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A T-cell-specific transcriptional enhancer element 3′ of C_α in the human T-cell receptor α locus

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ABSTRACT **A transcriptional enhancer element has been identified 4.5 kilobases 3′ of C_α (constant region α chain) in the human T-cell receptor (TCR) α-chain locus. This enhancer is active on both a TCR V_α (variable region α chain) promoter and the minimal simian virus 40 promotor in TCR α/β Jurkat and EL4 cells but is inactive on a V_α promoter in human TCR γ/δ PEER and Molt-13 cells, clone 13 B cells, and HeLa fibroblasts. The enhancer has been localized to a 116-base-pair *Bst*XI/*Dra* I restriction enzyme fragment, which lacks immunoglobulin octamer and κB enhancer motifs but does contain a consensus cAMP-response element (CRE). DNase I footprint analyses demonstrated that the minimal enhancer contains two binding sites for Jurkat nuclear proteins. One of these sites corresponds to the CRE, while the other does not correspond to a known transcriptional enhancer motif. These data support a model in which TCR α gene transcription is regulated by a unique set of cis-acting sequences and trans-acting factors, which are differentially active in cells of the TCR α/β lineage. In addition, the TCR α enhancer may play a role in activating oncogene expression in T-lymphoblastoid tumors that have previously been shown to display chromosomal translocations into the human TCR α locus.**

Human T lymphocytes can be divided into two distinct subsets based on their cell-surface expression of antigen receptor molecules. The majority of peripheral blood T cells, including most cells of both the helper and cytotoxic phenotypes, express a CD3-associated α/β T-cell receptor (TCR) (reviewed in ref. 1). A second smaller subset of T cells of unknown function express a CD3-associated γ/δ TCR (2, 3). Studies of murine thymic development have revealed that TCR γ/δ cells appear in the thymus several days before TCR α/β cells (4, 5), but it remains unclear whether these two cell types represent distinct lineages or, alternatively, whether TCR α/β cells are derived from cells that have failed to productively rearrange their TCR γ and δ genes.

While a great deal is known about the structure of the TCR α, β, γ, and δ genes (reviewed in ref. 6), relatively little is known about the molecular mechanisms that control the rearrangement and expression of these genes during T-cell ontogeny. Previous studies have identified a transcriptional enhancer element located 5–7.5 kilobases (kb) 3′ of the constant region $C_\beta 2$ gene segment in the murine TCR β locus (7, 8). In addition, one previous report has described a transcriptional enhancer element located directly 5′ of the first exon of the human TCR C_α gene (9). Despite this previous study, there were several reasons to suspect the presence of additional transcriptional regulatory elements in the TCR α locus. Specifically, T cells from transgenic mice made with TCR α constructs containing the previously reported TCR α transcriptional enhancer element but lacking sequences 3′ of the TCR C_α gene expressed relatively low levels of TCR α mRNA (10). Moreover, expression of the transgene in these mice was also observed in B cells, lung, heart, kidney, brain, and intestine (10). In contrast, T cells from mice made with TCR α transgenes containing 14 kb of 3′ flanking DNA expressed high levels of TCR α transcripts, and TCR α gene expression in these mice was relatively T-cell specific (11).

In this report, we describe the identification and characterization of a potent transcriptional enhancer element located 4.5 kb 3′ of C_α in the TCR α locus. This enhancer is necessary for transcription from a TCR V_α promoter and is also active on several heterologous promoters. Moreover, the enhancer is active in TCR α/β Jurkat and EL4 T cells but not in TCR γ/δ PEER and Molt-13 T cells, clone 13 B cells, or HeLa fibroblasts. DNA sequence and DNaseI footprint analyses have suggested that the enhancer is regulated by at least two sets of cis-acting sequences and trans-acting factors, one of which contains a consensus cAMP-response element (CRE) and both of which are distinct from the previously described enhancer motifs which have been shown to regulate the expression of the immunoglobulin heavy- and light-chain genes.

MATERIALS AND METHODS

Isolation and Characterization of Human TCR α Genomic Clones. Genomic clones containing the C_α gene and a V_α promoter were isolated from a human genomic library (12) by hybridization to a C_α probe from the previously described L17 TCR α cDNA clone (13) and to a V_α probe prepared from the previously described human pGA5 TCR α cDNA (14).

Plasmids. The pSV0CAT (15), pSV2CAT (15), and pSPCAT (16) plasmids have been described. The pVαCAT plasmid was constructed by cloning the pGA5 human TCR V_α promoter (as a 1.4-kb *Bam*HI/*Xho* II fragment with a 3′ end located at position +72 relative to the cap site) in a 5′ to 3′ orientation into the *Hin*dIII site immediately 5′ of the chloramphenicol acetyltransferase (CAT) gene in the promoterless pSV0CAT plasmid. A 1.4-kb *Kpn* I/*Bam*HI fragment containing the TCR α enhancer (see Fig. 1) was subcloned in both orientations into the *Bam*HI and *Sma* I sites immediately 3′ and 5′ of the CAT genes in pVαCAT and pSPCAT, respectively, to produce the pVα1.4CAT, pVαR1.4CAT, pSP1.4CAT, and pSPR1.4CAT plasmids (see Fig. 2 *Left*).

Transfections. Human Jurkat T cells, clone 13 Epstein–Barr virus-transformed B cells, and HeLa fibroblasts were transfected with 10 μg of DNA per 10^7 cells by a modification of the DEAE-dextran approach as described (12). Transfections of PEER cells were performed by electroporation as described by Chu *et al.* (17) using a Bio-Rad gene pulser set at 270 V, 960 μF.

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Abbreviations: TCR, T-cell receptor; C, constant; V, variable; CRE, cAMP-response element; CAT, chloramphenicol acetyltransferase; SV40, simian virus 40.

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CAT and Growth Hormone Assays. CAT activity was determined from cell extracts normalized for protein content by standard techniques and ascending thin-layer chromatography followed by liquid scintillation counting as described (12). Growth hormone levels were determined from aliquots of the same extracts used in the CAT assays with a commercially available kit (Nichols Institute Diagnostics, San Juan Capistrano, CA).

DNA Sequencing. DNA sequencing was performed directly from double-stranded plasmid DNA by the dideoxynucleotide chain-termination method as described (18).

DNase I Footprint Analyses. DNase I footprint analyses were performed as described (19) using Jurkat nuclear extracts prepared according to the protocol of Dignam *et al.* (20).

RESULTS

Identification of a Transcriptional Enhancer Element 3′ of C_α in the Human TCR α Locus. In an attempt to detect additional transcriptional regulatory elements in the human TCR α locus, we assayed a set of overlapping restriction endonuclease fragments spanning ≈10 kb 3′ of the human TCR C_α gene for transcriptional enhancer activity. These fragments were cloned into the *Bam*HI site 3′ of the bacterial CAT gene in the pSV2CAT plasmid and transfected into TCR α/β human Jurkat T cells. The pSV2CAT vector was chosen as the initial reporter plasmid because, although it contains the simian virus 40 (SV40) enhancer, we have previously shown that the SV40 promoter/enhancer is essentially inactive in Jurkat cells but can be activated by a heterologous transcriptional enhancer element (19). As shown in Fig. 1, a 6.0-kb *Bam*HI fragment containing 5.6 kb of DNA 3′ of the C_α gene segment was able to increase transcription from the SV40 promoter/enhancer 150-fold in Jurkat cells. Using restriction enzyme subfragments, we localized the enhancer activity to a 700-base-pair (bp) *Bst*XI fragment located 4.5 kb 3′ of the C_α gene segment (Fig. 1).

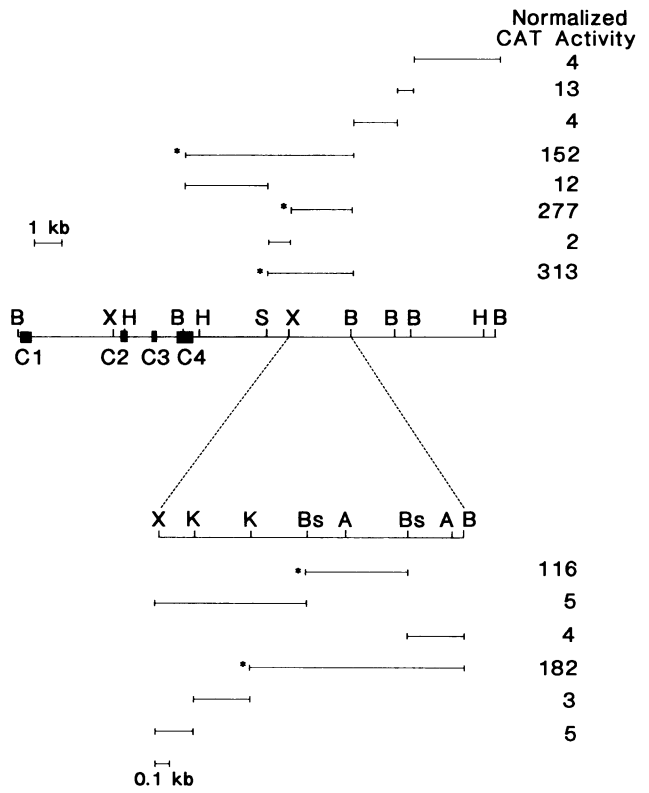


FIG. 1. Identification and mapping of a transcriptional enhancer element 3′ of the human TCR α locus. A partial restriction endonuclease map of a human genomic TCR α clone showing *Bam*HI (B), *Xba* I (X), *Hin*dIII (H), *Kpn* I (K), *Apa* I (A), *Sma* I (S), and *Bst*XI (Bs) restriction endonuclease sites is shown in the middle of the figure. The four exons of the TCR C_α gene are shown as solid blocks (C1–C4). Overlapping restriction enzyme fragments from this clone, as denoted by the bars, were subcloned into the *Bam*HI site 3′ of the CAT gene in the pSV2CAT vector (20) and assayed for transcriptional enhancer activity after transfection into human Jurkat T cells. The CAT activity produced by each plasmid after normalization to the CAT activity produced by transfection with the control pSV2CAT plasmid alone (which produced 0.08–0.16% acetylation) is shown on the right. Fragments that displayed significant transcriptional enhancer activity (>100-fold higher than control levels) are marked with an asterisk.

Promoter and Cellular Specificity of the Human TCR α Transcriptional Enhancer. To study the promoter specificity of this enhancer element, a 1.4-kb *Kpn* I/*Bam*HI fragment, which contained the enhancer (see Fig. 1), was cloned in both orientations into the *Sma* I site 5′ of the minimal SV40 promoter in the pSPCAT plasmid (11) and into the *Bam*HI site 3′ of the CAT gene in the pVαCAT plasmid in which CAT transcription is under the control of a human TCR V_α promoter (Fig. 2). The presence of the TCR α enhancer 5′ of the SV40 promoter in pSPCAT increased CAT activity 21- to 56-fold after transfection into Jurkat cells (Fig. 2). Interestingly, the TCR V_α promoter alone was essentially inactive in Jurkat cells. However, the addition of the TCR α enhancer fragment increased V_α promoter-dependent transcription 28- to 33-fold after transfection into Jurkat cells. In a separate set of experiments, the enhancer was also shown to be active on the herpes simplex virus thymidine kinase promoter in Jurkat cells (data not shown). However, in a negative control experiment, the enhancer was unable to increase transcription from a construct containing the TCR V_α promoter in a 3′ to 5′ orientation with respect to the CAT gene (data not shown). Taken together, these experiments demonstrated that the 1.4-kb *Kpn* I/*Bam*HI fragment contains a classical transcriptional enhancer, which can act in a position- and orientation-independent fashion on both a TCR V_α promoter and several heterologous promoters in TCR α/β Jurkat cells.

To define the cellular specificity of the TCR α enhancer element, the pVαl.4CAT plasmid containing the TCR α enhancer 3′ of the TCR V_α promoter and CAT gene was transfected into human clone 13 B-lymphoblastoid cells and human HeLa fibroblasts, and CAT activities were compared to those produced by the pVαCAT control plasmid (Fig. 3). In each case, the TCR α enhancer increased transcription <5-fold in these non-T-cell lines, indicating that the enhancer is relatively T-cell specific. In contrast, the enhancer was shown to be active on both the V_α and minimal SV40 promoters in murine EL4 TCR α/β cells (data not shown). To determine whether the enhancer is active in TCR γ/δ cells, the same plasmids were transfected into TCR γ/δ PEER and Molt-13 cells. In PEER cells, the TCR α enhancer increased transcription from the V_α promoter <2-fold as compared to the pVαCAT control plasmid (Fig. 3). Similar results were obtained in Molt-13 TCR γ/δ cells (data not shown). In parallel control transfections of PEER cells, the 4F2HC enhancer, which we have previously shown displays potent transcriptional enhancer activity in a variety of lymphoid and nonlymphoid cell types (19), was able to increase CAT activity from the TCR V_α promoter 100-fold (data not shown). These control experiments indicated that the transfections of PEER cells were efficient and that the V_α promoter can be activated by an appropriate enhancer in TCR γ/δ cells.

DNA Sequence Analysis of the TCR α Transcriptional Enhancer. To further characterize the TCR α enhancer element, the 700-bp *Bst*XI fragment was subjected to DNA sequence analysis (Fig. 4). Searches of this sequence with consensus sequences for the previously described octamer (24) and κB (25) immunoglobulin enhancer motifs failed to detect areas of significant sequence similarity. In contrast,

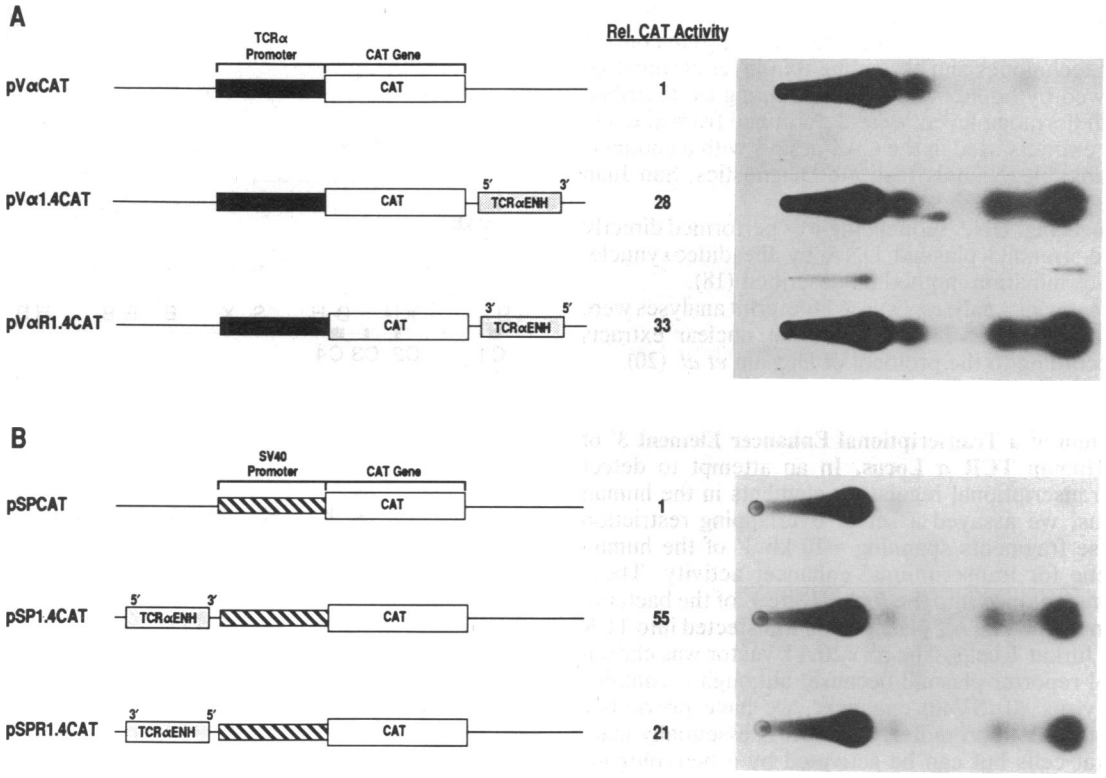
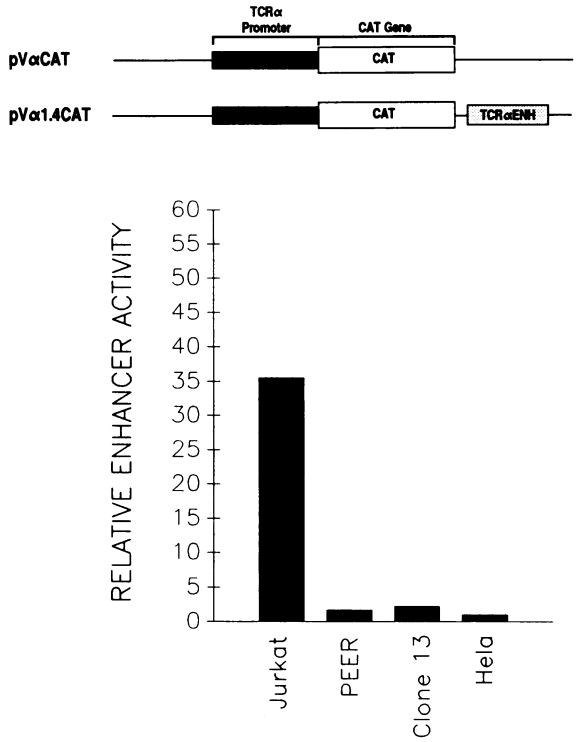


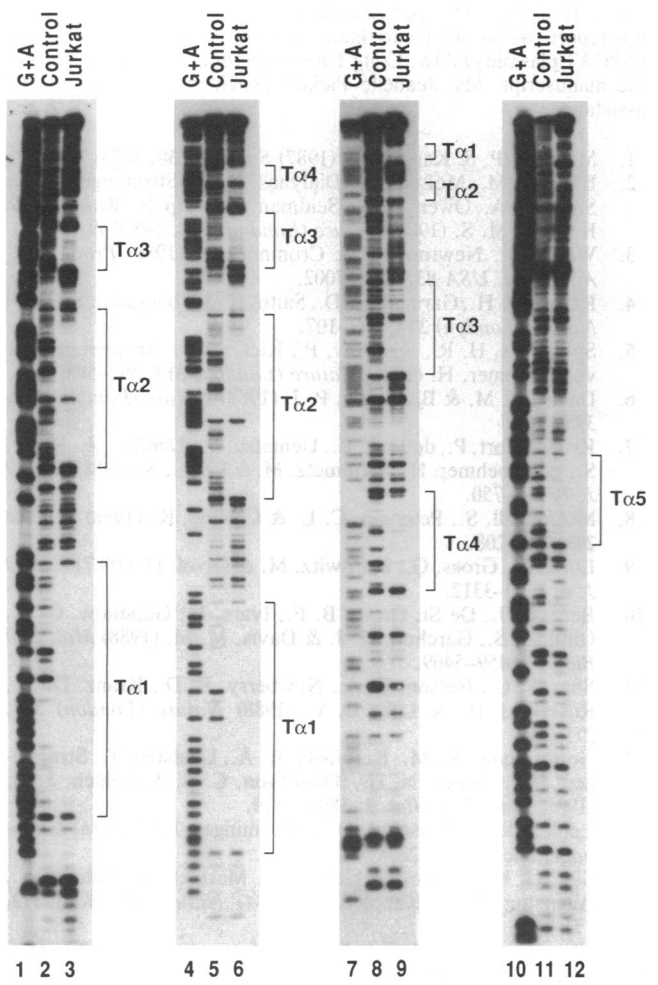
FIG. 2. Activity of the TCR α transcriptional enhancer on the minimal SV40 promoter and a TCR V_α promoter in Jurkat cells. (*Left*) The control pVαCAT and pSPCAT plasmids as well as the TCR α enhancer-containing pVα1.4CAT, pVαR1.4CAT, pSP1.4CAT, and pSPR1.4CAT plasmids are shown schematically. Ten micrograms of each of these plasmids along with 2 μg of the pRSVGH reference plasmid, which contains the human growth hormone gene under the control of the Rous sarcoma virus promoter/enhancer, were transfected into human Jurkat cells and CAT activities and growth hormone levels were determined. Relative CAT activities corrected for transfection efficiencies and normalized to those produced by the pVαCAT (which produced 0.07% acetylation) and pSPCAT (which produced 0.53% acetylation) control plasmids are shown on the left of the autoradiograms. Results were confirmed by at least three separate transfection experiments.

this fragment did contain a single sequence (GGCACGTG-GCC), which was identical at 9 of 10 bp to the previously described κE2 immunoglobulin enhancer element (26). In addition, it contained a consensus binding site (CCCCAGGC) for the AP-2 transcription factor (27) as well as a consensus CRE (28). The finding of a consensus AP-2 binding site in the TCR α enhancer was of interest because previous studies have shown that this site can confer phorbol ester inducibility (29) and because TCR α transcription has been shown to be significantly increased by phorbol ester treatment (30).



DNase I Footprint Analysis of the TCR α Transcriptional Enhancer. A variety of cellular and viral transcriptional enhancer elements have been shown to function by binding nuclear transcriptional regulatory proteins (reviewed in ref. 31). To determine whether the human TCR α enhancer contains nuclear protein binding sites, we performed a DNase I footprint analysis of the 700-bp *Bst*XI enhancer fragment with Jurkat nuclear extracts (Fig. 4). Five nuclear protein binding sites, Tα1–Tα5, were identified in these experiments. Each of these sites was protected on both strands. One of the sites (Tα1) corresponds to the CRE, while the Tα3 site corresponds to the κE2-like/AP-2 binding sequences. The other sites do not correspond to previously identified enhancer

FIG. 3. Activity of the TCR α enhancer in different human cell lines. Ten micrograms of the pVαCAT and pVα1.4CAT plasmids (Upper) along with 2 μg of the pRSVGH plasmid were transfected into Jurkat α/β T cells, PEER γ/δ T cells, clone 13 Epstein–Barr virus-transformed B cells, and HeLa fibroblasts, and both CAT activities and growth hormone levels were determined from cell extracts prepared 48–60 hr after transfection. Relative enhancer activity was calculated as the CAT activity produced by the pVα1.4CAT plasmid/CAT activity produced by the pVαCAT control plasmid (which produced 0.06–0.09% acetylation) after adjustment for transfection efficiencies using growth hormone levels. Results were confirmed by at least three separate transfection experiments.



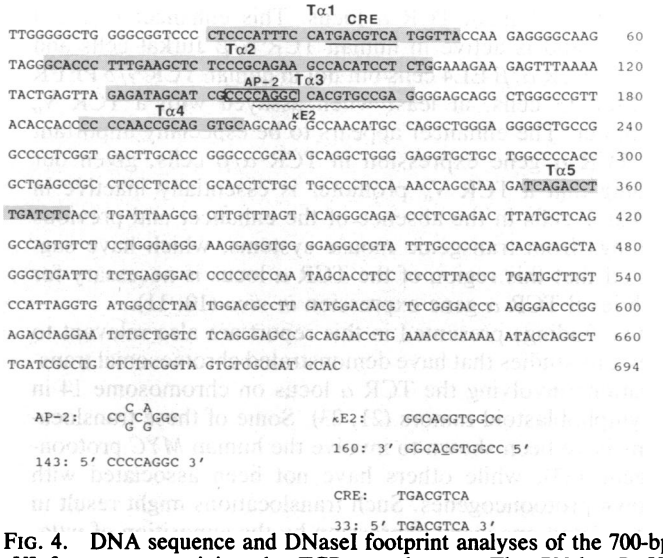


FIG. 4. DNA sequence and DNaseI footprint analyses of the 700-bp *Bst*XI fragment containing the TCR α enhancer. The 700-bp *Bst*XI fragment containing the TCR α enhancer (see Fig. 1) was subjected to DNase I footprint analyses using Jurkat nuclear extracts (*Left*) and to DNA sequence analysis (*Right*). For DNase I footprint analyses, the 403-bp *Bst*XI/*Xho* I fragment (bp 1–403) (lanes 1–3), the 266-bp *Bst*XI/*Apa* I fragment (bp 1–266) (lanes 4–6), the 266-bp *Apa* I/*Bst*XI fragment (bp 266–1) (lanes 7–9), or the 403-bp *Xho* I/*Bst*XI fragment (bp 403–1) were end-labeled and incubated in the absence (control) or presence (Jurkat) of Jurkat nuclear extract prior to partial digestion with DNase I. Standard Maxam and Gilbert purine sequencing reactions (G + A) of the same fragments were run in parallel. Protected sequences (Tα1–Tα5) are shown in brackets next to the autoradiograms. These sequences are shaded and labeled (*Right*). Sequences displaying similarity to the previously described AP-2 (21) (boxed), κE2 (22) (wavy underline), and cAMP-responsive (23) (solid underline) enhancer motifs are labeled. A comparison of these sequences to the consensus AP-2, κE2, and CRE sequences is shown (*Lower*). Differences between the TCR α enhancer sequence and the consensus sequences are underlined.

motifs or nuclear protein binding sites. Although we have labeled the Tα2 site as a single nuclear protein binding site, it may in fact be composed of two distinct binding sites as the middle of this sequence contains one or more bands that are not protected by Jurkat nuclear proteins on either strand.

Localization of TCR α Enhancer Activity to a 116-bp Fragment Containing the Tα1 and Tα2 Binding Sites. To determine the relative importance of each of the nuclear protein binding sites to enhancer function, subfragments of the 700-bp *Bst*XI fragment were assayed for enhancer function after subcloning into the *Bam*HI site 3′ of the minimal SV40 promoter/CAT cassette in pSPCAT and transfection into Jurkat T cells. As shown in Fig. 5, these experiments localized the enhancer activity to a 116-bp *Bst*XI/*Dra* I fragment (bp 1–116) and suggested that the Tα1 and Tα2 binding sites are sufficient for full enhancer function, while the Tα3, Tα4, and Tα5 sites are not required for TCR α enhancer activity, at least in Jurkat tumor cells.

DISCUSSION

In the studies described in this report, we have identified a transcriptional enhancer element located 4.5 kb 3′ of the C_α

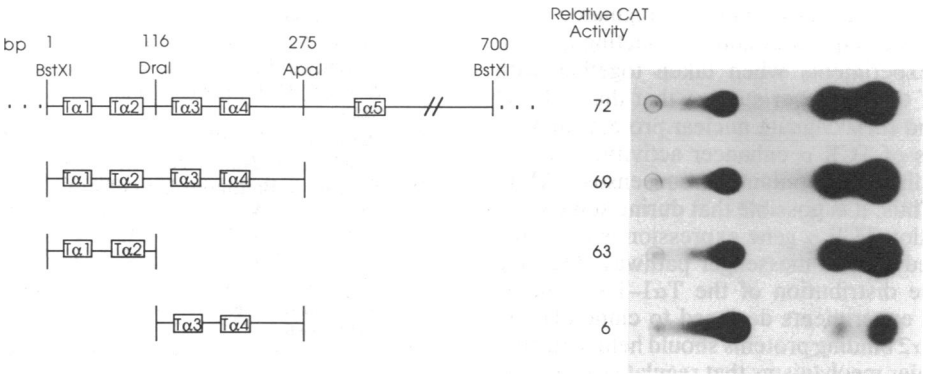


FIG. 5. Localization of the TCR α transcriptional enhancer. Overlapping restriction enzyme fragments from the 1.4-kb *Kpn* I/*Bam*HI TCR α enhancer fragment (shown in the top line; see Fig. 1) as denoted by the bars were subcloned into the *Bam*HI site 3′ of the CAT gene in the pSPCAT vector and were assayed for transcriptional enhancer activity after transfection into Jurkat T cells. The CAT activity produced by each plasmid after normalization to the CAT activity produced by transfection with the control pSPCAT plasmid alone (which produced 1.2% acetylation) is shown as relative CAT activity. The Tα1, Tα2, Tα3, Tα4, and Tα5 nuclear protein binding sites as determined by DNase I footprinting (Fig. 4) are shown schematically.

gene in the human TCR α locus. This enhancer is T-cell specific and is active in human TCR α/β Jurkat cells and murine TCR α/β EL4 cells but not in human TCR γ/δ PEER or Molt-13 cells, at least when assayed with a TCR V_α promoter. The enhancer appears to be especially important for TCR α gene expression in TCR α/β cells, given our finding that a TCR V_α promoter is essentially inactive in Jurkat T cells in the absence of the enhancer and previous findings from transgenic mouse systems, which have suggested that this region of the TCR α locus is necessary for high level TCR α gene expression *in vivo* (10, 11).

The findings presented in this report are also relevant to previous studies that have demonstrated chromosomal translocations involving the TCR α locus on chromosome 14 in T-lymphoblastoid tumors (21, 23). Some of these translocations have been shown to involve the human *MYC* protooncogene (23), while others have not been associated with known protooncogenes. Such translocations might result in deregulated oncogene expression by the apposition of putative oncogenes with the TCR α enhancer element. Because the TCR α enhancer is extremely potent, is active on a number of heterologous promoters, and can presumably act over relatively large distances, even translocations resulting in the repositioning of oncogenes relatively far away from the enhancer might still lead to such deregulated oncogene expression.

Because the human TCR δ locus is located between the unrearranged TCRα variable- and joining-region gene segments (32), it seemed possible that the TCR α transcriptional enhancer might activate TCR α gene expression in TCR α/β cells and TCR δ gene expression in TCR γ/δ cells. Our finding that the TCR α enhancer is active in TCR α/β Jurkat cells but fails to activate transcription from the TCR V_α promoter in TCR γ/δ PEER and Molt-13 cells suggests that the developmentally regulated activation of the TCR α transcriptional enhancer might be an important regulatory step in the differentiation of TCR α/β cells. The elucidation of the role of specific TCR α enhancer and promoter sequences in determining the TCR α/β cell specificity of TCR α gene expression awaits the results of ongoing transfection experiments testing the effects of different TCR α enhancer fragments on both V_α and heterologous promoters in additional TCR α/β and TCR γ/δ cell lines.

The fact that the TCR α enhancer does not contain the previously described octamer and κB enhancer motifs that have been shown to regulate immunoglobulin gene transcription suggests that TCR α transcription is regulated by a distinct set of cis-acting sequences and trans-acting factors. DNaseI footprint experiments when taken together with deletion analyses of the enhancer suggest that the Tα1 and Tα2 binding sites and their cognate nuclear proteins may be important regulators of TCR α enhancer activity. Interestingly, the Tα1 binding site contains a consensus cAMP-response element. Thus, it is possible that during some stage of T-cell differentiation TCR α gene expression is regulated by a cAMP-mediated second messenger pathway. Ongoing studies of the tissue distribution of the Tα1–Tα5 binding activities as well as experiments designed to clone cDNAs encoding Tα1 and Tα2 binding proteins should help to further elucidate the molecular mechanisms that regulate expression of the TCR α gene during T-cell development and activation.

Note. During the preparation of this manuscript, Winoto and Baltimore (33) described a highly homologous murine TCR α transcriptional enhancer, which is located 3′ of the murine TCR C_α gene segment.

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