

# REGULATION OF TRANSCRIPTION OF THE HUMAN T CELL ANTIGEN RECEPTOR $\delta$ CHAIN GENE

## A T Lineage-specific Enhancer Element

### Is Located in the J $\delta$ 3-C $\delta$ Intron

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T cells recognize antigens using a clonally expressed TCR, TCR- $\alpha/\beta$ , whose genes undergo somatic rearrangements during the early stages of thymic differentiation. The  $\alpha/\beta$  heterodimer is expressed in noncovalent association with the CD3 complex at the cell surface of the great majority of mature thymocytes and peripheral T cells. Another CD3-associated heterodimer,  $\gamma/\delta$ , was found to be expressed by a minority of typically CD4<sup>-</sup>CD8<sup>+/-</sup> thymocytes and peripheral T cells (1-7). It has been shown that TCR- $\gamma$  and - $\delta$  genes undergo rearrangement during the earliest phase of T cell differentiation. Although rearrangements of TCR- $\gamma/\delta$  genes are generally considered to be T cell specific, frequent inappropriate rearrangements of these genes have been documented in early B cell leukemias. Such findings are uncommonly found in chronic B cell proliferations (8, 9).

The organization of both TCR- $\gamma$  and - $\delta$  genes has been established. In both genes the germline-encoded repertoire appears to be limited, and most of the diversity is due to nucleotide additions and/or deletions at V-(D)-J junctions (10). To date, six V $\delta$ , three D $\delta$ , three J $\delta$ , and one C $\delta$  segments have been described (11-14). One V $\delta$  segment, V $\delta$ 3, is located 3' to C $\delta$  and rearranges by inversion (Fig. 1 A, top).

Considerable evidence has recently demonstrated that CD3- $\gamma/\delta$ , like the CD3- $\alpha/\beta$ , functions as an antigen receptor. The antigen repertoire of this TCR has not yet been fully defined, but it has been established that at least a subset of  $\gamma/\delta$ -expressing lymphoid cells are involved in the primary response against mycobacterial antigens (15). It is generally thought that  $\alpha/\beta$ - and  $\gamma/\delta$ -expressing cells belong to distinct T cell lineages.

The mechanisms involved in the choice between the  $\alpha/\beta$  vs.  $\gamma/\delta$  lineage remain

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<sup>1</sup> Abbreviation used in this paper: CAT, chloramphenicol acetyltransferase.

unknown, but mutual exclusion of  $\alpha/\beta$  and  $\gamma/\delta$  heterodimer expression has been documented in a number of cells. This may be due, at least in part, to the localization of the TCR- $\delta$  genes inside the TCR- $\alpha$  locus (16), which leads to TCR- $\delta$  deletion during V $\alpha$  to J $\alpha$  or other (17) TCR- $\alpha$  recombinations. Other mechanisms may theoretically be involved in the choice of lineage, including those involved in regulation of TCR- $\delta$  gene transcription.

In the present study, we have attempted to characterize potential transcriptional regulatory elements in the TCR- $\delta$  locus. By analogy with previous studies defining enhancers in Ig and TCR genes (18–25), we have searched for such elements upstream and downstream to the unique C $\delta$  C region. We show that two DNA fragments, OL3 and  $\delta$ A2, of 258 and  $\sim$ 300 bp, respectively, are able to induce transcriptional enhancement when inserted into a vector containing a reporter gene fused with a heterologous promoter and transfected in appropriate cells. We have also observed that OL3 enhance the transcription of the reporter gene when transfected into T cells, especially in the  $\gamma/\delta$ -expressing Peer cell line, but not in fibroblastic or B cell lines. The nucleotide sequence of this fragment contains some motifs sharing homology with previously described core enhancers.

### Materials and Methods

*Cell Lines.* The  $\gamma/\delta$ -expressing Peer cell line, the CD3- $\alpha/\beta$  expressing Jurkat line, the pre-B RPMI 1788 cell line, and the fibroblast Hela cell line were used. A polyclonal EBV-transformed B cell line was used in some experiments.

*Cloning of the TCR- $\delta$  Locus.* The J $\delta$ -C $\delta$  region was cloned from the DS6 genomic library (26).

*Construction of Chloramphenicol Acetyltransferase (CAT)<sup>+</sup> Expression Vectors.* The TpBLCAT2 expression vector was used (27). This vector contains a CAT gene fused to the heterologous herpes simplex tk promoter. The DNA fragments tested for a potential transcriptional enhancing activity were cloned in the Bam HI and/or Hind III restriction sites located in the polylinker 5' to the CAT gene. The RSV-CAT vector containing the ubiquitous rous sarcoma virus enhancer was used as a positive control for the presence of an enhanced transcription (28). Each experiment was performed at least twice.

*Transfection of Cells.* All cell lines were transfected by electroporation using standard methods (29).

*Cat Assays.* Cat assays were performed as described (29).

*DNA Sequencing.* Nucleotides sequences were performed according to the method of Sanger et al. (30).

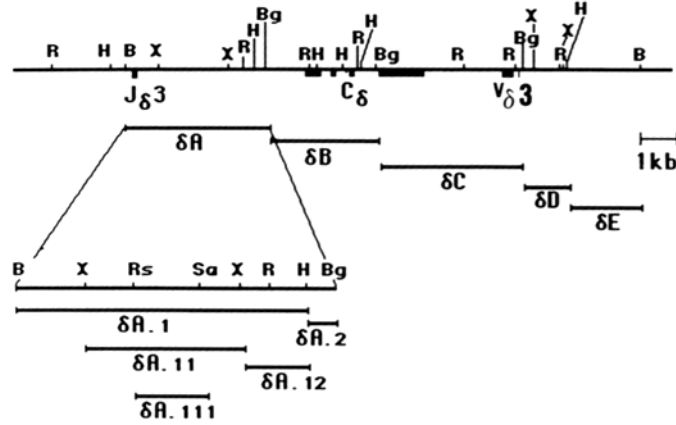
*In Vitro Gene Amplification by the PCR.* PCR amplification was performed as described (31). Oligonucleotides containing cloning sites were used as amplification primers: (a) GAAGCTTG-CAATGTAATATATTTGTGG; (b) GAAGCTTATGGATTCTATAAGACACTE; (c) CGGATC-CAAGTGTCTTATAGAATCCAT; (d) GAAGCTTCTTGAAAGTCAGCCAGAGTA; (e) CGGATCCTACTCTGGCTGACTTTCAAG; (f) CGGATCCGACAATTGAAAGGAT-TAGAC. For the location of the primers, see Fig. 3 A. A Hind III site is included in the 5' part of oligos a, b, and d, and a Bam HI site in the 5' end of oligos c, e, and f.

### Results and Discussion

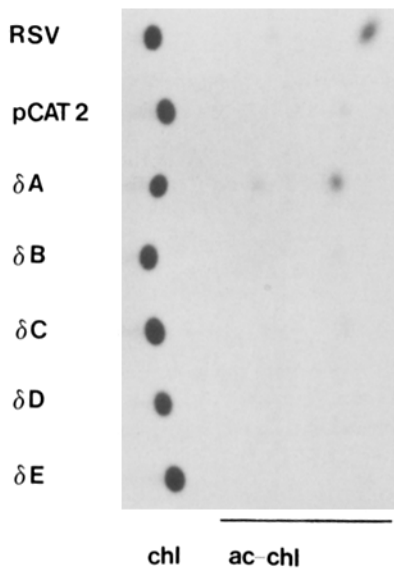
Enhancer elements have been described in rearranging genes of the Ig family, either in J-C introns or 3' to C regions (18–25). By analogy with these findings, we looked for the presence of a TCR- $\delta$  enhancer element in a 14-kb DNA segment extending from the most 3' described J $\delta$  segment, J $\delta$ 3, to a region located 6 kb 3' to the unique C $\delta$  region and containing the V $\delta$ 3 segment (Fig. 1 A).

DNA fragments were inserted in a CAT expression vector containing the heterol-

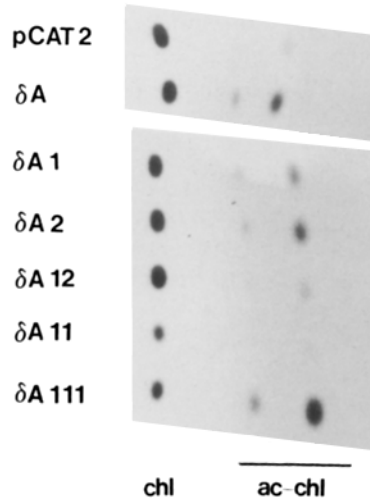
**A**



**B**



**C**



**FIGURE 1.** Localization of the TCR- $\delta$  enhancer by transfection of CAT vectors into the Peer cell line. (A) Restriction map of the 3' part of the TCR- $\delta$  locus and definition of the DNA fragments inserted in CAT expression vectors. (B) Demonstration of the presence of a transcriptional positive regulatory sequence inside the  $\delta A$  DNA fragment by transient transfection assays in the Peer cell line. The transfected cell extract was assayed for its ability to convert chloramphenicol (chl) to the acetylated form (ac-chl). The DNA fragment inserted in the polylinker located 3' to the CAT gene in the expression vector is indicated beside each lane. RSV-CAT (RSV) and pCAT2 constructions (see Materials and Methods) are used as positive and negative controls, respectively. A clear enhancement is observed in lane  $\alpha A$ , but not in lanes  $\delta B$ ,  $\delta C$ , and  $\delta E$ . All data shown in this panel were obtained in the same series of transfection experiments. (C) Study of the transcriptional activity associated with subfragments from  $\delta A$ .  $\delta A$  and  $\delta A.1$  fragments were cloned in opposite orientations in the CAT vector, showing that transcriptional enhancement is independent of the orientation in which the enhancer is cloned. Note that less extract was changed in the  $\delta A.11$  lane than in other lanes. In this lane, percentage of acetylation is 45%, compared with 4% in the negative control pCAT2 lane. For details, see text.

ogous tk promoter and transfected into the  $\gamma/\delta$ -expressing Peer cell line. By comparison with the construction containing the tk promoter alone (Fig. 1, pCAT2 vector), significantly enhanced CAT activity was observed when the Bam HI-Bgl2 3.2-kb  $\delta A$  fragment located between J $\delta 3$  and C $\delta$  was inserted in the construct (Fig. 1 B). No increased transcriptional activity was detected when the other restriction fragments,  $\delta B$ ,  $\delta C$ ,  $\delta D$ , and  $\delta E$ , were used in similar experiments (Fig. 1 B). This suggests that one or multiple positive regulatory *cis* elements are located in the intron extended from J $\delta 3$  to C $\delta$ .

To analyze the lineage specificity of such elements, the  $\delta A$ -containing CAT expression vector was transfected into the  $\alpha/\beta$ -expressing Jurkat T cell line, the 1788 RPMI B cell line, and into the Hela fibroblastic cell line. No enhanced CAT activity was observed after transfection of the B and fibroblastic cells. An enhanced activity was found when the Jurkat cell line was used (Fig. 2). In some experiments, the transcriptional enhancement appeared to be less intense than that observed for the Peer  $\gamma/\delta$  cell line. No enhanced CAT activity was observed when  $\delta B$ -,  $\delta C$ -,  $\delta D$ -, or  $\delta E$ -containing expression vectors were transfected in any cell line (data not shown).

To localize more exactly the regulatory elements, the  $\delta A$  fragment was further restricted and new vectors containing the restriction fragments were constructed. A more complex pattern of cell specificity was obtained (Figs. 1 C and 2). The Xba-Xba 1.8-kb fragment  $\delta A.11$  was able to enhance CAT activity in a T lineage-specific fashion similar to that observed for the whole  $\delta A$  fragment. Surprisingly, the 5'-located  $\delta A.2$  fragment contains a sequence that enhances the CAT activity in all cell lines tested, contrasting with that observed using the entire  $\delta A$  fragment (Fig. 2).

To further analyze the T cell-specific regulatory element located inside the  $\delta A.1$  region, this fragment was further digested, and the T cell-specific enhancer element was localized to the 880-bp  $\delta A.111$  fragment (Fig. 1 C). A DNA region containing this DNA fragment was therefore sequenced (data not shown), allowing the construction of specific oligonucleotides used in PCR experiments. Three DNA-amplified fragments, OL1, OL2, and OL3, encompassing the entire  $\delta A.111$  fragment, were obtained (Fig. 3 A) and cloned in CAT vectors (Fig. 3 B). After transfection in the PEER cell line, CAT expression enhancement was observed for the construction containing the OL3 fragment, but not for those containing OL1 or OL2 (Fig. 3 B). This allows the localization of the regulatory element in the 258-bp OL3 segment. The nucleotide sequence of OL3 is shown in Fig. 3 C. This sequence contains short nucleotide sequences sharing homology with previously described cellular or viral core enhancers, as well as a perfectly repeated nine mer sequence.

The localization of the IgH, Ig  $\kappa$ , and TCR- $\alpha$  or - $\beta$  enhancers with respect to the J and C regions vary. It has been shown that the human IgH and Ig  $\kappa$  enhancers are localized in the intron separating the most proximal J segment and the C region (18-22). The mouse TCR- $\alpha$  enhancer was localized 3 kb downstream to the C $\alpha$  region (25). The murine TCR- $\beta$  enhancer has been demonstrated to be localized 7.5 kb downstream to C $\beta 2$  (23, 24).

In this work, we have analyzed a DNA region extending from the J $\delta 3$  segment to 7 kb 3' to C $\delta$ . The V $\delta 3$  segment located 3' to C $\delta$  is included in the region studied. We have found that two DNA fragments supporting transcription-enhancing activity are located inside the J $\delta 3$ -C $\delta$  intron. We cannot, however, exclude that other *cis*-acting regulatory elements are present 5' to J $\delta 3$ , particularly between J $\delta 1$  and

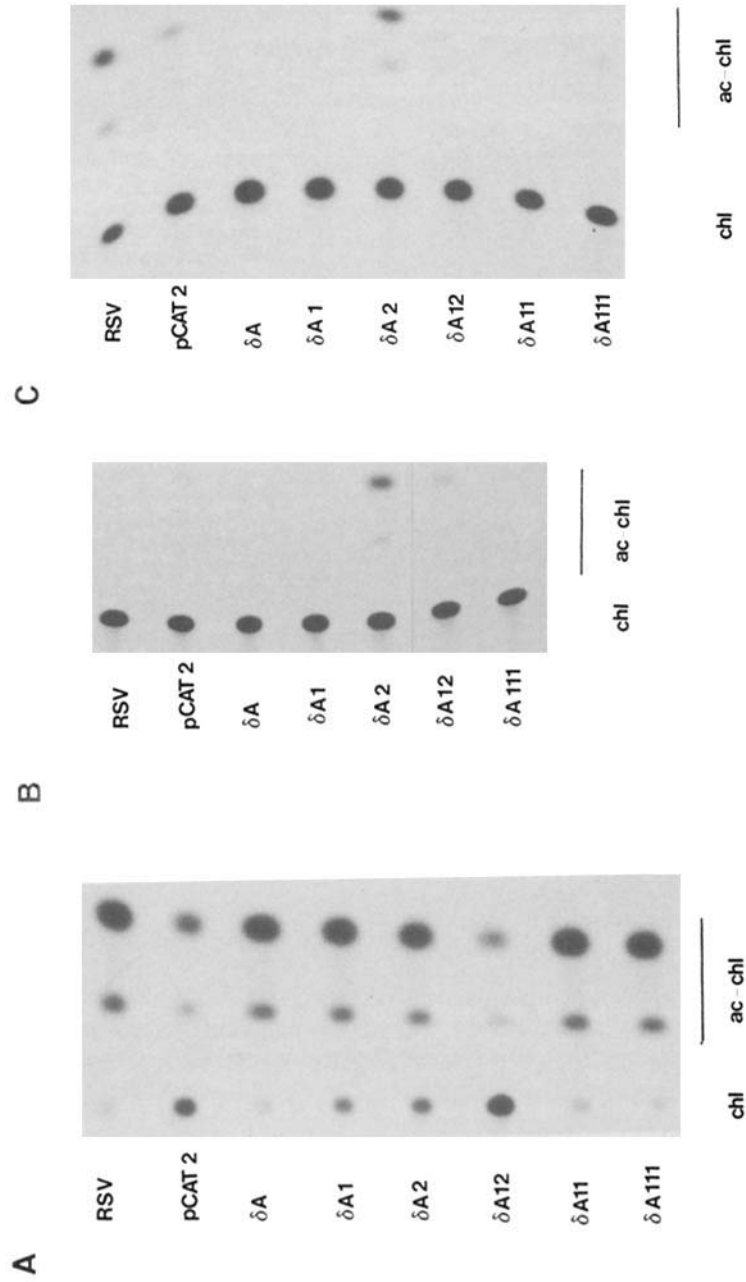
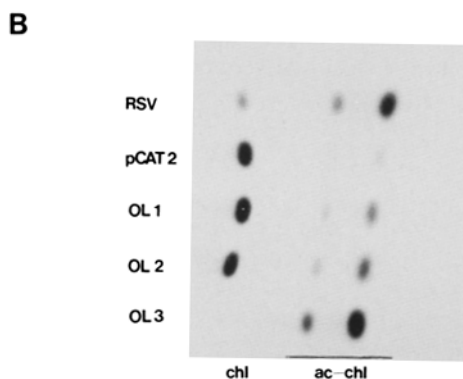


FIGURE 2. Specificity of the TCR- $\delta$ -enhancing sequences. The constructions were transfected in the  $\alpha/\beta$ -expressing T cell line Jurkat (A), the B cell line RPM1.1788 (B), and the fibroblast line HeLa (C). Note that in our hands, the enhancing activity of the RSV enhancer in the B cells used is weak.



**FIGURE 3.** Cloning of subfragments from  $\delta A111$  by PCR and analysis of associated transcriptional activity. OL1, OL2, and OL3 fragments have been sequenced after insertion into the CAT vector. (A) The positions of primers are shown with numbers indicating the most 5' nucleotide by reference with the first  $\delta A111$  nucleotide. (B) Study of the transcriptional activity associated with OL1, OL2, and OL3. Each fragment was cloned in the Hind III-Bam HI site of the *IpBLCAT2* vector. The inserts were analyzed by DNA sequencing. (C) Nucleotide sequence of the 248-bp OL3 fragment. Motifs sharing some sequence homology with previously described transcriptional regulatory sequences are tentatively indicated. A repeated nine mer sequence is boxed. The TCR- $\alpha$  enhancer (TCR- $\alpha$ ), polyoma virus enhancer (POL), and the  $\mu E3$  sequence from the IgH enhancer are described in references 25, 32, and 33, respectively.

**J $\delta 3$ .** Such a sequence would be retained by the V-D-J $\delta 1$  rearrangements, which represent the most frequently observed TCR- $\delta$  recombinations. Note that no enhancer element could be detected 3' to C $\delta$ , including the  $\delta C$  fragment, which contains the V $\delta 3$  segment along with 47 bp 5' and at least 2 kb 3' of this V segment. No enhancer element was detected in the 1-kb  $\delta D$  fragment 5' to V $\delta 3$ .

The localization of the regulatory elements inside the J $\delta 3$ -C $\delta$  intron may have biological implications. First, as J $\delta 3$  is the most proximal joining segment, this intron remains undeleted during V $\delta$ -D $\delta$ -J $\delta$  recombinations. This is the case even when the V $\delta 3$  segment located 3' to C $\delta$  is involved in the recombination as this segment rearranges by inversion. The location of the TCR- $\delta$  enhancer sequences allows them to regulate the transcription of rearranged TCR- $\delta$  genes from an as yet undescribed promoter located 5' to each V segment. Second, it has recently been shown that frequent translocations occur inside the TCR- $\delta$  locus, juxtaposing as yet unidentified

sequences to J $\delta$  segments in leukemic T cells (34). The regulatory sequences we have described in this report may be involved in the transcriptional control of the translocated genes.

We have also attempted to roughly define the cell specificity of the TCR- $\delta$  enhancer sequence activation. Our experiments suggest that activation of the OL3-enhancing activity may be restricted to T cells. This should, however, be extended by testing other lymphoid and nonlymphoid cell lines. Preliminary experiments show that OL3 is also inactive in polyclonal EBV-transformed mature cell lines (data not shown). A similar T cell specificity has been previously demonstrated for mouse TCR- $\alpha$  and - $\beta$  enhancers (24, 25). The data obtained with the  $\delta$ A.2 DNA fragment remain more puzzling. Enhancement of transcription observed in all cell lines was indeed abolished by inclusion of this fragment in a larger one, namely  $\delta$ A. This finding is reminiscent of that observed in the C $\beta$  locus (24). It is possible that a negative regulatory element is located 5' to  $\delta$ A.2. Experiments are in progress to explore this hypothesis. Finally, it should be stressed that the strategy used herein cannot completely characterize the TCR- $\delta$  enhancer(s). Determination of the nucleotide sequences involved in binding to *trans*-acting regulatory proteins and studies of chromatin accessibility with respect to cell lineage and differentiation, as recently performed in the study of murine V $\beta$  5' regulatory sequences (35), will aid our further understanding of this subject.

### Summary

We have defined transcriptional enhancing sequences inside the TCR- $\delta$  gene locus, using transient transfections with constructs containing DNA fragments cloned upstream to a reporter gene fused to a heterologous promoter. A 14-kb DNA region extending from the J $\delta$ 3 segment to 6 kb 3' to C $\delta$  was analyzed. We show the presence of positive regulatory sequences inside the J $\delta$ 3-C $\delta$  intron and have localized these sequences to two DNA fragments of  $\sim$ 300 and 258 bp. Analysis of cell specificity of the activation of such sequences demonstrates a T cell pattern for one of the two fragments. The nucleotide sequence of the T cell-specific element shows motifs sharing homology with previously described core enhancers.

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