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A Transcriptional Enhancer 3′ of $C_{\beta 2}$ in the T Cell Receptor β Locus

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Run-on transcription experiments were used to demonstrate that transcription of T cell receptor β chain V genes is activated by DNA rearrangement, in a manner similar to immunoglobulin genes. A transcriptional enhancer likely to be involved in this activation has been identified. A 25-kilobase region from $J_{\beta 1}$ to $V_{\beta 14}$ was tested for enhancer activity by transient transfections, and an enhancer was found 7.5 kilobases 3′ of $C_{\beta 2}$. The β enhancer has low activity relative to the simian virus 40 viral enhancer, does not display a preference for V_β promoters, has a T cell–specific activity, and binds two purified immunoglobulin heavy chain enhancer factors.

GENE PRODUCTS OF THE TWO MOST closely related members of the immunoglobulin gene superfamily, immunoglobulin (Ig) genes and T cell receptor (TCR) genes, perform the antigen recognition function in the humoral and cellular immune responses, respectively (*1, 2*). When Ig genes undergo joining of the variable (V), diversity (D), and joining (J) gene segments a transcriptional enhancer located between the J and constant (C) segments is brought within functional proximity of the rearranged V gene promoter and activates transcription (*3*). Like the Ig heavy chain locus, the TCR β locus is composed of V, D, J, and C gene segments (*4, 5*). Since TCR β VDJ joining is similar to Ig VDJ joining, it seemed possible that an enhancer might be located between TCR J_β and C_β gene segments that would activate rearranged V_β promoters. This possibility is strengthened by the recent report of an enhancer in the TCR α gene locus (*6*). However, it may be that TCR genes are never transcribed at as high a rate as Ig genes and an enhancer is not necessary for TCR β chain gene regulation. If so, TCR V_β promoters would be constitutively active and T cell–specific VDJ joining would be sufficient to ensure T cell–specific expression.

To distinguish these possibilities experimentally, we used run-on transcription in isolated nuclei, which quantitates polymerase loading on a particular region of DNA regardless of subsequent processing or degradation of the transcript (*7*). In initial experiments BO4H.H.9.1 was used. This hybridoma has rearranged its β-chain genes and expresses $V_{\beta 3}$ (*8*); genomic DNA blots showed that unrearranged $V_{\beta 1}$ was present (*9*). Quantitation of two experiments (Table 1) confirms that $V_{\beta 3}$ and $C_{\beta 1}$ are transcribed at similar levels but that transcription from the unrearranged $V_{\beta 1}$ gene is undetectable. Similarly, in the T lymphoma cell line SL3, which by RNA blot analysis expresses functional β mRNA but not $V_{\beta 3}$ (*9*), there is only a low level of transcription detected from the unrearranged $V_{\beta 3}$ gene, a level substantially below the $C_{\beta 1}$ level in this cell line (Table 1). Low transcription of $V_{\beta 3}$ may reflect enhancer-independent activity that precedes VDJ joining in the IgH locus (*10*). The unrearranged $V_{\beta 3}$ gene was shown to be transcriptionally silent in the NIH 3T3 fibroblasts as well as in the plasmacytomas S107 and P3X63-Ag8, although appropriate controls were positive (Table 1). Therefore, the run-on transcription experiments indicate the following. (i) Although there may be a low level of transcription from unrearranged V_β genes in some T cells, rearrangement is necessary to activate transcription fully. (ii) Unrearranged V_β gene segments are transcriptionally silent in non-T cells. Thus we searched the β locus for a transcriptional enhancer.

We considered it possible that a putative β chain enhancer might act preferentially on V_β promoters in a manner analogous to the Ig κ enhancer (*11*). Therefore, two vectors were used for this study: (i) pA10CAT2, which contains the SV40 early promoter 5′ of the chloramphenicol acetyl transferase (CAT) gene but no enhancer (*12*), and (ii) pV_βCAT, which contains the $V_{\beta 3}$ gene promoter 5′ of the CAT gene and no enhancer (*13*). The entire region from $D_{\beta 1}$ to $V_{\beta 14}$ was subcloned into one or both vectors and tested by transient transfection into T cells for enhancer activity. Both orientations of most inserts were tested. We found that the efficiency of T cell transfection was 15 times higher when electroporation was used rather than DEAE dextran (*9*); thus all the results presented here were obtained from electroporation of EL4 T cells (*14*). To correct for differences in transfection efficiency we cotransfected a plasmid expressing β-galactosidase (*15*).

There was no detectable enhancer activity in pA10CAT2 constructs in the region between $J_{\beta 1}$ and $C_{\beta 1}$ (Fig. 1A). Since a T cell–specific deoxyribonuclease (DNase) I hypersensitive site has been reported in the $J_{\beta 2}$ to $C_{\beta 2}$ intron (*16*) and enhancer regions are usually hypersensitive to DNase I, we tested this region by using both vectors. No enhancer activity was detected in any of our constructs after multiple transfections. Three additional T cell lines were also transfected with constructs containing this re-

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gion, but enhancer activity was not detected (*9*).

Finally, we tested the 10-kb region between $C_{\beta 2}$ and $V_{\beta 14}$. Different portions of the region were cloned, in both orientations, into $pV_\beta CAT$ as well as pA10CAT2, and several constructs showed enhancer activity (Figs. 1B and 2). Although this activity was only about 10% of the positive control, pSV2CAT, it is evident that significant and linear CAT activity was present (Figs. 1B and 2 and Table 2). The smallest fragment that contains enhancer activity is an 800-bp Pvu II–Nco I fragment (TCR 11 and 12); larger constructs that contain this region show activity (TCR 1, 2, 3, 6, 9, and 10), although those lacking it do not (TCR 4, 5, 7, and 8). Both promoters were enhanced by the same fragments. The maximum activity detected from this region is approximately 14% of pSV2CAT activity. Constructs of $pV_\beta CAT$ containing either the SV40 enhancer or β enhancer are less active than pA10CAT2 constructs, probably reflecting the relative strengths of the $V_{\beta 3}$ and SV40 promoters.

It seems reasonable to expect that the β enhancer is involved in the activation of V_β transcription, which accompanies VDJ joining. The V_β genes arrange to either the first or second DJ clusters; however, because the enhancer is located 3′ of $C_{\beta 2}$, it is not deleted by either rearrangement and it could activate transcription from V_β gene segments that rearrange to either $D_{\beta 1}J_{\beta 1}$ or $D_{\beta 2}J_{\beta 2}$. Given its location, the enhancer must be able to work over a large distance, about 12 kb for $D_{\beta 2}J_{\beta 2}$ or about 20 kb for $D_{\beta 1}J_{\beta 1}$ rearrangements. Since the IgH enhancer can activate IgH promoters located as far away as 17.5 kb (*17*), it is not unreasonable to expect that the TCR β enhancer can act over similar distances. Our results do not rule out the possibility that changes of transcription factors may be required, in addition to VDJ joining, to activate β chain transcription, a possibility suggested by a report of trans-acting negative regulators of

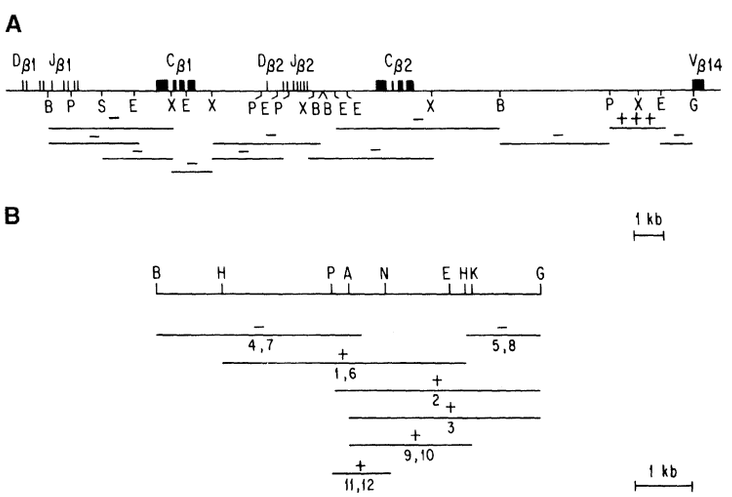

Fig. 1. Analysis of the β chain locus for a transcriptional enhancer. (**A**) Map of the 25-kb region analyzed for enhancer activity. The lines depict fragments tested for enhancer activity; plus signs denote strong enhancer activity as determined by CAT activity; minus signs denote lack of activity. (**B**) Enlarged map of the 7-kb region in which strong enhancer activity was found. Clones 1 to 5, 9, and 11 use the SV40 promoter and clones 6 to 8, 10, and 12 use the $V_{\beta 3}$ promoter (*12, 13*). Restriction fragments were cloned 3′ of the CAT gene into either the Bam HI or Xba I sites of pA10CAT2 (*12*) or the Xba I or Hind III sites of $pV_\beta CAT$ (*13*) to test for enhancer activity. Both orientations of most constructs were tested, giving the same results. The restriction enzymes used were A, Hpa I; B, Bam HI; E, Eco RI; G, Bgl I; H, Hind III; K, Kpn I; P, Pvu II; S, Sst I; and X, Xba I.

Table 1. Radioactivity obtained from run-on synthesis in isolated nuclei. Each number represents the average radioactivity on duplicate dot filters, as determined by scintillation counting. The counts per minute have been corrected for the plasmid background and normalized for the length of the probe. Linearized DNA probes (5 μg) were applied to nitrocellulose and hybridized with 20×10^6 to 100×10^6 cpm of labeled RNA synthesized in nuclei from approximately 10^8 nuclei (*17*). Since $C_{\beta 1}$ and $C_{\beta 2}$ have 96% homology (*4*), the $C_{\beta 1}$ probe hybridizes to transcripts from either gene segment. The B cell P3X/63-Ag8 does not secrete heavy chain, which presumably accounts for the observed low level of IgH transcription. Abbreviations: β_2M, β 2-microglobulin; GAPDH, glyceraldhyde 3-phosphate dehydrogenase; $C_{\beta 1}$, $V_{\beta 1}$, and $V_{\beta 3}$ are the respective genes; V_{H14B}, heavy chain V_{14b} gene; IGHE, IgH enhancer; and ND, not determined.

	DNA probes (cpm)						
	$V_{\beta 3}$	$V_{\beta 1}$	$C_{\beta 1}$	β_2M	GAPDH	V_{H14B}	IGHE
Labeled RNA from:							
T cells							
BO4H.H.9.1	95	9	104	146	434	ND	ND
	30	0	31	91	203	ND	ND
SL3	11	ND	61	62	55	0	ND
	27	ND	73	78	ND	0	ND
Non-T cells							
NIH 3T3	0	ND	0	69	55	0	ND
	0	ND	0	63	35	0	ND
S107	0	ND	0	89	423	0	381
P3X63-Ag8	0	ND	ND	129	149	0	11

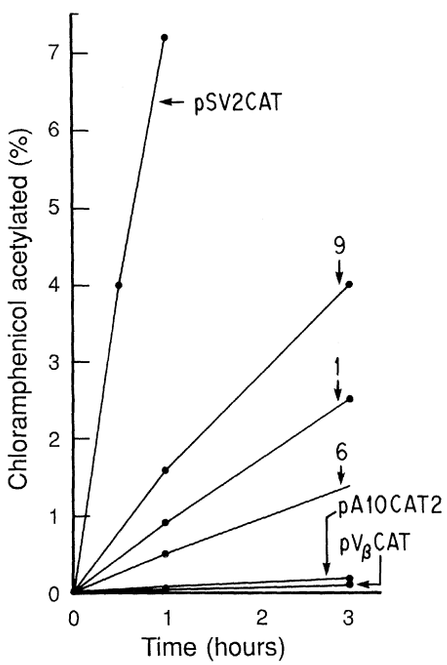

Fig. 2. CAT enzyme activity in transfected T cells. The EL4 cells were transfected by electroporation (*14*). Cells (2×10^7) in 0.4 ml of HBS (140 m*M* NaCl, 0.75 m*M* Na_2HPO_4, 25 m*M* Hepes, *p*H 7.1) and sheared salmon sperm DNA (500 μg/ml) were transfected with equimolar amounts of the supercoiled plasmid constructs corresponding to 30 μg of a 4.9-kb plasmid. The transfection efficiency was normalized by cotransfecting with 50 μg of a β-galactosidase–expressing plasmid, pCH110 (*15*). An ISCO 494 power supply was used, set to 2.0 kV and minimum current and wattage. The cells were harvested 42 hours after transfection and divided in half; half were assayed for CAT activity (*26*) and half for β-galactosidase activity (*15*). A representative CAT assay was counted, and the percentage of [^{14}C]chloramphenicol converted to the acetylated form was plotted as a function of time. Construct pSV2CAT was assayed for 0 to 1 hour; constructs pA10CAT2, $pV_\beta CAT$, 1, 6, and 9 were assayed for 0 to 3 hours.

TCR β transcription in some T cell lines (*18*).

The $V_{\beta 3}$ promoter did not display T cell specificity in our assays; when it was coupled with the non-T cell–specific SV40 enhancer, the activity was similar in T, B, and L cells (Table 2). In contrast, the TCR β enhancer was strictly T cell–specific; when it was coupled with the non-T cell–specific SV40 promoter, significant activity was seen only in T cells. Thus the β chain enhancer appears to be more tissue specific than the IgH or TCR α enhancers since these elements are active in T as well as B cells (*6, 19*). Relative to the SV40 promoter and enhancer elements, both the TCR $V_{\beta 3}$ promoter and the TCR β enhancer had weak activity. The low activity of the $V_{\beta 3}$ promoter was poorly rescued with the strong SV40 enhancer in all cells, suggesting that V_{β} promoters may not be capable of high levels of transcription.

To determine if proteins that are known to be necessary for IgH enhancer function also bind to the β enhancer, we used affinity-purified proteins in a series of gel shift analyses. Three nuclear proteins, octamer-binding factor (*20, 21*), u-EBP-E (*21, 22*), and u-EBP-C2 (*21, 22*), were tested for binding to seven probes spanning the 800-bp Pvu II–Nco I fragment (Fig. 3). Results are shown for three probes; protein binding was observed for two probes (Fig. 3); other fragments did not bind any of the proteins tested. Octamer-binding factor, which is required for both IgH enhancer and promoter function, did not bind to the β enhancer in the region tested. However, u-EBP-E and u-EBP-C2 were able to bind to the β enhancer (Fig. 3). The protein u-EBP-E has one high-affinity site and one low-affinity site relative to the IgH enhancer control, and u-EBP-C2 has one high-affinity site (Fig. 3). Site-directed mutagenesis will be necessary to demonstrate formally that uEBP-E and uEBP-C2 are required for β enhancer function. However, since these proteins are required for IgH function, it seems likely that they may also be important for β enhancer function, suggesting some conservation in the mechanism of action of the two enhancers.

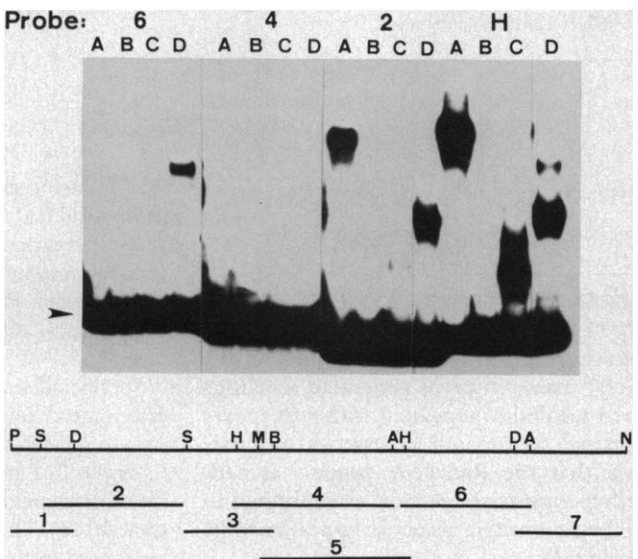

Fig. 3. Gel retardation assay with purified Ig heavy chain enhancer factors. The gel retardation assays were performed essentially as described in (*22*). Approximately 0.35 ng of affinity-purified proteins (*21*) and 200 ng of the non-specific competitor poly[d(I-C) · d(I-C)] were used. The probe numbers correspond to the map below. Probe 2 is 190 bp, 4 is 210 bp, and 6 is 160 bp; H is a 200-bp Hinf I control probe from the Ig heavy chain enhancer that binds octamer-binding factor, u-EBP-E, and u-EBP-C2 (*22*). The arrowhead indicates the position of free probe. Lanes labeled A have u-EBP-C2, B have no added protein, C have octamer-binding factor, and D have u-EBP-E. The restriction sites shown on the 800-bp Pvu II–Nco I fragment are not complete; only those used to generate the end-labeled probes are shown. The restriction enzymes used were A, Alu; B, Mbo II; D, Dde I; H, Hinf I; M, Mse I; N, Nco I; P, Pvu II; and S, Sau 3A.

Table 2. TCR β enhancer activity and tissue specificity in T, B, and L cells. Results from multiple experiments (*n* shown in parentheses) were corrected for transfection efficiency and presented as the mean ± SD; when the construct was transfected two times, the result is given for both transfections. The enhancer numbers correspond to the constructs in Fig. 1B. The construct pV_{β}CATSVE has the SV40 enhancer cloned 3′ of the CAT gene in pV_{β}CAT. The P3X63-Ag8 and L cells were transfected by calcium phosphate coprecipitation (*24*). To normalize for transfection efficiency in EL-4 and P3X63-Ag8 cells, cotransfection of pCH110 and β-galactosidase assays were used (*15*); in L cells, cotransfection of a human growth hormone–expressing plasmid and an ELISA assay for growth hormone were used (*25*).

Promoter	Enhancer	Percentage of pSV2CAT activity		
		T	B	L
SV	TCR 4	0 (6)		
SV	TCR 7	0 (4)		
SV	TCR 5	0 (2)		
TCR	TCR 8	0 (3)		
SV	TCR 2	5.4 ± 3.4 (6)		
SV	TCR 3	9.0 ± 2.2 (3)	0, 0.9 (2)	0,0 (2)
SV	TCR 1	9.0 ± 2.1 (3)	0, 1.4 (2)	
TCR	TCR 6	2.7 ± 1.5 (3)	0, 0 (2)	
SV	TCR 9	13.7 ± 3.6 (4)		0,0 (2)
TCR	TCR 10	1.6 ± 0.6 (5)		
SV	TCR 11	4.4 ± 0.8 (4)		
TCR	TCR 12	2.9 ± 0.7 (4)		
TCR	SV	3.4 ± 1.0 (6)	2.5, 4.5 (2)	2.7, 10.3 (2)

The Ig and TCR genes are members of the Ig supergene family and are thought to be closely related in evolution through duplication and divergence of primordial V-C gene regions (*1*). The presence of enhancers in Ig and TCR loci suggests that it is evolutionarily important for these genes, which generate antigen-recognition diversity through combinatorial mechanisms depending on DNA rearrangement, to conserve the ability to activate rearranged gene transcription. There is insufficient information to allow speculation on the genetic events responsible for the appearance of an enhancer 3′ of $C_{\beta 2}$ at a location dissimilar from enhancers in Ig and TCR α loci. The presence of the $V_{\beta 14}$ gene segment 3′ of $C_{\beta 2}$ and the enhancer indicates that this region may have experienced an unusual level of DNA inversions or other rearrangements. However, the location of the β enhancer is particularly important in an evolutionary context because it demonstrates that even if enhancer activity in the J-C region is lost during evolution, there must be strong pressure for an enhancer to activate rearranged genes.

Note added in proof: An enhancer in the β chain gene has also been recently reported by Krimpenfort *et al.* (*23*).

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Cell-Autonomous Recognition of the Rust Pathogen Determines *Rp1*-Specified Resistance in Maize

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The *Rp1* gene of maize determines resistance to the leaf rust pathogen *Puccinia sorghi*. X-ray treatment of heterozygous (*Rp1 Oy/rp1 oy*) maize embryos generated seedlings with yellow sectors lacking *Rp1*. Yellow sectored seedlings inoculated with rust spores gave rust pustule formation in yellow (*Rp1*-lacking) sectors and hypersensitive resistance in green tissues, thereby demonstrating that the *Rp1* gene product is cell-autonomous in its action. In cases where the hypersensitive reaction was initiated in green (*Rp1*) tissue next to a yellow sector, the hypersensitive response appeared to be propagated poorly, if at all, through *Rp1*-lacking cells.

NUMEROUS GENES PROVIDING resistance to plant pathogenic microorganisms have been identified in a wide variety of plant species. Many of these disease resistance genes are dominant and specify plant resistance to a particular race or races of a specific pathogen (*1*). Often, the resistance phenotype is associated with the hypersensitive response (HR), a local necrosis of plant tissue initiated by and surrounding the site of pathogen contact with the plant host (*2*). This localized cell death presumably isolates the pathogen from host nutrients and is associated with the release of various toxic compounds from the dying plant cells (*2*). Initiation of the hypersensitive response by the infected plant is generally specified by an interaction between a dominant host resistance gene and a dominant recognition factor (or "avirulence locus") in the plant pathogen (*1, 3*). The molecular nature of the events that initiate and propagate disease resistance and the hypersensitive response are not known.

The experiments described herein were designed to investigate the cell autonomy of the HR-associated disease resistance specified by the *Rp1* locus of maize. The results demonstrate that the initiation of the hypersensitive response and resistance to the leaf rust pathogen *Puccinia sorghi* require a cell-autonomous, nondiffusible factor specified by *Rp1*.

Specific alleles of the *Rp1* locus of maize determine dominant, HR-associated resistance to specific races of the fungal pathogen *P. sorghi*. *Rp1* maps 25 centimorgans from the centromere on the short arm of chromosome 10 (*4*). The allele of the oil yellow (*oy*) locus used in this study specifies a recessive yellow plant color trait (*5*) and maps approximately midway between the centromere and *Rp1* on the short arm of chromosome 10. Chromosome breakage between *Oy* and the centromere in developing embryos with the genotype *Rp1 Oy/rp1 oy* should uncover the recessive *oy* phenotype

Table 1. Sector analysis in 2215 21-day-old seedlings from x-irradiated *Rpl Oy/rpl oy* embryos. The median size of yellow sectors on the first to third leaves is given as a fraction of leaf size. DAP, days after pollination.

Irradiated ear	Time of irradiation (DAP)	Median yellow sector size	Number of seedlings with			
			Yellow sectors per total screened	Pustules in yellow sectors	HR in yellow sectors	Pustules in green tissue
1	5	1/16	2/151	0	0	0
2	6	1/32	2/66	1	0	0
3	7	1/16	11/360	3	0	0
4	7	1/16	13/337	2	1	0
5	9	1/32	8/88	0	0	0
6	9	1/32	16/253	1	0	0
7	10	1/32	21/459	1	0	0
8	12	1/16	11/199	0	0	1*
9	12	1/32	24/302	1	0	0

*Fully susceptible seedling.

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