Active T-Cell Receptor Genes Have Intron Deoxyribonuclease Hypersensitive Sites

Ethan Bier, Yasuhiro Hashimoto, Mark I. Greene, Allan M. Maxam

Abstract. *The T-cell receptor β-chain gene has a nuclease hypersensitive site in several kinds of T cells, which does not appear in B cells expressing immunoglobulins. Conversely, the κ immunoglobulin gene shows a known hypersensitive site at its enhancer element in B cells, as expected, but this site is absent in T cells. As is the case with immunoglobulin genes, the T-cell receptor site lies within the gene, in the intron separating joining and constant region segments. These nuclease hypersensitive DNA configurations in the introns of active T-cell receptor and immunoglobulin genes may arise from control elements that share ancestry but have diverged to the extent that each normally acts only in lymphoid cells which use the proximal gene product.*

Immunoglobulin genes have lymphocyte-specific transcription enhancers (*1–3*) and nuclease hypersensitive sites (*4–7*) in the introns separating segments that encode joining (J) and constant (C) regions. As in other developmentally regulated genes, these immunoglobulin hypersensitive sites correlate with actual or potential gene expression in differentiating cells. In at least one B lineage cell, a signal from the surface induces a deoxyribonuclease I (DNase I) hypersensitive site in the κ immunoglobulin gene (Igκ). Treatment of the pre–B cell line 70Z/3 with bacterial lipopolysaccharide (LPS), a B-lymphocyte mitogen, induces both a DNase I hypersensitive site in the JC intron of the κ light-chain gene and transcription of that gene (*4*). This LPS-inducible DNase I site is tightly linked to the lymphoid κ-enhancer element (*3*). A lymphoid enhancer also resides in the JC intron of the murine immunoglobulin heavy-chain gene (IgH) (*1, 2*), and there is a DNase I hypersensitive site in the putative human IgH enhancer region in a human B cell (*7*).

T-cell receptor genes, like immunoglobulin genes, are assembled from clusters of germline V (variable), D (diversity), and JC DNA segments (*8–11*). This structural similarity of the two lymphocyte gene systems, plus the marked developmental relatedness of B cells and T cells, suggests that sequences controlling immunoglobulin and T-cell receptor genes could also be related. Accordingly, we planned experiments to ascertain whether the expression of T-cell receptors, like immunoglobulins, involves elements in JC introns. Because of the implications for lymphoid development, it was important to know whether T-cell receptor control elements (wherever they are located) are normally active only in T cells and immunoglobulin elements are active only in B cells, or if both are active in lymphocytes generally.

Nuclease hypersensitive sites. Changes in local chromatin structure often reflect the developmental regulation of genes expressed in tissue-specific patterns (*12*). A common alteration of a gene undergoing developmental activation is the appearance of sites sensitive to certain endonucleases in the immediate vicinity of that gene in chromatin (*4–7, 13–16*). Although the physical basis and biological meaning of nuclease hypersensitive sites are not well understood (*17*), it has been proposed that such sites are in or nearby DNA regulatory elements interacting with tissue-specific gene control factors (*18, 19*). Some sequences that contain transcriptional enhancers encompass nuclease sites that are strongly correlated with expression of adjacent genes (*1–4, 7, 20*). As noted above, LPS induces both a DNase I site at the κ enhancer and transcription of the κ gene in 70Z/3 pre-B cells. In a few cases, mutations that occur very close to expression-associated sites reduce or eliminate transcription of the gene and also abolish the hypersensitivity of the sites (*14, 20, 21*).

Nuclease hypersensitive sites near any gene for which one has a probe can be detected by treating purified nuclei from a tissue with DNase I at concentrations that cleave chromosomal DNA about once every 10 to 20 kilobases (kb) (*13–15*). When DNA from lightly digested nuclei is purified, cut with an appropriate restriction enzyme, subjected to electrophoresis, blotted (*22*), and then hybridized with a cloned specific sequence probe, a DNase I hypersensitive site is revealed by a subband beneath the corresponding unbroken restriction fragment. The location of such a hypersensitive site can be mapped after double digestion with restriction enzymes. We examined the β T-cell receptor and κ immunoglobulin genes for DNase I hypersensitive sites in mouse lymphoid cells that do and do not express each of these genes. Many of these experiments involved a β_2 T-cell receptor gene in the T lymphoma BW5147 (*23*) and the κ immunoglobulin genes of the pre-B cell line 70Z/3 (*4*) (Fig. 1).

The T-cell receptor gene in T cells. Initially, we looked at the DNase I sensitivity of the β_2 T-cell receptor gene region in the BW5147 T lymphoma and in a reovirus-specific cytolytic T-cell hybridoma 87-4, for which BW5147 was the fusion partner (*24*). Nuclei were prepared from BW5147 and 87-4 and digested lightly with DNase I (*13–15*), and the DNA was isolated and purified. These DNA preparations were then cut with Pvu II, blotted (*22*), hybridized with a probe for the β_2 constant region ($C_{\beta 2}$), and the nitrocellulose membrane was processed for autoradiography.

Two bands are present in the BW5147 lanes—a 6.5-kb Pvu II fragment carrying the single rearranged β_2 JC region ($JC_{\beta 2}$), and a weaker 4.3-kb fragment below. In the 87-4 hybridoma lanes, there are three bands—the 6.5-kb BW5147 $JC_{\beta 2}$ fragment, a 5.6-kb Pvu II fragment bearing a rearranged $JC_{\beta 2}$ allele contributed by the fusion partner, and the same 4.3-kb frag-

Ethan Bier is a graduate student in the Cell and Developmental Biology Program at Harvard Medical School and the Dana-Farber Cancer Institute, Boston, Massachusetts 02115. Yasuhiro Hashimoto is a postdoctoral fellow in the Department of Pathology, Harvard Medical School and the Dana-Farber Cancer Institute, Boston, Massachusetts 02115. Mark I. Greene is a professor in the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111. Allan M. Maxam is an assistant professor in the Department of Biological Chemistry and Committee on Cell and Developmental Biology, Harvard Medical School and the Dana-Farber Cancer Institute, Boston, Massachusetts 02115.

ment below. In general, as the DNase I concentration increases in the BW5147 and 87-4 experiments (Fig. 2), the upper bands grow weaker and the lower one becomes more intense. We conclude that this lower 4.3-kb fragment arises from DNase I cleavage at a hypersensitive site in the β_2 T-receptor gene of these T cells. This fragment is produced by a DNase I cut at one end and by Pvu II at the other, and we refer to it as the DP1 subfragment (Fig. 1).

To ascertain whether the hypersensitive site just described is an idiosyncrasy of BW5147 and the related hybridoma 87-4 or is a general feature of β_2 genes in T cells, we performed the same experiment on several T-cell lines of unrelated origin. These include EL-4 (*25*), YAC-1 (*26*), and a radiation-induced thymoma (Rad-T) (*27*) (Table 1). Treatment of nuclei with DNase I followed by Southern blotting gave the patterns displayed in Fig. 3. Thus, all of these T cells exhibit the 4.3-kb DP1 subfragment seen in BW5147 and hybridoma 87-4.

Further indication that the DP1 subfragment comes from an expression-linked hypersensitive site was obtained with thymocytes activated by 24-hour incubation in the presence of concanavalin A (con A) and interleukin-2 (IL-2) (*28*). When nuclei from such thymocytes are treated with DNase I and the DNA is analyzed by Southern blotting as before, a light subband appears at the same position as that in BW5147 (Fig. 2). In a control experiment on thymocytes cultured for 24 hours without exogenous T-cell activating factors, the band is not visible, revealing con A or IL-2 (or both) dependence. Also, con A plus IL-2 induced a small increment in the level of $C_{\beta 2}$ region transcription in these thymocytes (*29*). The thymocyte DP1 subfragment in Fig. 2 is considerably weaker than those found in BW5147 or 87-4. This may be so for any of several reasons. Less DNA (60 percent) was loaded on the gel in the thymocyte lanes than in the BW5147 or 87-4 lanes. Only a fraction of the total thymocytes may be competent to induce the DNase I hypersensitive site in response to con A plus

Table 1. Characteristics of T cells and T-cell receptor genes. Regions of the β-chain locus of the T-cell receptor which are known to be germline or rearranged are shown as (---) and (...), respectively. V implies the presence of a V_β gene segment, while X indicates an unidentified segment. The sizes of expressed RNA's are entered where known. The + sign indicates that β RNA is present in about the same amount as in EL-4; the question mark (?) means that RNA expression is unknown. T-cell β-chain receptor observations from our work are indicated by (†). NA, not applicable.

Cells	β T-cell receptor genes	RNA (kb)	References		
			Cell	Genes	RNA
Nonlymphoid (germline)					
Strain, any	$D_{\beta 1}$--$J_{\beta 1}$ $C_{\beta 1}$----$D_{\beta 2}$--$J_{\beta 2}C_{\beta 2}$	None	NA	(*11–19*)	NA
Induction, NA	$D_{\beta 1}$--$J_{\beta 1}$ $C_{\beta 1}$----$D_{\beta 2}$--$J_{\beta 2}C_{\beta 2}$	None			
BW5147 T-lymphoma					
Strain, AKR	.....$XJ_{\beta 2}C_{\beta 2}$	1.0	(*23*)	†	†
Induction, spontaneous					
87-4 Tc hybridoma					
Strain, AKR × C3H/Hej	.....$VD_nJ_{\beta 2}C_{\beta 2}$	1.3	(*24*)	†	†
Induction, BW5147 fusion	.....$XJ_{\beta 2}C_{\beta 2}$	1.0			
EL-4 T-lymphoma					
Strain, C57BL/6	.....$VD_nJ_{\beta 2}C_{\beta 2}$	1.3	(*25*)	(*31*)	(*31*)
Induction, carcinogen	.....$D_{\beta 1}J_{\beta 1}C_{\beta 1}$----$D_{\beta 2}$--$J_{\beta 2}C_{\beta 2}$	None			
YAC-1 T-lymphoma					
Strain, A/Sn	.....$XJ_{\beta 2}C_{\beta 2}$	+	(*26*)	†	†
Induction, Moloney MuLV	.....$XJ_{\beta 2}D_{\beta 2}$	?			
RAD-T T-lymphoma					
Strain, B.10A(3R)	.....$XJ_{\beta 2}C_{\beta 2}$	+	(*27*)	†	†
Induction, RadLV	.....$XJ_{\beta 2}C_{\beta 2}$	?			

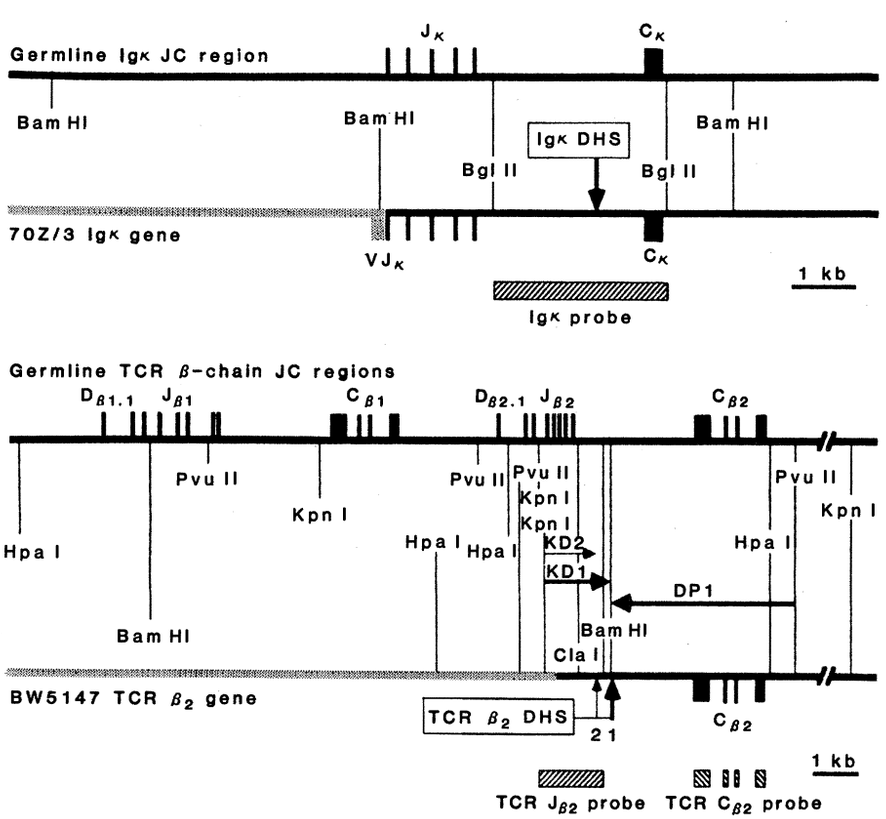

Fig. 1. Maps of the mouse κ-immunoglobulin gene and T-cell receptor β-chain genes. Solid horizontal lines indicate germline configurations and stippled lines represent other sequences brought in by rearrangements. (Top) Configurations of the germline and rearranged κ-gene alleles present in the murine pre-B-cell tumor cell line 70Z/3 (*4*). (Bottom) The T-cell receptor β-chain genes as they are arranged in the mouse germline and in BW5147. Most of the map of the germline β locus was compiled from published maps (*11*). Southern blots (some not shown) were used to map the leftmost Hpa I, Pvu II, and Kpn I sites of the rearranged BW5147 β_2 gene of the C region ($C_{\beta 2}$). The BW5147 rearrangement breakpoint is located somewhere between the germline Pvu II and Cla I sites. The identification and placement of a second Bam HI site in the $JC_{\beta 2}$ intron was achieved by fine structure mapping of a pUC9 subclone (pCl-2) of the Hind III genomic restriction fragment that carries the germline $J_{\beta 2}$ region cloned from BALB/c liver cells (*34*). These two Bam HI sites are separated by 150 bp.

IL-2. Finally, only a fraction of thymocytes may harbor a rearranged β_2 T-receptor gene (other thymic cells could express β_1, which is less evident under our hybridization conditions, and yield a β_1 subband of a different size from that of the β_2 DP1 band).

Location of DNase I hypersensitive sites in the JC intron. According to restriction maps (*11*), a 6.1-kb germline Pvu II fragment carries most of the $JC_{\beta 2}$ gene region (Fig. 1). Although some of the J region at the left end of this fragment has been replaced in the BW5147 lymphoma and 87-4 hybridoma, the JC intron in the middle and C region at the right are unchanged. We first mapped Pvu II, Hpa I, and Kpn I sites in the rearranged end of the BW5147 β_2 gene (legend to Fig. 1). Then, knowing these BW5147 sites on the left and germline sites in the unrearranged intron and the constant region, we used double digestion with Pvu II and several other restriction enzymes to map the DNase I hypersensitive site (Fig. 4). These mapping experiments on DNA from DNase I treated nuclei position the DNase I site very near the 3′ member of a pair of Bam HI sites in the JC intron of the β_2 gene (Fig. 1).

Close examination of Fig. 2 reveals another, faint DNase I generated fragment (most noticeable in 87-4 lanes) migrating a little behind the DP1 subfragment. This suggests that a second weaker DNase I hypersensitive site exists just 5′ to the DP1 cleavage site. To map DNase I hypersensitive sites in this region more precisely, Pvu II and Kpn I digests of DNase I–treated BW5147 DNA were blotted (*22*) and hybridized with a probe from the $J_{\beta 2}$ region (Fig. 1). Two DNase I subfragments separated by 0.4 kb then appeared in BW5147 with both Pvu II and Kpn I (Fig. 5). The larger and darker of the pair of DNase I subbands is due to DNase I cleavage at the same site that yields the DP1 fragment. Thus, the large Kpn I–DNase I subfragment seen with the $J_{\beta 2}$ probe (hereafter referred to as the KD1 subfragment) is roughly 150 bp longer than the BW5147 Kpn I–Bam HI double digest restriction fragment (Fig. 5). This observation is consistent with the center of the strong $JC_{\beta 2}$ DNase I hypersensitive site being at the most 3′ of the two β_2 intron Bam HI sites (Fig. 1). The center of the smaller and more diffuse DNase I subband seen in Southern blots of Kpn I–digested BW5147 DNA (hereafter referred to as the KD2 subfragment) is about 400 bp 5′ to the KD1 site (legend to Fig. 5). Resolution of the DNase I hypersensitive domain in the $JC_{\beta 2}$ intron into two nearby sites with the $J_{\beta 2}$ region probe may be attributable to two factors. If a strong DNase I hypersensitive site resides between a weak site and the probe in a Southern blot (such as the T-cell DNA samples that were digested with Pvu II and probed with $C_{\beta 2}$), the weak site will be unobserved if cleavage at the strong proximal site has also occurred (*30*). The $J_{\beta 2}$ probe is nearest to the weak DNase I site and therefore reveals the full magnitude of that site. The smaller size of the DNase I generated subfragments as seen with the $J_{\beta 2}$ probe also makes resolution of the small distance between the two sites easier.

Just as the $C_{\beta 2}$ probe detected the DP1 subfragment in several other T cells (Fig. 3), the $J_{\beta 2}$ probe also detects KD1 and KD2 in EL-4 and Rad-T (*29*). The EL-4 cell contains two different β_2 alleles; one that has an unrearranged $J_{\beta 2}$ region (this allele does, however, carry a joined $DJ_{\beta 1}$ region), and an allele with a rearrangement to $J_{\beta 2}$, which is presumed to be transcribed to yield a full-sized β_2 messenger RNA (mRNA) (*31*). Both EL-4 alleles are DNase I hypersensitive in the $JC_{\beta 2}$ intron (*29*) although no transcripts have been detected from the allele that retains the $J_{\beta 2}$ germline configuration (*31*).

Developmental specificity of "immunogene" DNase I sites in lymphocytes. T cells and B cells are much closer to each other developmentally than they are to other tissues. Most tissues differ one from the next by 10 to 15 percent of the total mRNA complexity, whereas T cells are separated from B cells by only a 2 percent mRNA difference (*8*). Developmental specificity of DNase I hypersensitive sites in "immunogenes" (genes that rearrange to specify antigen recognition) can be addressed by examining

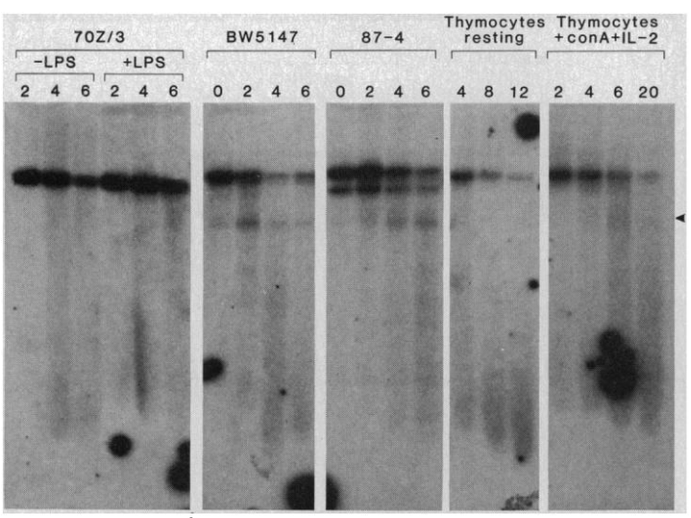

Fig. 2. DNase I hypersensitivity of the T-cell receptor β-chain gene in T cells and B cells. The C_β region was tested for DNase I sensitivity in a pre-B cell (70Z/3 ± LPS stimulation) (*47*) and in four kinds of T cells (BW5147 lymphoma, 87-4 hybridoma, resting thymocytes, and thymocytes stimulated with con A plus IL-2). Nuclei from each of these cells were treated with DNase I at several concentrations from 0 to 20 unit/ml, as indicated. Then the DNA was purified, cut with a restriction enzyme, subjected to electrophoresis, blotted (Southern), hybridized with a cloned T-cell receptor probe, and autoradiographed. The intense upper bands present in all lanes are JC_β region Pvu II restriction fragments (germline $JC_{\beta 1}$ and $JC_{\beta 2}$ in 70Z/3, rearranged $JC_{\beta 2}$ in BW5147, two different rearranged $JC_{\beta 2}$'s in 87-4, and germline $JC_{\beta 1}$ and $JC_{\beta 2}$ in thymocytes) (Fig. 1). DNase I cleavage of these Pvu II restriction fragments at a hypersensitive site produces the lower weak band in BW5147, 87-4, and con A plus IL-2 thymocyte lanes. The weak subband appearing in the absence of added DNase I is likely to be the result of an endogenous nuclease activity which, like DNase I, preferentially cleaves at this site in nuclei. The undigested β_2 Pvu II restriction fragment in BW5147 is 6.5 kb in length, and the corresponding fragments in 87-4 are 6.5 and 5.6 kb long. The 70Z/3 cells were grown with and without LPS (*4*). Resting thymocytes and con A plus IL-2 activated thymocytes were incubated for 24 hours in RPMI plus fetal calf serum (5 percent) in the absence or presence of con A (2 μg/10^6 cells) plus culture supernatant (20 percent) from con A–stimulated rat spleen cells as in (*28*). The 87-4 cells were grown (at 10 percent CO_2) in Dulbecco's minimum essential medium (DMEM) plus the following supplement: fetal calf serum (10 percent), penicillin and streptomycin (each at 100 units per milliliter), and glutamine (2 m*M*); nonessential amino acids (1 percent) and sodium pyruvate (1 percent) were also present. All other T-cell lines were grown in RPMI 1640 with the above supplement (at 5 percent CO_2). Cultured cell lines were grown to a density of 10^6 cells per milliliter and then harvested. The live thymocytes were separated by centrifugation through Ficoll (*48*). Nuclei were then isolated, treated with DNase I, and the DNA was purified (*4*). The purified DNA (6 μg from thymocytes and 10 μg from all other cells) was restricted with Pvu II, subjected to electrophoresis (40 V; 17 hours) through 1 percent agarose gels, and then Southern blotted (*22*); the hybridization probe used was the $C_{\beta 2}$ complementary DNA clone of mRNA expressed by the β_2 gene in the helper T-cell hybridoma B3C6 (*23, 49*).

both T cells and B cells for both immunoglobulin and T-cell receptor DNase I sites.

To determine whether T-cell receptor genes are hypersensitive in B cells, we analyzed the DNase I sensitivity of the $JC_{\beta 2}$ intron in both resting and LPS-stimulated 70Z/3 pre-B cells. [LPS induces a DNase I hypersensitive site in the κ immunoglobulin gene in 70Z/3 cells (*4*).] When the $C_{\beta 2}$ hybridization probe was used as before, these B-cell DNase I experiments produced no fragment corresponding to the T-receptor hypersensitive site (Fig. 2, 70Z/3 resting or LPS stimulated). The same result was obtained when the nitrocellulose membranes (Fig. 2) were rehybridized with the $J_{\beta 2}$ probe (*29*). The absence of any DNase I hypersensitive site in the $JC_{\beta 2}$ region of both resting and LPS-activated 70Z/3 cells indicates that conditions sufficient for induction of the κ-gene hypersensitive site are inadequate for generating β_2-gene hypersensitivity (Fig. 2). This observation, although limited to the 70Z/3 cell, forecasts that β_2 T-receptor genes lack the observed DNase I hypersensitive sites in B lymphocytes.

Since the β_2 T-cell receptor intron DNase I sites in T cells are not present in B cells, it seems natural to ask whether known B-cell immunoglobulin gene hypersensitive sites are also observable in T cells. A partial answer was provided when we looked for the κ-immunoglobulin hypersensitive site in several kinds of T cells (Fig. 6). We prepared Southern blots from DNase I–treated nuclei of T cells, as before, and hybridized them with a probe for the κ-chain JC region. As a positive control, we also analyzed the κ-chain gene in 70Z/3 pre-B cells grown with and without LPS. These experiments revealed the known DNase I hypersensitive site in both germline and rearranged 70Z/3 κ immunoglobulin genes (Fig. 6, 70Z/3 with and without LPS), but not in the κ-chain genes of T cells (Fig. 6, BW5147, 87-4, resting thymocytes, and thymocytes activated with con A and IL-2). Weak bands appear in some T-cell lanes below the germline κ-chain restriction fragment (Fig. 6). However, these minor T-cell subfragments are not the same size as the strong ones in the pre-B cell. Thus, these weak bands do not arise from DNase I cuts at the pronounced hypersensitive site in LPS-stimulated 70Z/3 cells. That major DNase I site is constitutively present at the κ enhancer in myelomas (*5, 6*), and is therefore not an idiosyncrasy of 70Z/3 pre-B cells.

Comparison of the relative amount of κ and β_2 restriction fragments remaining for each identical DNase I sample in Figs. 2 and 6 reveals that the β_2 gene in its entirety is more DNase I sensitive than the κ-gene region in T cells. Conversely, the whole κ-gene region is more DNase I sensitive in the pre-B cell line 70Z/3 than is the β_2-gene region. The greater general DNase I sensitivity of the κ gene relative to the β_2 gene is also seen in the myeloma SP2 (*32*) and a derivative hybridoma 87.92.6 (*33*) (Fig. 7). Thus, in addition to hypersensitive sites, these experiments appear to detect differences in the regional T-receptor and immunoglobulin gene nuclease sensitivities in T cells and B cells that are characteristic of active versus inactive chromatin (*5, 12*).

Relationship of DNase hypersensitive

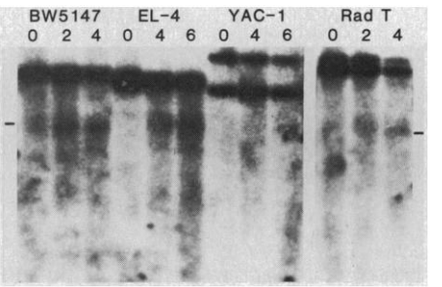

Fig. 3. DNase I hypersensitivity of the JC intron in four independent T-cell tumors. Nuclei from BW5147, EL-4, YAC-1, and Rad-T cells were prepared and treated with DNase I, and the DNA was purified as in Fig. 2. All samples were digested with Pvu II, subjected to electrophoresis through 1 percent agarose gels, blotted (*22*) and then hybridized with the $C_{\beta 2}$ probe as in Fig. 2.

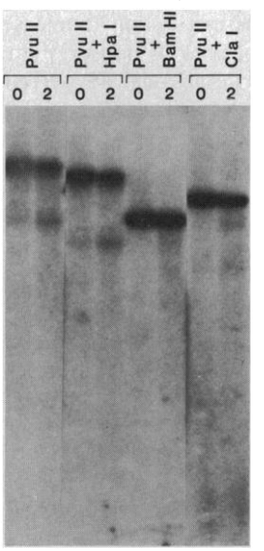

Fig. 4. Mapping the DNase I hypersensitive site in BW5147. To map the hypersensitive site defined by the DNase I–Pvu II subband in the BW5147 Southern blot of Fig. 2, DNA from DNase I–treated BW5147 nuclei was double-digested with Pvu II plus restriction enzymes that cut either in the 5′ half (Cla I and Bam HI) or the 3′ half (Hpa I) of the Pvu II fragment containing the $C_{\beta 2}$ gene. Southern blot analysis of these double digests (as in Fig. 2) with the $C_{\beta 2}$ complementary DNA clone as probe, positions the T-cell–specific DNase I hypersensitive site. Since neither Cla I nor Bam HI affects the size of the 4.3-kb DNase I–Pvu II subfragment, but Hpa I does reduce the size of that subfragment (to 3.8 kb), the DNase I hypersensitive site must be located in the 5′ half of the β_2 Pvu II fragment. The virtual comigration of the DNase I–Pvu II and the Bam HI–Pvu II fragments localizes the DNase I hypersensitive site to the vicinity of the 3′-most of the two Bam HI sites in the JC intron, as indicated on Fig. 1. The sizes of the undegraded restriction fragments are: Pvu II, 6.5 kb; Pvu II plus Hpa I, 6.0 kb; Pvu II plus Bam HI, 4.3 kb; and Pvu II plus Cla I, 5.1 kb.

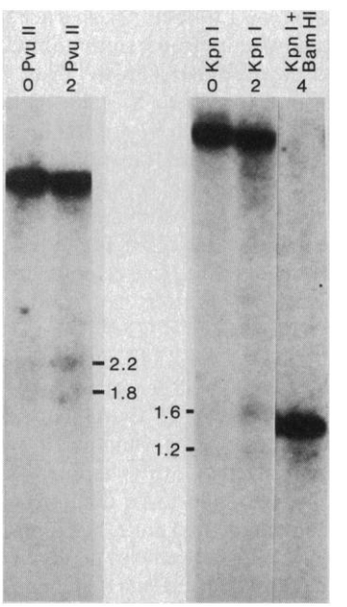

Fig. 5. Resolution of the $JC_{\beta 2}$ DNase I sites with a $J_{\beta 2}$ probe. Samples of the BW5147 DNA described in Fig. 2 were digested with Pvu II, Kpn I, or Kpn I plus Bam HI, subjected to electrophoresis through either a 1 percent agarose gel (Pvu II samples) or a 1.5 percent agarose gel (Kpn I and Kpn I–Bam HI samples), blotted (*22*), and then hybridized with the $J_{\beta 2}$ probe shown in Fig. 1. The $J_{\beta 2}$ DNA used as probe was prepared by gel isolation of the 1.6-kb Kpn I–Bam HI fragment from pCl-2. These Southern blots of BW5147 display two DNase I generated subbands with Pvu II (at 2.2 kb and 1.8 kb) and two also with Kpn I (1.6 kb represents KD1 and 1.2 kb represents KD2). Digestion of a DNase I treated BW5147 sample with Kpn I plus Bam HI yielded a 1.45-kb restriction fragment and the KD2 DNase I subfragment. As in the legend to Fig. 1, the Bam HI sites in the $JC_{\beta 2}$ intron are 150 bp apart. The KD1 fragment is therefore generated by DNase I cleavage centered at the 3′ member of the pair of intron Bam HI sites. Thus, the KD1 site is the same as the DP1 site mapped in Fig. 4. Since Southern blots of DNase I treated BW5147 digested with Bam HI alone do not reveal a 1.2-kb DNase I subband (*29*), the 1.2-kb Kpn I–DNase I fragment cannot be the result of a possible DNase I to DNase I cleavage between two DNase I sites spaced 1.2 kb apart. Thus, the center of the KD2 site must lie about 400 bp 5′ to the KD1 site.

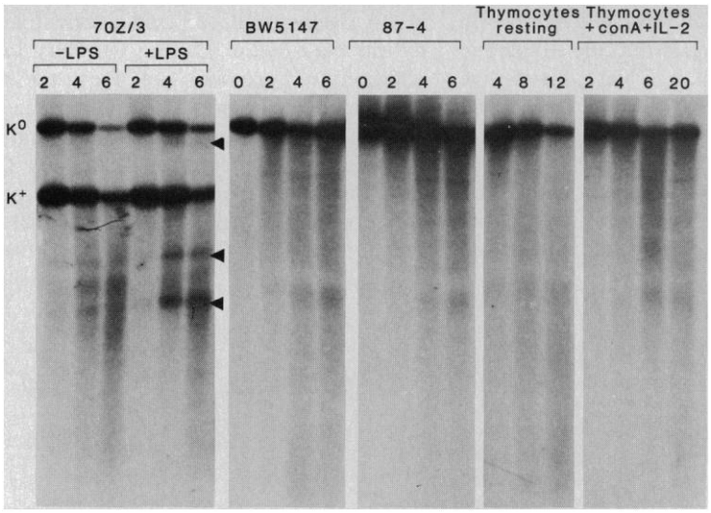

Fig. 6. DNase I hypersensitivity of the κ-immunoglobulin gene. Bam HI digests of the same DNA samples used in Fig. 2 were blotted (*22*) and hybridized with a cloned Bgl II genomic restriction fragment containing the intron C region of the rearranged κ gene in the mouse myeloma LPC-1 (*1*). The upper K^0 dark band in all lanes is a germline JC_κ region Bam HI restriction fragment, and the additional K^+ restriction fragment in 70Z/3 lanes bears a functionally rearranged (VJC) κ-chain gene (see Fig. 1). DNase I cleavage of these two Bam HI fragments at hypersensitive sites produces the lower weak bands in 70Z/3 lanes, which are most apparent in LPS-stimulated cells. The sizes of the undegraded κ-chain Bam HI restriction fragments are K^0 = 11.5 kb and K^+ = 5.6 kb. DNase I–Pvu II derived fragments of 9.3 kb, 4.3 kb, and 2.2 kb are marked with arrowheads. The 2.2-kb band (which is generated by DNase I cleavage of both germline and rearranged κ-chain alleles) together with the 3.4-kb band account for the specific cleavage of the 5.6-kb K^+ allele, and sums with the 9.3-kb K^0 specific fragment to 11.5 kb.

sites and transcription. As yet no unequivocal experiment or strong hypothesis has established a molecular mechanism linking nuclease hypersensitive sites with transcription or protein-DNA interactions. Hypersensitive sites may arise from a variant DNA configuration that activates gene transcription or, conversely, transcription could induce vicinal hypersensitive sites. Alternatively, such sites might appear in gene control elements when the latter encounter regulatory proteins. There is little evidence on which to determine the direction of causality of DNase I hypersensitivity and transcription if such a relation exists; however, certain patterns provide clues.

The Northern blot of Fig. 8 reveals that the BW5147 line we used contains a very small amount of a 1.0-kb β_2 mRNA, but the 87-4 hybridoma expresses a significant amount of a 1.3-kb β_2 mRNA. The 1.3-kb β_2-gene transcripts are typical for functional VDJC mRNA's, whereas 1.0-kb transcripts are thought to originate by transcription initiation from weak promoters upstream of joined DJ segments. Since 87-4 has no β_1 genes (*29*), the 1.3-kb 87-4 mRNA presumably reflects β_2 gene transcription. This same blot reveals no β gene transcription in resting or LPS-stimulated 70Z/3 cells. As already stated, LPS induces a major increment in the transcription of the κ gene in 70Z/3. We do not find κ RNA in BW5147 or 87-4 (*29*).

Several BW5147 sublines are being used in T-cell receptor gene studies. These BW5147 variants are distinguished by the number of differently rearranged JC_β they harbor. Clones with one, two, and three different C_β restriction fragments have been observed (*8, 34*). Whether or not a given BW5147 makes β RNA seems to depend on which of these genes are present. Thus, the isolation of β complementary DNA clones from RNA expressed by one or both BW5147 β genes in a helper T-cell hybridoma (*8, 9*) contrasts with the absence of a 1.3-kb mRNA from the single β_2 gene in the BW5147 line of our experiments. A plausible hypothesis for these associations between T-cell receptor genes and transcripts is the loss or gain of chromosomes carrying β genes, one of which is barely, if at all, transcribed and the only copy present in our strain (Fig. 8).

The close correlation between DNase I hypersensitivity and transcription of κ immunoglobulin genes in 70Z/3 (*4, 35*) combined with the restriction of both the expression and DNase I hypersensitivity of κ genes and β genes to B cells and T cells, respectively, suggests that a nuclease hypersensitive DNA configuration is either a prerequisite or a consequence of expression of these immunogenes. Our strain of BW5147 contains much less β_2 RNA than the hybridoma 87-4 does, yet the β_2 DNase I hypersensitive site in 87-4 is no stronger than that in BW5147. Similarly, both alleles of the EL-4 $JC_{\beta 2}$ intron contain DNase I hypersensitive sites, yet β-gene transcription seems to be confined to the allele that is rearranged at $J_{\beta 2}$ (*31*) (Table 1). Therefore, it is unlikely that the degree of DNase I hypersensitivity in the $JC_{\beta 2}$ intron depends on the magnitude of β_2 transcription. The occurrence of DNase I hypersensitive sites in the absence of transcription has precedent for genes that either were previously active (*36*) or are poised to become active (*16*).

Changes in the chromatin structure surrounding the c-myc gene have been observed in association with nearby retroviral insertions (*37*), and with chromosomal translocations of the c-myc locus in Burkitt lymphomas (*19*). Similarly, changes in the pattern of DNase I hypersensitive sites flanking the *Drosophila* Sgs4 glue protein gene are apparent in a strain with a transposon inserted just 5′ to the coding region (*38*). It is thus possible that the DNase I hypersensitive site we observed in transformed T-cell lines (such as BW5147, EL-4, YAC-1, and Rad-T) is due to some chromosomal rearrangements that bring extrinsic activating elements into the β T-receptor locus. However, the light DNase I subband at the same position in the DNA of total thymocytes activated by con A plus IL-2 suggests that this site is of significance in nontransformed T cells.

In contrast to the restriction of κ-gene activity to B cells observed by us and others (*4, 35*), heavy chain (H-chain) genes have been found rearranged, transcribed, and DNase I hypersensitive in some T cells. Immunoglobulin H-chain DJ joining, which proceeds early in B-cell development, has also occurred during the ontogeny of many T lymphomas (*40–44*) and some, but not all, T-cell clones (*45*). Likewise, transcripts of the μ immunoglobulin heavy chain gene region are present in many T-cell tumors and some thymus cells (*40, 41, 43, 44*), but not in most helper and cytolytic cell lines (*45*). These T-cell IgH transcripts come from germline DJCμ or partially rearranged DJCμ alleles, but usually differ in size from the corresponding transcripts in B cells because of variations in RNA splicing and possibly even the transcription initiation point (*43*). The level of IgH transcription in T cells ranges between 0.1 to 50 molecules per cell (*40, 41*), which is low in comparison to about 10,000 molecules per cell in fully differentiated B cells (*35, 44*). That IgH RNA in T cells is such a small fraction of mature B-cell levels suggests that H-

chain gene activation in T lymphocytes is far from complete.

The genes coding for the two chains of the immunoglobulin molecule differ in developmental control as well as in whether or not they are expressed or rearranged in T cells. The developmental course of H-chain rearrangement and expression precedes that of the light (L) chain in pre-B cells. For example, the regulation of H- and L-chain transcription in 70Z/3 is different in that LPS treatment is required to induce transcription of the L-chain gene whereas the H-chain gene is constitutively expressed (*35*). Thus, there may be an intrinsic difference in the way the heavy- and light-chain immunoglobulin control elements function developmentally.

Immunoglobulin heavy chain genes have DNase I hypersensitive sites in some T cells as well as in B cells. Storb and colleagues (*39*) mapped sites in BW5147 and in thymocytes to JC_μ intron positions which differ in the two kinds of cells. While showing these multiple, alternative DNase I sites in the thymoma and thymocytes, they noted that in B cells the mouse JC_μ intron has just one site. Mills *et al.* (*7*) located two DNase I hypersensitive sites in the JC_μ intron of the human genes in the B-cell line RPMI 1788. These human IgH DNase I sites were not found, however, in nonlymphoid controls such as an erythroleukemia line or HL60 cells. Of the sites in RPMI 1788, the major one is in the region that carries the putative human IgH enhancer and has strong homology with a mouse sequence encompassing the mouse IgH enhancer (*1, 2*) which has specific protein contacts in B lymphocytes (*46*). The location of the T-cell IgH DNase I sites with respect to the B-cell sites is difficult to determine since the T-cell studies were done in mouse cells and the B-cell studies were carried out with human cells. The high degree of polymorphism seen in the length of the mouse H chain JC intron further confounds the alignment of DNase I hypersensitive sites in B cells and T cells.

Why IgH genes are rearranged, DNase I hypersensitive, and transcribed in T cells which do not make immunoglobulins is an obvious question, and provokes speculation along several lines. Perhaps leukemic dedifferentiation of T cells reactivates B-lineage controls, which in turn act on immunoglobulin genes, or if leukemogenesis involves an error-prone DNA repair system, this may trigger the recombination system for immunoglobulin DJ joining. Alternatively, the agents that T cells use for forming and expressing T-receptor genes might also be used by B cells for IgH genes. Two further observations emphasize such possibilities. One by Herr *et al.* (*42*) is that clonal AKR thymomas contain several distinct T-cell subpopulations, each defined by different joined DJ segments of IgH and that these IgH genes seem to have rearranged during the first few cell divisions after transformation of thymocytes. The other by Adams, Cory, and colleagues (*40*) is that not only T lymphomas, but myeloid tumors as well contain transcripts of the JC_μ region, and in equivalent, if not greater, amounts. The activity of H-chain genes in T-lymphoid and myeloid tumors could

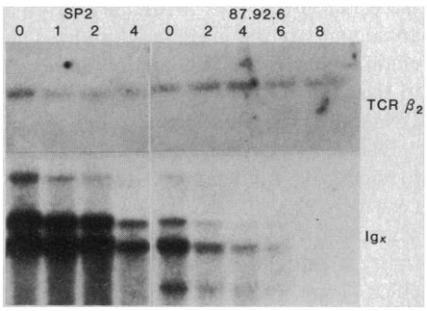

Fig. 7. General DNase I sensitivity of κ versus β_2 genes in B cells. DNA samples from the B-cell myeloma SP2 (*32*) and B-cell hybridoma 87.92.6 (*33*) were prepared from DNase I–treated nuclei as in Fig. 2. DNA samples in the top panels were digested with Pvu II, blotted (*22*), and probed with the $C_{\beta 2}$ gene sequence. Identical DNA samples in the lower panels were digested with Bam HI, blotted, and hybridized with the κ-chain gene probe (see Fig. 1). Examination of the relative amounts of restriction enzyme fragments remaining after treatment with increasing concentrations of DNase I reveals that T-receptor β-chain gene restriction fragments disappear less rapidly as a function of DNase I concentration than κ-chain gene restriction fragments in SP2 and 87.92.6. The κ-chain gene Bam HI restriction fragment common to SP2 and 87.92.6, which is second from the bottom, is virtually the same size (6.2 kb) as the Pvu II β-chain gene fragments. Direct comparison of the disappearance of these two restriction fragments is therefore controlled for target size. Thus, as a whole, the T-receptor β_2 genes are less DNase I–sensitive than the κ genes in these B cells. 87.92.6 was constructed by a fusion of SP2 with immune B cells having anti-idiotype specificity for antibodies to reovirus (*33*).

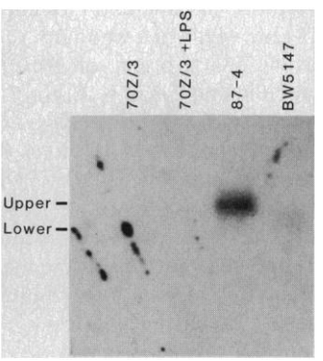

Fig. 8. Northern blot of T-cell and B-cell RNA probed with the $C_{\beta 2}$ region. Total cellular RNA was extracted from T cells in the presence of guanidine thiocyanate (*50*) and cytoplasmic RNA was isolated according to the Maniatis NP40 protocol (*51*) from 70Z/3 cells. Poly(A)$^+$ RNA was purified from 70Z/3 cytoplasmic RNA by two passages over oligo(dT) cellulose. Total T-cell RNA (15 μg) and poly(A)$^+$ RNA (1.5 μg) from resting or LPS-stimulated 70Z/3 cells were denatured with glyoxal, and subjected to electrophoresis through a 1 percent agarose gel, transferred to nitrocellulose (*52*), and hybridized with the $C_{\beta 2}$ probe. Upper and lower indicate the positions of 1.3 kb and 1.0 kb β T-cell receptor transcripts, respectively; on this gel, 18*S* ribosomal RNA also migrated to position upper.

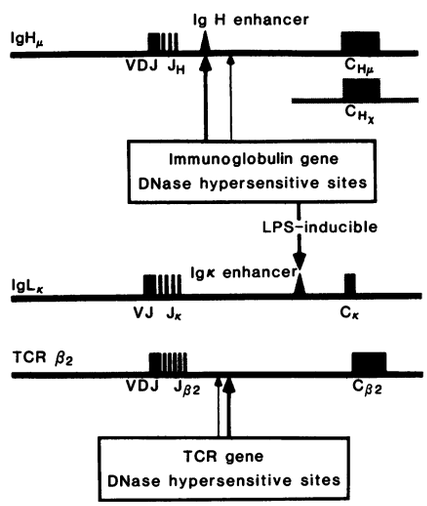

Fig. 9. The arrangement of DNase I hypersensitive sites (↑) and enhancer elements (▲) in typical immunoglobulin and T-cell receptor gene JC introns. All gene maps are normalized to make the JC interval constant in length. The DNA sequence around the enhancer element described in the mouse immunoglobulin H-chain JC intron (*1, 2*) is well conserved in humans and mice, and is the location of a major DNase I hypersensitive site in the human B-cell line RPMI 1788 (*7*). A minor DNase I hypersensitive site between the H-chain enhancer and the μ switch region is also found in RPMI 1788. The DNase I hypersensitive site that LPS induces in the κ immunoglobulin gene JC intron in 70Z/3 pre-B cells (*4*) is closely linked to a lymphocyte-specific enhancer (*3*). The DNA sequence encompassing this κ-gene enhancer and DNase I hypersensitive site is strongly conserved between mice, rabbits, and humans (*53*). The DNase I hypersensitive sites in the JC intron of the β_2 T-cell receptor gene in murine T cells (in our work described here) are presented diagramatically for comparison to the immunoglobulin sites.

be a manifestation of common or wayward developmental regulation. In any case, it is apparent both in terms of rearrangement and expression that IgH gene regulation is less tissue-specific than the regulation of light-chain genes. Our data on the κ genes and the β T-cell receptor genes, taken in the context of the work just summarized concerning the immunoglobulin loci, suggests that the β T-cell receptor locus may be more analogous to the immunoglobulin light chain loci than the H-chain loci in regard to tissue specificity of transcription and DNase I hypersensitivity.

DNase I hypersensitive sites, enhancers, or both have now been observed in the introns between J and C segments of three genes that encode subunits of antigen-specific immune molecules. Thus, like κ L-chain and H-chain genes, T-cell receptor β-chain genes have expression-associated nuclease hypersensitive sites in their JC introns. In Fig. 9 we align these three genes to illustrate the relative positions of their DNase I sites and (where known) their transcription enhancers. The unusual location of these potential regulatory elements inside genes is consonant both with the combinatorial joining of V- and C-gene segments, and with the common evolutionary origin of B-cell immunoglobulin and T-cell receptor C-region sequences (*8–11*).

It would be of obvious interest to know whether β_1, α, and γ T-cell receptor genes have nuclease hypersensitive sites in T cells that express those chains. The extent to which the activities of regulatory elements for immunoglobulin genes, T-cell receptor genes, and other genes of this superfamily are tissue-specific is important for understanding the network that controls their expression. Insights into the function of these elements may also be gained when it has been determined whether DNA regions around hypersensitive sites in immunogenes are positive (or negative) control elements and, if so, what their distinguishing features are.

References and Notes

1. S. Gillies, S. Morrison, V. Oi, S. Tonegawa, *Cell* **33**, 717 (1983).
2. J. Banerji, L. Olson, W. Shaffner, *ibid.*, p. 729; M. Neuberger, *EMBO J.* **2**, 1373 (1983).
3. C. Queen and D. Baltimore, *Cell* **33**, 741 (1983); M. Mercola, X. Wang, J. Olson, K. Calame, *Science* **221**, 663 (1983); D. Picard and W. Schaffner, *Nature (London)* **307**, 80 (1984); C. Queen and J. Stafford, *Mol. Cell. Biol.* **4**, 1042 (1984).
4. T. Parslow and D. Granner, *Nature (London)* **299**, 449 (1982); *Nucleic Acids Res.* **11**, 4775 (1983).
5. W. Weischet, B. Glotov, H. Schnell, H. Zachau, *Nucleic Acids Res.* **10**, 3627 (1982).
6. S. Chung, V. Folsom, J. Wooley, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2427 (1983).
7. F. Mills, L. Fisher, R. Kuroda, A. Ford, H. Gould, *Nature (London)* **306**, 809 (1983).
8. S. Hedrick, D. Cohen, E. Neilsen, M. Davis, *ibid.* **308**, 149 (1984).
9. Y. Chien, N. Gascoigne, J. Kavaler, N. Lee, M. Davis, *ibid.* **309**, 322 (1984).
10. Y. Yanagi *et al.*, *ibid.* **308**, 145 (1984); S. Hedrick, E. Neilsen, J. Kavaler, D. Cohen, M. Davis, *ibid.*, p. 153; G. Siu *et al.*, *Cell* **37**, 393 (1984); H. Saito *et al.*, *Nature (London)* **309**, 757 (1984); J. Kavaler, M. Davis, Y. Chien, *ibid.* **310**, 421 (1984).
11. N. Gascoigne, Y. Chien, D. Becker, J. Kavaler, M. Davis, *Nature (London)* **310**, 387 (1984); M. Malissen *et al.*, *Cell* **37**, 1101 (1984).
12. H. Weintraub and M. Groudine, *Science* **193**, 848 (1976); A. Garel and R. Axel, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3966 (1976); A. Garel, M. Zolan, R. Axel, *ibid.* **74**, 4867 (1977); U. Storb, R. Wilson, E. Selsing, A. Wakefield, *Biochemistry* **20**, 990 (1981); E. Mather and R. Perry, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4689 (1983).
13. C. Wu, P. Bingham, K. Livak, R. Holmgren, S. Elgin, *Cell* **16**, 797 (1979).
14. A. Shermoen and S. Beckendorf, *ibid.* **29**, 601 (1982).
15. J. Stalder, M. Groudine, J. Dogson, J. Engel, H. Weintraub, *ibid.* **19**, 973 (1980).
16. J. Burch and H. Weintraub, *ibid.* **33**, 65 (1983); H. Fritton *et al.*, *Nature (London)* **311**, 163 (1984).
17. A. Larsen and H. Weintraub, *Cell* **29**, 609 (1982); H. Weintraub, *ibid.* **32**, 1191 (1983); A. Nordheim and A. Rich, *Nature (London)* **303**, 674 (1983); H. A. F. Mace, H. R. B. Pelham, A. A. Travers, *ibid.* **304**, 555 (1983); S. Elgin, *ibid.* **309**, 213 (1984).
18. B. Emerson and G. Felsenfeld, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 95 (1984); C. Parker and J. Topol, *Cell* **37**, 273 (1984); C. Wu, *Nature (London)* **311**, 81 (1984).
19. U. Siebenlist, L. Hennighausen, J. Battey, P. Leder, *Cell* **37**, 381 (1984).
20. J. Jongstra *et al.*, *Nature (London)* **307**, 708 (1984).
21. M. Muskavitch and D. Hogness, *Cell* **29**, 1041 (1982); W. McGinnis, A. Shermoen, J. Heemskerk, S. Beckendorf, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1063 (1983); K. Nasmyth, *Cell* **30**, 567 (1982).
22. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
23. Our strain of BW5147 was obtained from L. Glimcher. This line of BW5147 contains only one β_2 allele in contrast to other lines in which two or three different β alleles have been found [L. Glimcher and E. Shevach, *J. Exp. Med.* **156**, 640 (1982)].
24. B. Whitaker, M. C. Kauffman, R. Finberg, *J. Immunol.* **129**, 900 (1982). We thank Robert Finberg for the helper T-cell hybridoma 87-4.
25. P. Gorer, *Brit. J. Cancer* **4**, 372 (1950); R. Herberman, *J. Natl. Cancer Inst.* **48**, 265 (1972).
26. M. Cikes, S. Friberg, G. Klein, *J. Natl. Cancer Inst.* **50**, 347 (1973).
27. D. Meruelo, M. Lieberman, N. Ginzton, B. Deak, H. McDevitt, *J. Exp. Med.* **146**, 1079 (1977). We thank Daniel Meruelo for the radiation-induced leukemia cell line (Rad-T).
28. J. Shaw, V. Monticone, V. Paetkau, *J. Immunol.* **120**, 1967 (1978).
29. E. Bier, unpublished data.
30. _____, T. Smith, W. Ralph, in preparation.
31. M. Kronenberg, J. Goverman, R. Haars, M. Malissen, E. Kraig, L. Phillips, T. Delovitch, N. Sucin-Foca, L. Hood, *Nature (London)* **313**, 647 (1985).
32. M. Shulman, C. Wilde, G. Kohler, *ibid.* **276**, 269 (1978).
33. J. Noseworthy *et al.*, *J. Immunol.* **131**, 2533 (1983).
34. Y. Hashimoto, unpublished data.
35. R. Perry and D. Kelley, *Cell* **18**, 1333 (1979); N. Sakaguchi *et al.*, *J. Immunol.* **125**, 2654 (1980); K. Nelson, E. Mather, R. Perry, *Nucleic Acids Res.* **12**, 1911 (1984).
36. M. Groudine and H. Weintraub, *Cell* **30**, 131 (1982).
37. W. Schubach and M. Groudine, *Nature (London)* **307**, 702 (1984).
38. W. McGinnis, A. Shermoen, S. Beckendorf, *Cell* **34**, 75 (1983).
39. U. Storb, B. Arp, R. Wilson, *Nature (London)* **294**, 90 (1981).
40. D. Kemp, A. Harris, S. Cory, J. Adams, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2876 (1980); D. Kemp, A. Harris, J. Adams, *ibid.* **77**, 7400 (1980); J. Adams *et al.*, *Immunol. Rev.* **59**, 5 (1981).
41. D. Kemp, A. Wilson, A. Harris, K. Shortman, *Nature (London)* **286**, 168 (1980); S. Cory, J. Adams, D. Kemp, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4943 (1980); Y. Kurosawa *et al.*, *Nature (London)* **290**, 565 (1981); R. Near and U. Storb, *Biochemistry* **20**, 2386 (1981).
42. W. Herr, A. Perlmutter, W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7433 (1983).
43. F. Alt, N. Rosenberg, V. Enea, E. Siden, D. Baltimore, *Mol. Cell. Biol.* **2**, 386 (1982).
44. M. Zuniga, P. D'Eustachio, N. Ruddle, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3015 (1982).
45. A. Forster, M. Hobart, H. Hengartner, T. Rabbits, *Nature (London)* **286**, 897 (1980); M. Kronenberg, M. Davis, P. Early, L. Hood, J. Watson, *J. Exp. Med.* **152**, 1745 (1980); Y. Cayre, M. Palladino, K. Marcu, J. Stavnezer, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3814 (1981).
46. A. Ephrussi, G. Church, S. Tonegawa, W. Gilbert, *Science* **227**, 134 (1985); G. Church, A. Ephrussi, W. Gilbert, S. Tonegawa, *Nature (London)* **313**, 798 (1985).
47. C. Paige, P. Kincade, P. Ralph, *J. Immunol.* **121**, 641 (1978).
48. J. DeVries, M. van Benthem, P. Rumke, *Transplantation* **15**, 409 (1973).
49. A. M. Maxam *et al.*, unpublished data.
50. J. Chirgwin, A. Przybyla, R. MacDonald, W. Rutter, *Biochemistry* **18**, 5294 (1979).
51. T. Maniatis, E. Fritsch, J. Sambrook, *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982).
52. P. Thomas, *Methods Enzymol.* **100B**, 255 (1983).
53. L. Emorine, M. Kuehl, L. Weir, P. Leder, E. Max, *Nature (London)* **304**, 447 (1983).

54. We thank Steve Gillies and Susumu Tonegawa for the κ-immunoglobulin gene clone, Antonella Spadaro, Maryellen Ruvolo, Kut-Nie Tan, and Linda Clayton, who made the β_2 T-receptor complementary DNA clone, and Karen Gruskin for help on the manuscript and figures. Supported by NIAID grant AI 19901 (M.I.G.) and NCI grant CA 22427 (A.M.M.).

2 February 1985; accepted 15 June 1985