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Immunology

Identification of a T-cell-specific transcriptional enhancer located 3′ of *Cγ1* in the murine T-cell receptor γ locus

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ABSTRACT **A transcriptional enhancer element has been localized 3 kilobases 3′ of the murine T-cell receptor *Cγ1* locus using a chloramphenicol acetyltransferase reporter gene construct. As a monomer the enhancer functions only in PEER $\gamma\delta$ cells and Jurkat $\alpha\beta$ cells of the T-cell lines tested. However, a tetramer of the enhancer functions in virtually all T-cell lines tested, including $\alpha\beta$ T-cell lines, but not in other cell types. These results suggest that elements other than the enhancer are responsible for the failure of rearranged *Cγ1* genes to be expressed in $\alpha\beta$ T cells. The enhancer has been localized to a 200-base-pair *Rsa* I restriction fragment, which contains sequence motifs similar to those found in the other T-cell receptor enhancers but not in the immunoglobulin enhancers.**

Whereas the majority of T cells express a CD3-associated T-cell antigen receptor (TCR) composed of α and β chains (1, 2), a smaller subset expresses the more recently characterized CD3-associated $\gamma\delta$ TCR. Although the function of $\gamma\delta$ T cells is still speculative, they possess several features distinct from $\alpha\beta$ T cells (3–5). γ and δ rearrangements are the first to occur in murine ontogeny, as early as embryonic day 13 or 14 (6–8), to generate the first T cells to appear in ontogeny. The early fetal thymic $\gamma\delta$ cells home to the epidermal epithelium and constitute the Thy-1^+ dendritic epidermal cells (9). Later waves of $\gamma\delta$ T cells home to different epithelial tissues as well as the secondary lymphoid organs (10–12).

Strikingly, within each wave of migrating T cells, distinct sets of *Vγ* and *Vδ* gene segments are utilized (13–16). It is possible that differential *V*-gene usage by discrete sets of $\gamma\delta$ cells is regulated, at least in part, at the level of gene rearrangement. Studies of immunoglobulin genes suggest that prior transcription of unrearranged gene segments may regulate the frequency of rearrangement (17). Hence, an understanding of the control of γ gene transcription may lead to insights into the programed rearrangement of *Vγ* and *Vδ* genes and the genesis of distinct sublineages of $\gamma\delta$ cells.

Moreover, the developmental decision to differentiate along the $\gamma\delta$ lineage or $\alpha\beta$ lineage may be controlled at least partly at the level of transcription. Although *Cγ1* (and other *Cγ*) genes are rearranged in most peripheral $\alpha\beta$ T cells, the corresponding transcripts are usually absent (6, 13). Thus, transcriptional regulation may play an important role in the developmental decision between $\alpha\beta$ and $\gamma\delta$ lineages.

Toward the goal of understanding γ gene transcriptional regulation, we have isolated a tissue-specific transcriptional enhancer located 3 kilobases (kb) downstream of *Cγ1*. The minimal enhancer was active in some, but not all, transformed T-cell lines. However, multimers of the enhancer, or of a specific site within the enhancer, were active in virtually all T-cell lines tested but not in B cells or nonlymphoid cells. Potential roles of the *Cγ1* enhancer in regulating T-cell development are discussed.

MATERIALS AND METHODS

Murine TCR γ Genomic Clones. A genomic clone containing the 17-kb *Vγ2-Jγ1Cγ1 Eco*RI fragment was described previously (13). An overlapping genomic clone containing an additional 12.5 kb downstream was isolated from a BALB/c genomic library in EMBL3 by hybridization with the 1.7-kb *Bgl* II–*Sal* I fragment 2.5 kb 3′ of *Cγ*.

Plasmids. Plasmids J21 and J21MoEn have been described (18). In pβ-GAL-A the chicken β-actin promoter was cloned 5′ of the β-galactosidase gene and a simian virus 40 (SV40) poly(A) sequence in Bluescript pKS (Stratagene). In the remaining plasmids, the various γ gene fragments were subcloned into the downstream polylinker of J21.

Cell Lines. All cells were maintained in 10% fetal calf serum, RPMI 1640 medium, 50 μM 2-mercaptoethanol, and antibiotics.

Transfections. All cells were transfected using the DEAE-dextran method as described (19) with the following modifications. For all $\gamma\delta$ T cells and two $\alpha\beta$ T cells, Hut78 and S49.1, 4×10^7 cells were used per transfection, whereas $\approx 10^7$ NIH 3T3 cells were used per transfection. For the remaining cell lines 2×10^7 cells per transfection were used. For all transfections, ≈ 3 μg of DNA per 10^7 cells was used. Equal molar amounts of DNA, adjusted to equal weight using sonicated herring sperm DNA, were used. All transfections were done in duplicate and experiments were repeated at least twice.

In some experiments, 3 μg of pβ-GAL-A was cotransfected along with the J21 constructs as an internal control for transfection efficiency. The cytoplasmic extracts were prepared as before and β-galactosidase activity was assayed by a standard protocol.

Chloramphenicol Acetyltransferase (CAT) Assays. The CAT assay was performed essentially as described (19) with the following modifications. After a 40- to 48-hr incubation following transfection, the cells were harvested and 50–200 μg of proteins of cell extract (depending on transfection efficiency) was assayed for its ability to convert [^{14}C]chloramphenicol to the acetylated form in a 1- to 2-hr incubation period. In the case of some poorly transfected cell lines (PEER, Molt-13 $\gamma\delta$ cells, and the DN $\gamma\delta$ hybridomas), the incubation period was extended to 4–6 hr and extra acetyl-CoA was added at 2-hr intervals. Quantitation of acetylation ratios was performed by liquid scintillation spectroscopy in Ecolume (NEN) fluor of the excised spot from thin-layer chromatography sheets.

DNase I Footprinting. Footprinting was done using a standard protocol (20) with heparin fractions of the nuclear extracts indicated. Briefly, nuclear extracts in buffer C/0.1 M KCl (20) were bound to heparin-Sepharose CL-6B (Pharmacia), eluted with 0.5 M KCl, and dialyzed against buffer C/0.1 M KCl.

Abbreviations: CAT, chloramphenicol acetyltransferase; TCR, T-cell antigen receptor; SV40, simian virus 40; MLV, murine leukemia virus.
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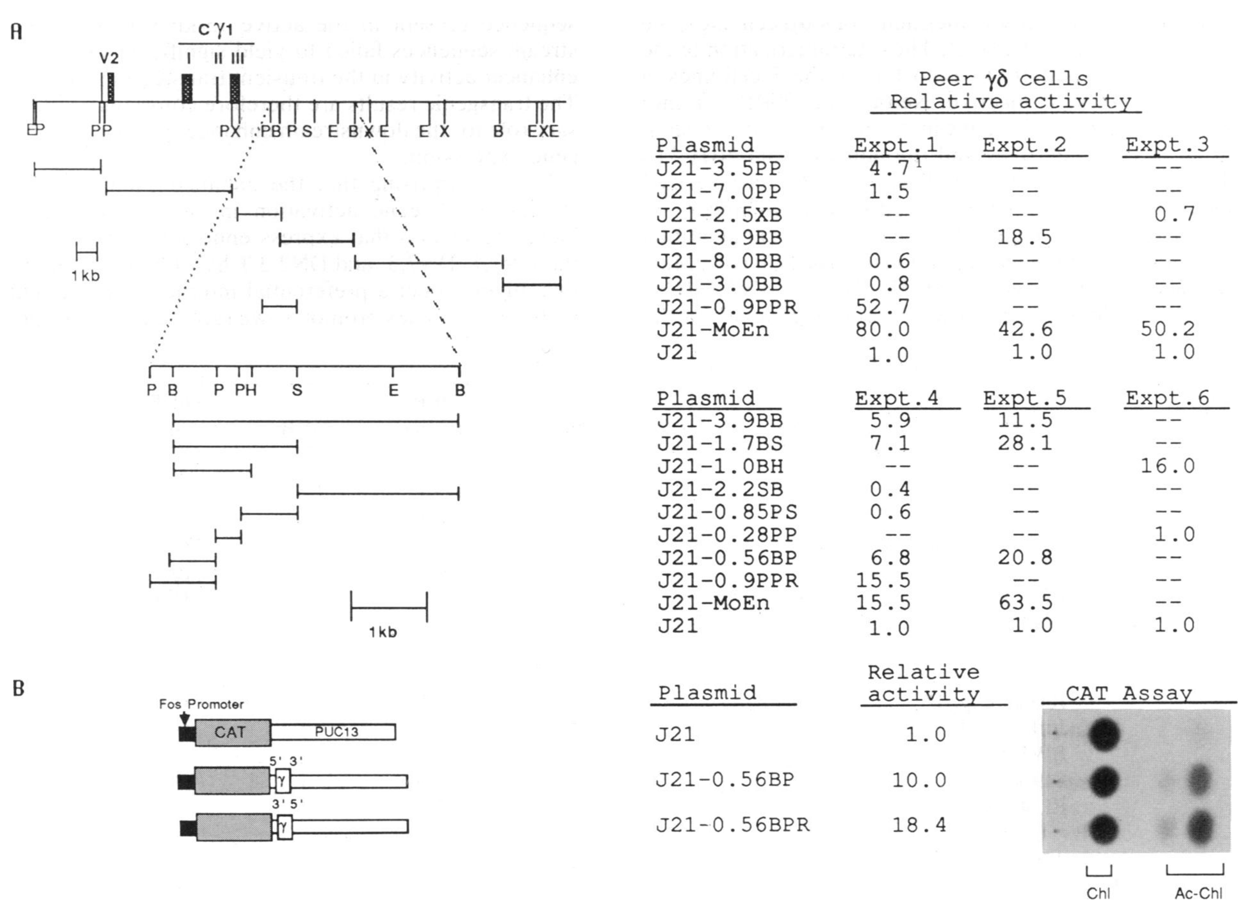
RESULTS

We used a transient transfection assay to screen the *Cγl* locus for enhancer activity. Supercoiled plasmids carrying the reporter gene, CAT, and fragments of the *Cγl* locus were transfected into cell lines. Two days later cell extracts were tested for CAT activity. Restriction fragments from 2.5 kb to 8 kb in length spanning 30 kb of a rearranged *Vγ2-JγlCγl* gene were subcloned into the downstream polylinker of the test plasmid J21. In J21, a minimal *fos* promoter (−71 to +109) drives CAT expression. Stimulation of CAT activity indicates the presence of a transcriptional enhancer. We found only one fragment, a 3.9-kb *Bgl* II–*Bgl* II fragment located 2.5 kb downstream of *Cγl*, with reproducible enhancer activity in the human γδ cell line PEER (Fig. 1*A*). By testing restriction fragments of the 3.9-kb fragment, we found that most of the activity resided in a 560-bp *Bgl* II to *Pvu* II fragment (.56BP) (Fig. 1*A*). This fragment could be further trimmed to a 200-bp *Rsa* I–*Rsa* I minimal enhancer fragment (.20RR), which had indistinguishable activity from .56BP (Fig. 2). Further removal of the 5′ or 3′ half of .20RR reduced the activity severalfold (Fig. 2). The enhancer functioned in both orientations, although the activity was slightly stronger when the enhancer was subcloned downstream of the CAT gene in the reverse transcriptional orientation (Fig. 1*B*).

To define the cellular specificity of the γ enhancer, we transfected a number of T and non-T cells with J21 constructs that include the minimal enhancer (Table 1). The enhancer was active in the PEER γδ cell line and the Jurkat αβ cell line and inactive in the three B cells tested (A20, M12.1, and BJAB), in the fibroblast NIH 3T3 cell line, and in the cervical carcinoma HeLa cell line. These results suggested that the enhancer is T-cell specific. Surprisingly, however, the enhancer had little or no activity in several other γδ cell lines (Molt-13, DN7.1, DN7.3, DN2.3, and DN1.1) or in several other αβ cell lines (BW5147, S49.1, EL-4, and Hut78). Larger fragments containing the enhancer (3.9BB and 1.0BH) were also inactive in Molt-13 and DN7.1 cell lines (not shown). This pattern of expression was highly reproducible in numerous transfection experiments.

Previous studies have shown that multiple tandem copies of enhancers generally yield stronger activity than enhancer monomers (21). We therefore tested a tetramer of the .20RR minimal enhancer fragment, subcloned downstream of the CAT gene in J21, for activity in several cell lines. The tetramer was active in all four T-cell lines tested, including



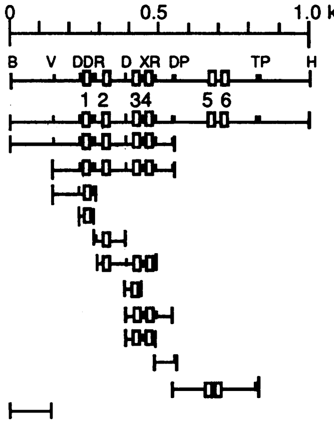
Peer γδ cells
Relative activity

Plasmid	Expt.1	Expt.2	Expt.3
J21-3.5PP	4.7[1]	--	--
J21-7.0PP	1.5	--	--
J21-2.5XB	--	--	0.7
J21-3.9BB	--	18.5	--
J21-8.0BB	0.6	--	--
J21-3.0BB	0.8	--	--
J21-0.9PPR	52.7	--	--
J21-MoEn	80.0	42.6	50.2
J21	1.0	1.0	1.0

Plasmid	Expt.4	Expt.5	Expt.6
J21-3.9BB	5.9	11.5	--
J21-1.7BS	7.1	28.1	--
J21-1.0BH	--	--	16.0
J21-2.2SB	0.4	--	--
J21-0.85PS	0.6	--	--
J21-0.28PP	--	--	1.0
J21-0.56BP	6.8	20.8	--
J21-0.9PPR	15.5	--	--
J21-MoEn	15.5	63.5	--
J21	1.0	1.0	1.0

Plasmid	Relative activity
J21	1.0
J21-0.56BP	10.0
J21-0.56BPR	18.4

FIG. 1. Identification and mapping of a transcriptional enhancer element at the 3′ end of the murine *Cγl* locus. (*A*) Partial restriction endonuclease map using *Eco*RI (E), *Pvu* II (P), *Xba* I (X), *Bgl* II (B), *Sal* I (S), and *Hin*dIII (H). Not all of the *Pvu* II, *Hin*dIII, and *Xba* I sites are shown. The five exons of *Vγ2-JγlCγl* are shown as stippled boxes. Test fragments shown as bars were subcloned into the polylinker 3′ of the CAT gene in the J21 vector and appear across from their relative activity in representative experiments. Average activity values of duplicate transfections were normalized to those produced by transfection with the control plasmid J21, which were 0.3%, 1.5%, 0.3%, 0.4%, 0.1%, and 0.5% for experiments 1–6, respectively. The 900-base-pair (bp) *Pvu* II fragment in J21-0.9PPR is in reverse orientation. (*B*) Representative CAT assay displaying the activity of the TCR γ enhancer when cloned downstream of the minimal *fos* promoter (−71 to +109) and CAT reporter gene and transfected into PEER cells. The control J21 as well as the TCR γ enhancer-containing plasmids are shown schematically. In J21-BP.56 and J21-BP.56R, the 560-bp *Bgl* II to *Pvu* II fragment (*A*) is cloned downstream of CAT into the unique *Bgl* II site of J21 in the orientations indicated. Relative CAT activities were calculated as before relative to that of J21 (1.5% conversion). The thin-layer chromatography spots corresponding to [^{14}C]chloramphenicol (Chl) and acetylated [^{14}C]chloramphenicol (Ac-Chl) are indicated. Superscript 1, this enhancement was not reproduced in other experiments.



Relative CAT Activity

Plasmid	Expt.1	Expt.2	Expt.3	Expt.4
J21-1.0BH	--	--	--	9.5
J21-.56BP	12.8	--	6.8	7.3
J21-.42EP	8.5	--	--	--
J21-.14VR	0.2	--	--	--
J21-.05DD	0.5	--	--	--
J21-.11DD	--	1.7	--	--
J21-.20RR	11.5	8.5	7.8	--
J21-.06DB	--	2.0	--	--
J21-.16DD	3.8^1	--	2.7	--
J21-.10DR	--	2.1	1.7	--
J21-.08RV	0.6	--	--	--
J21-.28PP	--	--	0.7	--
J21-.14EV	--	--	0.9	--
J21	1.0	1.0	1.0	1.0
J21-MoE	20.0	--	26.1	24.3

FIG. 2. Deletional analysis of the γ enhancer. Fragments are shown schematically by bars and named on their right by size (in kb) and restriction endonuclease endpoints. The restriction enzymes used are *Bgl* II (B), *Dde* I (D), *Eco*RV (V), *Hin*dIII (H), *Pvu* II (P), *Rsa* I (R), *Sty* I (T), and *Bst*BI (X). The regions that correspond to NFγ1-6 are numbered and represented schematically by open boxes. Activity normalized to activity of cells transfected with J21 (0.70%, 0.8%, 0.28%, and 0.55% for experiments 1–4, respectively). Superscript 1, the 160-bp *Dde* I fragment was in reverse orientation in experiment 1.

the Molt-13 and DN7.1 cell lines, in which the monomer was inactive (Table 1). We also tested a tetramer of an internal 60-bp fragment of the enhancer, .06DB, which had little or no activity by itself (Fig. 2) but includes a major protein-binding site as determined by DNase I footprinting analysis (see below). The .06DB tetramer is strongly active in 9 of 10 T-cell lines, including 6 of 6 $\gamma\delta$ cell lines and 3 of 4 $\alpha\beta$ cell lines, the exception being EL-4 (Table 1). Thus, tetramerization of the enhancer leads to activity in almost all of the T-cell lines in which the monomer is inactive. Neither the .20RR tetramer nor the .06DB tetramer is active in the non-T-cell lines tested, including three B-cell lines and the nonlymphoid NIH 3T3 and HeLa cell lines (Table 1). Therefore, the tetramerized enhancer functions as a strong T-cell-specific enhancer.

Transgenic studies suggest that the *Cγ1* enhancer identified herein is important for gene activation in $\gamma\delta$ cells *in vivo*. Mice transgenic for a rearranged 15-kb *Eco*RI–*Sal* I *Vγ2-Jγ1Cγ1* fragment, including the enhancer, express high levels of the transgene in $\alpha\beta$ and $\gamma\delta$ T cells (22). In contrast, three independent lines of mice transgenic for an 11.7-kb *Nco* I–*Nco* I *Vγ2-Jγ1Cγ1* fragment, which ends 1 kb upstream of the enhancer, fail to express the transgene in either $\gamma\delta$ or $\alpha\beta$ cells (D.M.S., E. Selsing, and D.H.R., unpublished results). Although the 11.7-kb fragment also lacks 2.3 kb of upstream sequence present in the active 15-kb fragment, these upstream sequences failed to yield significant or reproducible enhancer activity in the transient transfection assay (Fig. 1). The transgenic results are therefore consistent with a necessary role for the downstream enhancer described here in *Cγ1* gene expression.

It was surprising that the enhancer monomer failed to support CAT gene activation in several $\gamma\delta$ T-cell lines, including several that express endogenous *Cγ1* genes [i.e., the DN7.1, DN7.3, and DN2.3 T hybridomas (16)]. To ask if this might reflect a preferential interaction of the enhancer with a homologous promoter, we replaced the c-*fos* promoter

Table 1. Activity of DNA segments dissected from γ enhancer

Cell line	Moloney	.42EP	.20RR	.20RR tetramer	.06DB	.06DB tetramer
$\gamma\delta$ T						
PEER	22.2	—	6.8	135.1	1.8	83.4
Molt-13, exp. 1	25.1	—	1.0	10.9	1.0	50.2
Molt-13, exp. 2	91.8	1.0	—	—	—	183.2
DN7.1	9.2	1.2	—	4.1	—	10.2
DN2.3	62.8	—	—	—	—	31.4
DN7.3	73.2	—	—	—	—	17.3
DN1.1	28.6	1.0	—	—	—	12.2
$\alpha\beta$ T						
Jurkat, exp. 1*	15.8	—	3.5	31.6	1.5	31.0
Jurkat, exp. 2	14.3	—	5.0	42.2	3.1	43.9
BW5147	13.4	—	—	—	—	9.7
S49.1	16.8	—	—	—	—	15.3
EL-4	11.0	—	—	—	—	1.3
B						
A20, exp. 1*	—	—	1.0	0.5	—	—
A20, exp. 2	20.0	—	—	—	—	1.0
M12.4.1	6.0	0.6	—	—	—	0.6
Nonlymphoid						
HeLa, exp. 1	17.7	—	—	1.1	—	—
HeLa, exp. 2	9.0	0.7	—	—	—	1.0
NIH 3T3	12.2	0.6	—	—	—	1.0

The constructs containing single or four-tandem copies of the enhancer-containing fragments (see Fig. 2) were transfected into different cell lines. Relative enhancer activity was determined quantitatively by scintillation spectroscopy of the excised spot from thin-layer chromatography sheets. The stimulation of transcription by the fragments is normalized to that of the J21 vector, which is set to 1. —, Not determined.

*pβGAL-A was cotransfected with CAT constructs in the same experiment (see text). The CAT activity was then normalized to the β-galactosidase activity.

of J21 with a *Vγ4* promoter (a 470-bp *Bgl* II to *Bst*NI fragment). Use of the *Vγ4* promoter did not reveal enhancer activity in the Molt-13 γδ T-cell line or in the murine $\gamma\delta^+$ hybridomas, even in those hybridomas that express the endogenous *Vγ4-Jγ1Cγ1* gene (e.g., DN7.1) (data not shown). This was not due to the inactivity of the *Vγ4* promoter in these cells, since it functioned well with the Moloney murine leukemia virus (MLV) enhancer.

It is possible that sites flanking .06DB, within .20RR, partially inhibit activity in some T-cell lines, as measured in the transient transfection assay. The relative activity of the tetramer of .20RR (the minimal enhancer) in the Molt-13 and DN7.1 cell lines was lower than that of the tetramer of the smaller .06DB fragment. In contrast, the .20RR tetramer was as active as or more active than the .06DB tetramer in PEER and Jurkat cell lines (Table 1). Further studies will be necessary to determine the significance of this partial inhibition.

To further characterize the enhancer, a 420-bp *Eco*RV to *Pvu* II fragment containing the enhancer was assayed for nuclear protein binding using the DNase I footprinting technique. Of six sites (NFγ1–NFγ6) that were reproducibly protected from DNase I digestion when incubated with the nuclear extracts from T cells, three (NFγ2–NFγ4) are within the minimal enhancer; of these, the most extensively protected site was NFγ3 (Fig. 3). NFγ1, NFγ5, and NFγ6 are outside of the minimal enhancer fragment and are therefore inessential for maximal enhancer activity.

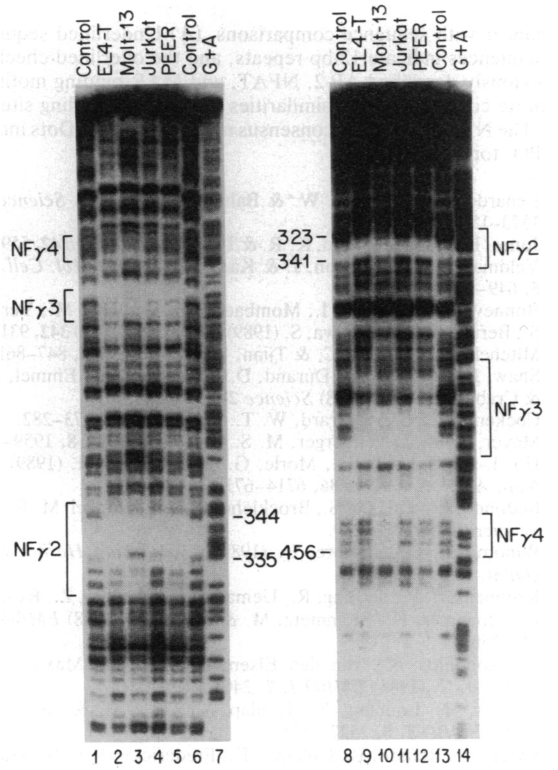


FIG. 3. DNase I footprinting of the γ enhancer. For the positive strand (lanes 1–7), DNA was labeled at the *Eco*RV site and then cut by the *Pvu* II restriction enzyme. The reverse was performed for the negative strand (lanes 8–14). A ladder of G + A Maxam–Gilbert sequencing reactions was run in parallel (lanes 7 and 14). The control samples were not incubated with nuclear extracts (lanes 1, 6, 8, and 13). The cited positions correspond to the *Bgl* II–*Hin*dIII sequence (see Fig. 4). Lanes 2–5, NFγ3 is strongly protected by all T-cell extracts tested as is position 344 (NFγ2). Position 335 is protected in all extracts except Molt-13 (lane 3), in which it is hypersensitive. Lanes 9–13, NFγ3 is strongly protected by all T-cell extracts as is position 456 (NFγ4). Position 323 (NFγ2) is clearly protected by the Molt-13 and PEER extracts (lanes 10 and 12, respectively). Positions 341 (NFγ2) and 387 (NFγ3) are hypersensitive sites. These patterns of protection and hypersensitivity were reproduced in each of three experiments.

The 1.0-kb *Bgl* II to *Hin*dIII fragment containing the enhancer was sequenced (Fig. 4*A*). Within the minimal enhancer fragment we discerned various motifs (Fig. 4*B* and legend). Notably, an 11-bp motif within NFγ3 and a similar motif within NFγ4 are similar to sequences previously identified in the other three TCR genes' enhancers (i.e., δE3, Tα2/NFα5, and β core), in the CD3 δ and ε enhancers, and in the core region of several viral enhancers, including those of SV40, polyoma, and Moloney MLV (27–34). Mutational analysis of the viral core sequences and the corresponding sequence in the TCR α gene demonstrates that those sequences are essential for full enhancer function (29, 33, 35).

DISCUSSION

Similar to many other T-cell-specific genes, including TCR α and β and CD3 δ and ε, a T-cell-specific enhancer is found at the 3′ end of the TCR *Cγ1* gene segment. The immunoglobulin genes, IgH and Igκ, are also known to contain 3′ enhancers as well as weaker enhancers in their joining-constant (J-C) introns. An enhancer appears in the J-C intron of the TCR δ gene but intronic enhancers are not detected in the other TCR genes; the report of an intronic TCR α enhancer has not been confirmed in a more recent study (18, 36). The location of enhancers controlling TCR and immunoglobulin genes 3′ of the J gene segment fits with the model that nonrearranged variable gene segments must be brought into the "activation domain" of a downstream enhancer for full activity (37–40).

Unexpectedly, the γ enhancer monomer was not demonstrably active in many of the T-cell lines tested, but tetramerization of the enhancer revealed activity in virtually all T-cell lines. The TCR δ enhancer monomer also fails to function in some γδ cells (A. Winoto, personal communication). Given that at least two enhancers appear to control the IgH and Igκ gene loci, it is possible that additional γ enhancer elements, outside the regions assayed, are required for activity in some γδ cells. However, transgenic data (see *Results*) suggest that the enhancer described here is sufficient for expression in many γδ cells *in vivo*.

Alternatively, the γ enhancer may simply be weak in some γδ cells, though of sufficient strength to support the accumulation of the relatively low levels of γ mRNA (41). This possibility is supported by our finding that tetramerization of the minimal enhancer or a site within the minimal enhancer leads to strong enhancer activity in all γδ T-cell lines tested. Finally, it is possible that sites within the minimal enhancer are the target of factor(s) that inhibit enhancer activity in some T-cell lines, at least when the sequences are introduced into cells by the transient transfection procedure. Determination of the relative contribution of these and other mechanisms to the pattern of cell-type specificity we have observed requires further investigation.

When tetramerized, the enhancer displayed T-cell specificity but not γδ T-cell specificity—i.e., it functioned in γδ and αβ T-cell lines. Thus, there is no evidence that the specificity of the enhancer accounts for the fact that rearranged *Cγ1* genes are not expressed in αβ T cells. Cis-acting regulatory elements flanking the enhancer may suppress γ expression in αβ T cells (42). Similar cis-acting elements that prevent α gene expression in γδ T cells have been reported (43). Such elements may play a role in the separation of the γδ and the αβ lineages during ontogeny.

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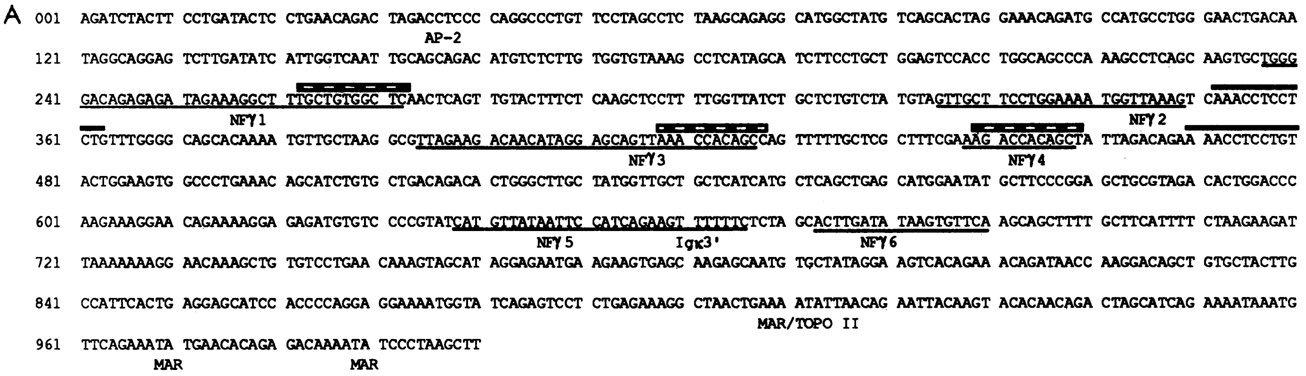

A

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001 AGATCTACTT CCTGATACTC CTGAACAGAC TAGACCTCCC CAGGCCCTGT TCCTAGCCTC TAAGCAGAGG CATGGCTATG TCAGCACTAG GAAACAGATG CCATGCCTGG GAACTGACAA
                                         AP-2
121 TAGGCAGGAG TCTTGATATC ATTGGTCAAT TGCAGCAGAC ATGTCTCTTG TGGTGTAAAG CCTCATAGCA TCTTCCTGCT GGAGTCCACC TGGCAGCCCA AAGCCTCAGC AAGTGCTGGG

241 GACAGAGAGA TAGAAAGGCT TTGCTGTGGC TCAACTCAGT TGTACTTTCT CAAGCTCCTT TTGGTTATCT GCTCTGTCTA TGTAGTTGCT TCCTGGAAAA TGGTTAAAGT CAAACCTCCT
                    NFγ1                                                                                            NFγ2
361 CTGTTTGGGG CAGCACAAAA TGTTGCTAAG GCGTTAGAAG ACAACATAGG AGCAGTTAAA CCACAGCCAG TTTTTGCTCG CTTTCGAAAG ACCACAGCTA TTAGACAGAA AACCTCCTGT
                                                               NFγ3                                    NFγ4
481 ACTGGAAGTG GCCCTGAAAC AGCATCTGTG CTGACAGACA CTGGGCTTGC TATGGTTGCT GCTCATCATG CTCAGCTGAG CATGGAATAT GCTTCCCGGA GCTGCGTAGA CACTGGACCC

601 AAGAAAGGAA CAGAAAAGGA GAGATGTGTC CCCGTATCAT GTTATAATTC CATCAGAAGT TTTTTCTCTA GCACTTGATA TAAGTGTTCA AGCAGCTTTT GCTTCATTTT CTAAGAAGAT
                                                     NFγ5           Igκ3'              NFγ6
721 TAAAAAAAGG AACAAAGCTG TGTCCTGAA CAAAGTAGCAT AGGAGAATGA AGAAGTGAGC AAGAGCAATG TGCTATAGGA AGTCACAGAA ACAGATAACC AAGGACAGCT GTGCTACTTG

841 CCATTCACTG AGGAGCATCC ACCCCAGGA GGAAAATGGTA TCAGAGTCCT CTGAGAAAGG CTAACTGAAA ATATTAACAG AATTACAAGT ACACAACAGA CTAGCATCAG AAAATAAATG
                                                                             MAR/TOPO II
961 TTCAGAAATA TGAACACAGA GACAAAATA TCCCTAAGCTT
            MAR                  MAR
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B

								TcRγ	NFγ5	658 to 667	AGTTTTTTCT
				TcRγ	Enh.	352 to 363	AAACCTCCTCTG	Igκ	3'Enh.	117 to 126	...A......
TcRγ	NFγ3	418 to 428	AAACCACAGCC	TcRγ	Enh.	470 to 481	.........T.A				
TcRγ	NFγ4	449 to 459	.G........T	TcRα	NFα4	187 to 198	CC..A.......	TcRγ	NFγ6	670 to 681	AGCACTTGATAT
TcRγ	NFγ1	272 to 262	G.G.......A					TcRα	Tα5	355 to 366	..AC......C.
TcR δ	E3	1252 to 1242	........TG.	TcRγ	NFγ2	338 to 347	AAATGGTTAA				
TcR α	Tα2	75 to 85	..G.....T..	TcRα	Enh.	17 to 26	......G...	TcRγ	Enh.	38 to 45	CCCCAGGC
TcR α	NFα5	184 to 194	..G.....T..	TcR δ	Enh.	1374 to 1383	.....TC...	AP2	SV40		....
TcR β	Enh.	634 to 624	........T..								
CD3 ε	Enh.		T..........	TcRγ	Enh.	907 to 921	GAAAATATTAACAGA	TcRγ	Enh.	915 to 908	AATATTTT
CD3 δ	Enh.		TTG.....CAA	TOPO II		Consensus	.T.........T...			990 to 983	....
Polyoma	Enh.		.........TG							971 to 964	......
SV40	Enh.		TTT.....C..	TcRγ	Enh.	446 to 428	CGAAAGCGAGCAAAAACTG			-13 to -19	....
Moloney	MLV		TT.......AT	NFAT		Consensus	A.....*...G........	MAR		Consensus	....

FIG. 4. Sequence of the 1-kb *Bgl* II–*Hin*dIII fragment containing the γ enhancer with sequence comparisons. (*A*) Underlined sequences indicate regions protected from DNase I by nuclear proteins. Thick overlined sequences indicate 12-bp repeats, and the overlined checkered boxes indicate 11-bp "core" repeats. Sequences displaying homology to the previously described AP-2, NFAT, and MAR binding motifs are labeled (23–25). (*B*) Sequence similarities with other enhancers. In addition to those cited in the text, similarities to the AP-2 binding site (23), the Igκ 3′ enhancer (26), and the human TCR α enhancer (Tα5) (27) are presented. The NFAT and MAR consensus motifs are listed. Dots indicate identity, and the asterisk indicates the absence of a base. Enh., enhancer; TOPO, topoisomerase.

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