electroporation was carried out at a capacitance of 960 microfarads and 250 V. After electroporation, the cells remained at room temperature for 10 min and then were resuspended in 10 ml of complete RPMI 1640 medium, placed in wells (3 ml/bottle) of a 12-well plate, and incubated for 48–72 h at 37 °C in 5% CO2.

Luciferase Assay—5×10^5 luc stock buffer, which contains 125 µM glycyllglycin, 75 mM MgSO4, and 20 mM EGTA, was premade and used later in preparation of lysis solution, assay solution, and luciferin substrate solution. The transfected cells were washed twice with 1× phosphate-buffered saline, pelleted, and lysed with 500 µl of lysis solution (1× luc buffer, 1× Triton X-100, 1 mM of dithiothreitol added immediately prior to use). 100 µl of cell lysate was added to a test tube that contained 500 µl of assay solution (1× luc buffer, 1 mM KH2PO4, 1 mM dithiothreitol, and 2 mM of ATP). The tube was loaded into the measurement chamber, and 100 µl of luciferin substrate solution (1× luc buffer, 0.4 mM d-luciferin-sodium salt, and 2 mM dithiothreitol) was injected automatically. The relative light units (RLUs) were measured on a Monolight 2001 Luminometer (Analytical Luminescence Laboratory, San Diego, CA). The background RLUs from cells transfected with vector pGL-2 only were measured in each experiment. The luciferase assay data were calibrated with β-galactosidase activity and are presented as averages in triplicate measurements.

β-Galactosidase Assay—The β-galactosidase activity was measured by using a Galacto-Light Plus Chemiluminescent Reporter assay kit following the manufacturer’s instructions (Tropix, Bedford, MA).

RESULTS

Decamer Motif and TCR Vβ Promoter Activities—To determine the role of decamer sequence motifs in promoter activity, we have searched for this regulatory element in promoter regions among 54 functional TCR Vβ genes based on their genomic DNA sequences (Hood et al., GenBank accession no. L38092). The consensus sequence for the decamer is AGTGACATCA. However, we observed some discrete differences in different TCR V gene subfamilies, such as AGTGAACATCA in Vβ6, AGTGCACATCA in Vβ5, and TGANNNTCA in Vβ13 subfamilies (7). Therefore, we have screened two general patterns of the decamer motif, NNTGANNNTCA and TGANNNTCA, in the promoter regions ~500 bp 5’ to the translation initiation

| Vβ gene | 5’ Sense | 3’ Antisense |
|---------|---------|-------------|
| 3.1     | ttaagctggatgcctctcctccag | ttgctcagcggctgtggttagt |
| 5.1     | aacctcagagctagccgagttctta | ctgctctgtctggctggaza |
| 5.2     | ttaagctggagctgcctatcagtag | ttgctcagggacacgtctcatctg |
| 5.7     | ttgctcagacagcagctctcttg | atgtcagcggcagctcagcactct |
| 6.1     | aacctcaggagacacgtctaggt | ataaagttctctcagcagatgta |
| 6.3     | ggtcagcttggtcctggggcagtcc | ggaaagttctcgacagacgggctc |
| 6.7a    | ggtcagctgatctcagctatgcttaa | ggaaagttctcgacagacgggctc |
| 6.7b    | ggtcagcttggtcctggggcagtcc | ggaaagttctcgacagacgggctc |
| 8.1     | agtctcgagaaagacsgacggctaccc | aggtcagcaggcagtcgctc |
| 9.1     | aagtctcagagctagccgagttctta | caagctcagagctagccgagttctta |
| 12.2    | agtctcagatcagctctatcagagt | caagctcagagctagccgagttctta |
| 14.1    | agtctcagtgggagctagggcgtc | ttgctcagttgctgggggctc |
| 17.1    | ttaagcttcccagggagtgtacc | ttgctcagctttcagcgggcttc |
| 21.4    | ggtcagcttggtcctggggcagtcc | ggaaagttctcgacagacgggctc |

*The same 5’ sense and 3’ antisense primers were used to amplify 5’-flanking sequences for Vβ 6.1, 6.3, 6.7a, and 6.7b.*
from genomic DNA. The promoter fragments were ligated into the promoter segments for other Vβ genes. They are Vβ5 promoter activity using a transient transfection system. The promoters contain these general patterns (Table II). 22 Vβ promoters may be divided into three groups in terms of their constitutive activities. The weak promoter group includes Vβ3.1, 5.7, 9.1, 12.2, 14.1, and 21.4. The promoters with moderate constitutive activity are those from Vβ5.1, 5.2, 8.1, and 17.1. The strong promoters are those from Vβ6 subfamilies, Vβ6.1, 6.3, and 6.7. Of them, the promoter of Vβ6.7 is the strongest.

 Allelic Variations in Promoter Region of Vβ6.7—We previously identified two alleles of the human TCR Vβ6.7 gene, Vβ6.7a and Vβ6.7b (14). They differ at two amino acid positions within the coding region: Vβ6.7a encodes Ser58 and Gly72, whereas Vβ6.7b encodes Arg58 and Glu72. An allele-specific monoclonal antibody, OT145, can recognize the product of 6.7a but not 6.7b (15). Later, Vissera et al. (16) found that the peripheral expression of these two alleles in heterozygous individuals was skewed, indicating that an allelic polymorphism in the coding region can have a significant impact on gene expression in the peripheral repertoire. However, other polymorphisms in the TCR β locus, such as those in the promoter region, may also affect gene expression. To address this possibility, we analyzed the 5′-flanking sequences of Vβ6.7 alleles a and b. First, a 463-bp sequence upstream of Vβ6.7a in phase λ DNA clone 5–2 (7) was obtained. The promoter segment of Vβ6.7 allele b was then PCR amplified from the genomic DNA of a b/b homozygous individual. Sequence analyses revealed three point mutations between alleles a and b within the 463-bp 5′-flanking region that were confirmed in a total of 24 plasmid clones derived from three Vβ6.7 a/a and three Vβ6.7 b/b homozygous individuals (Fig. 2). These mutations represent allelic variations in the promoter region of Vβ6.7. We expected that no significant difference in promoter activities would be detected between Vβ6.7 alleles a and b because no destruction of any major regulatory elements was caused by these mutations. Our expectation was confirmed by luciferase reporter assay (data not shown).

 Transcription Initiation Site of Vβ6.7—To determine the transcription initiation site of Vβ6.7, primer extension analysis was performed. An antisense oligonucleotide primer, 5′-TGT-GATCTGCCACAGGAGA-3′, was specifically designed. In this primer, 12 nucleotides match the 3′-end sequence of the first exon, and the remaining 6 nucleotides match the 5′-beginning codon ATG, using the FINDPATTERNS computer program. Among 54 functional human TCR Vβ gene families, 42 Vβ promoters contain these general patterns (Table II). 22 Vβs each contain one NNTGANNNTCA element. 8 Vβs each contain two such elements, and the remaining 5 Vβs each contain three such elements. Of these 5 Vβs, 4 come from the Vβ6 subfamily. They are Vβ6.3, 6.7, 6.11, and 6.14. All Vβ13 subfamilies, except Vβ13.1, contain the TGANNNTCA pattern.

 We randomly selected 13 TCR Vβ genes and compared their promoter activity using a transient transfection system. The 5′-flanking sequences of human TCR Vβ6.3 and 6.7 were PCR amplified from λ phage genomic DNA clones 4-1 and 5-2 (7). The promoter segments for other Vβ genes were PCR amplified from genomic DNA. The promoter fragments were ligated into a luciferase reporter vector pGL-2/Basic. These constructs were then used to transfect a human Jurkat T-cell line. The promoter activities were measured as luciferase activities in transfected cells. To eliminate the influence of transfection efficiency, all data from luciferase assays were calibrated with β-galactosidase activities and are presented as averages of triplicate measurements. Fig. 1 shows the reporter luciferase activities, expressed as RLUs, for 13 human TCRVβ promoters. Human TCR Vβ promoters may be divided into three groups in terms of their constitutive activities. The weak promoter group includes Vβ3.1, 5.7, 9.1, 12.2, 14.1, and 21.4. The promoters with moderate constitutive activity are those from Vβ5.1, 5.2, 8.1, and 17.1. The strong promoters are those from Vβ6 subfamilies, Vβ6.1, 6.3, and 6.7. Of them, the promoter of Vβ6.7 is the strongest.

### Table II

| Vβs  | NNTGANNNTCA | TGANNNTCA |
|------|-------------|-----------|
| 1.1  | +           | +         |
| 2.1  | +           | +         |
| 3.1  | +           | +         |
| 4.1  | +           | +         |
| 5.1  | +           | +         |
| 5.2  | ++          |           |
| 5.3  | +           | +         |
| 5.6  | +           | +         |
| 5.7  | +           | +         |
| 5.8  | +           | +         |
| 6.1  | ++          |           |
| 6.2  | ++          |           |
| 6.3  | ++          |           |
| 6.5  | +           | +         |
| 6.7  | ++          |           |
| 6.9  | ++          |           |
| 6.11 | +           | +         |
| 6.14 | ++          |           |
| 7.1  | +           | +         |
| 7.2  | +           | +         |
| 8.1  | +           | +         |
| 8.2  | +           | +         |
| 8.3  | +           | +         |
| 8.5  | +           | +         |
| 9.1  | +           | +         |
| 9.2  | +           | +         |
| 10.1 | —           | —         |
| 11.1 | —           | —         |
| 12.1 | —           | —         |
| 12.2 | +           | +         |
| 12.3 | +           | +         |
| 12.4 | +           | +         |
| 13.1 | —           | —         |
| 13.2 | —           | —         |
| 13.3 | —           | —         |
| 13.4 | —           | —         |
| 13.5 | +           | +         |
| 13.6 | +           | +         |
| 13.7 | +           | +         |
| 13.8 | +           | +         |
| 13.9 | +           | +         |
| 14.1 | —           | —         |
| 15.1 | +           | +         |
| 15.1 | +           | +         |
| 15.1 | +           | +         |
| 17.1 | +           | +         |
| 18.1 | +           | +         |
| 19.1 | —           | —         |
| 20.1 | —           | —         |
| 21.3 | +           | +         |
| 21.4 | +           | +         |
| 22.1 | —           | —         |
| 23.1 | +           | +         |
| 24.1 | —           | —         |
| 25.1 | —           | —         |
| 26.1 | +           | +         |
| 29.1 | —           | —         |
| 31.1 | —           | —         |

*The nomenclature of human TCR Vβ genes used in this study is consistent with that of Hood et al. (GenBank™ accession number L36092). The number of plus signs indicates the number of decamer-like motifs identified in the promoter region.*

**TABLE II**

*Decamer motifs in human TCR Vβ gene promoters*

![Figure 1](image-url)
of the second exon of Vβ6.7 (see the coding sequence of Vβ6.7 in Fig. 2). Only cDNA, but not the genomic DNA, can anneal to this primer. In Fig. 3, lanes C, T, A, and G were sequencing products of a plasmid DNA with known sequence, shown here as the size marker. Lane 1 was the primer extension product incubated with RNA isolated from Vβ6.7 T-cells. Lane 2 was the primer extension product incubated with RNA isolated from mouse thymoma cell line BW5147, included here as a negative control. Two intense bands were observed in lane 1. The higher one is 63 bp, and the lower one 60 bp. We assumed that the 63-bp band represents the full length extension product. This product aligns with an A 26 bp 5’ to the ATG codon. Given that adenine is a favored base for eukaryotic transcription initiation (17), we defined it as the initiation site for human Vβ6.7.

To determine whether the constitutive activity of the promoter element in Vβ6.7 was specific to the T-cell lineage, the RLUs for fragment V were low, and the RLUs for fragment VI were high. However, fragment VI was unable to reach the same level of activity as that induced by fragment IV. Therefore, the 147-bp of fragment IV represents the basic promoter for Vβ6.7.

No T-cell Lineage Specificity for Promoter Element in Vβ6.7—To determine whether the constitutive activity of the promoter element in Vβ6.7 was specific to the T-cell lineage, several cell lines, including human T-cells (Jurkat), Burkitt’s lymphoma cells (Ramos), fibroblast cells (cos 7), mouse thymoma cells (BW5147), and human embryonic kidney cells (293) were used. The luciferase reporter constructs containing TCR Vβ6.7 promoter segment and a nonspecific Rous sarcoma virus promoter were used to transfect these different cell lines. When compared with the nonspecific Rous sarcoma virus promoter, the RLUs were relatively high for Vβ6.7 promoter in lymphocyte cell lines (Jurkat, Ramos, and BW5147) but low in fibro-
Dodecamer, Rather Than Decamer, Is Critical to V\textsubscript{b}6.7 Promoter Activity—To further evaluate the role of decamer D1 and its surrounding nucleotides in promoter activity, site-directed mutagenesis was performed. When D1 (AGTGATGTCA) was mutated to D1 m1 (AGAGATGCCA), in which the two Ts that form the core element of TGA–TCA were substituted, one by an A and the other by a C, the luciferase activity was totally abolished (Fig. 6), suggesting that the palindromic TGA–TCA is critical. We further mutated two nucleotides immediately 3' of D1, which changed AGTGATGTCACT to AGTGATGTCAAA (Fig. 6, D1 m2). This mutation destroys the reverse complementation between the first two nucleotides, AG, to the last two nucleotides, CT, in the original sequence. As shown in Fig. 6, the luciferase activity was decreased to two-thirds that of the original, nonmutated sequence, indicating that these two nucleotides are also important to promoter activity. Therefore, the regulatory motif in V\textsubscript{b}6.7 should be considered a dodecamer (12-bp) rather than a decamer.

There is a dodecamer-like motif, AGTGACATCACA, with relatively the same location as the dodecamer in V\textsubscript{b}6.7, in the promoter of V\textsubscript{b}5.2. Although the luciferase assay showed that the original weak V\textsubscript{b}5.2 promoter became strong when this imperfect dodecamer was corrected (Fig. 7, pVb5.2/-5*), the transcription activity was still far below that of V\textsubscript{b}6.7. Only when a fragment from −85 to −1 of the V\textsubscript{b}6.7 promoter, which contains the dodecamer, was replaced at its 3'-end (Fig. 7, pVb5.2/−85) did the V\textsubscript{b}5.2 promoter become very strong and comparable in activity to V\textsubscript{b}6.7. This observation led us to speculate that additional regulatory elements may exist 3' behind the dodecamer of V\textsubscript{b}6.7. To test this hypothesis, constructs containing the fragment from −85 to −1 of V\textsubscript{b}6.7 and corresponding fragments from V\textsubscript{b}6.1 and V\textsubscript{b}6.3, were prepared and used in transient transfection. As shown in Fig. 8, although V\textsubscript{b}6.1 and 6.3 also contain the same dodecamer motif as V\textsubscript{b}6.7, their activities were below that of V\textsubscript{b}6.7. Sequence alignment showed that the 3'-ends of the promoters among V\textsubscript{b}6.7, 6.1, and 6.3 are highly homologous to each other and that they all have the same dodecamer motif. The most distinct differences occur in the area from −67 to −43. We have screened for transcription factor sites and found at least three additional sites that are present in V\textsubscript{b}6.7 but absent in V\textsubscript{b}6.1 and 6.3. They are Sp1-U2snR.2 at −49, SIF\_core\_RS at −53, and BS15 at −66 (Fig. 9). Future site-directed mutagenesis
These sites are listed in the bottom panel.

The process of T-lymphocyte development is similar to that of B-cell maturation. Transcriptional activation of rearranging Ag receptor gene segments has been hypothesized to regulate their accessibility to the recombinational machinery. Several pieces of evidence support this prerequisite in both B-cells and T-cells (1–4). Therefore, an understanding of transcriptional regulation may be important in elucidating the mechanisms controlling the lineage-specific patterns of rearrangement of the TCR genes during thymocyte differentiation.

Human TCR Vβ gene promoters themselves, which control the transcription, were reported to be essentially inactive. It was the enhancer, located 3.5–5.0 kilobase pairs 3′ to the Cβ2 gene segment, that conferred the transcriptional activity and T-cell lineage specificity to Vβ promoters (18–20). However, in this study, we found that Vβ promoters varied in terms of their activities. Although most of the Vβ promoters that we tested were very weak, some were constitutively strong, such as those from Vβ subfamilies. The promoter of Vβ6.7 was found to be the strongest when compared with others. The 5′-flanking sequence analyses revealed that there are three decamer-like sequence motifs in the promoter region of Vβ6.7. There seems to be a correlation between the promoter activity and decamer-like motifs. For example, the strong promoters, such as Vβ6.3 and 6.7, all contain three decamer-like sequences. The most highly conserved portion in the decamer is an inverted repeat with the core sequence TGA–TCA. We identified the decamer as AGTGATGTCA in Vβ6.7, AGTGACATCA in Vβ5.2, and TGANNNNTCA in Vβ8.1 subfamilies (7). The general idea is that the core regulatory element is composed of TGA–TCA. Those nucleotides in front of TGA, after TCA, or between TGA and TCA may be not so important. However, our results show that it is not so simple. Certainly, TGA–TCA is critical. When this element was destroyed by site-directed mutagenesis, the promoter activity was totally abolished (see Fig. 6, Construct 3). However, TGA–TCA alone is not sufficient to confer promoter activity. For instance, the decamer-like motif D3 in the 5′-distal segment of the Vβ6.7 promoter was found to have essentially no effect on promoter activity (Fig. 4, II). Motif D2 in Vβ6.7 also showed a very low contribution to the promoter activity (Fig. 4, V). The surrounding nucleotides 5′ and 3′ to TGA–TCA, AG and CT, were also important. When the CT at the 3′-end was mutated to AA, the promoter activity dropped to two-thirds of the original activity (see Fig. 6, Construct 4). These data support our hypothesis that the most critical regulatory element is a 12-nucleotide motif with a core sequence AGTGACATCA–TCTGACGTCA. Therefore, it is a decamer, rather than a decamer. Further evidence to support this idea comes from the study of promoter activities for Vβ5.1 and 5.2. Although these promoters also contain a decamer-like motif at the same location as the decamer in the Vβ6.7 promoter, their activities were found to be relatively low (Fig. 1). Not surprisingly, the decamer-like motif in the promoters of Vβ5.1 and 5.2 has the sequence AGTGACATCA–TCTGACGTCA, which is not a perfect decamer as in the Vβ6 genes. When this imperfect decamer in Vβ5.2 was corrected by site-directed mutagenesis, the promoter activity was found to be increased. However, only when its 3′-end was replaced by a fragment from −85 to −1 of Vβ6.7 did the Vβ5.2 promoter become very strong, with activity comparable to Vβ6.7 (see Fig. 7).

The palindromic features lead us to propose that the decamer may form a stem and loop structure by reverse complementation. Such a stem-loop structure will exert the regulatory function by binding with homo- or heterodimers of transcription factors, such as members of the cyclic AMP response element binding protein or activating transcription factor families (21–23). To test this hypothesis, we simply compared the light outputs for constructs in which the 147 bp of fragment IV were inserted in sense or antisense orientations.
(Fig. 6, Constructs 1 and 2). No significant difference in luciferase activity was observed between the two constructs, suggesting that this stem-loop model may stand. Together, these results indicate that a perfect dodecamer motif, which can form a stem-loop structure, plays an important role in TCR Vb promoter activity.

In addition to the dodecamer motif, there are other elements, such as the TATA box (at −123) and CACCC box (at −140), that also contribute to the promoter activity of Vb.7. In murine TCR Vb genes, the decamer motifs were identified in the promoter region 10–40 bp upstream of the TATA box (6). In human TCR Vb genes, the locations of the decamer motif are different. The decamer in the Vb subfamily, similar to murine TCR Vbs, is located −20 bp upstream of the TATA box. Interestingly, the dodecamer in the promoters of Vb 6.1, 6.3 and 6.7 were located 36 bp downstream of the TATA box. It was unclear whether these different locations might affect the promoter activity. The CACCC box, identified in human Vb8.1 and mouse TCR α gene silencer (24), was found to be another transcription factor binding site with T-cell lineage specificity (25, 26). We found the CACCC box in the 150-bp basic promoter fragment from Vb 6.7. The fragment that contains the dodecamer only, without TATA and CACCC boxes, showed partial promoter activity (Fig. 4, VI), indicating that TATA and CACCC boxes may play a synergistic role with the dodecamer and contribute to the high constitutive promoter activity of Vb genes. Other motifs identified 3′ behind the dodecamer of Vb 6.7, such as BS15, SIF_core_RS, and Sp1-U2snR:2, are also important but have not been characterized. Further analyses will help us to evaluate the contributions of these binding sites.

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