Measuring the Human T Cell Receptor γ-Chain Locus

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The human T cell receptor γ locus, including eleven variable-region, five joining-region, and two constant-region segments, is contained in 160 kilobases. During T cell somatic development these genes undergo rearrangement by deletion of the sequences separating the variable and joining regions. The molecular map of this locus was completely defined by deletion mapping and restriction mapping. Restriction fragments were resolved by standard agarose electrophoresis and field inversion electrophoresis. These studies demonstrate that the deletions in this locus, which occur during the formation of a functional T cell receptor γ-chain gene, range from 50 to 145 kilobases in length. These studies also provide a structural basis for understanding the development of the γ-chain peptide repertoire, and extends the potential of the emerging pulsed-field electrophoretic technology.

THE HUMAN T CELL RECEPTOR γ (TCRγ) locus encodes the immunoglobulin-like γ-chain, which comprises one subunit of the γδ T cell receptor. This locus undergoes rearrangement during the somatic development of T cells to produce a functional TCR γ-chain gene (*1*). The structure of functional TCR γ-chain genes and the germline TCR γ-chain gene segments have been analyzed. However, the precise mechanism by which a variable (Vγ) and a joining (Jγ) segment, which are normally located at great distance from one another, are joined remains uncertain. In general, the mechanism by which any immunoglobulin-like gene is formed during somatic development of a lymphocyte remains unknown. To understand this process by which pieces of DNA located at great distances from one another join together, we needed to know the precise distances separating Vγ, Jγ, and constant (Cγ) region segments in germline DNA.

Previous studies directed toward defining the germline diversity of γ-chain genes have identified most of the DNA segments that can undergo rearrangement in the γ-chain locus. The human genome contains two closely related Cγ genes associated with five Jγ segments arranged in the germline DNA in the order Jγ1.1, Jγ1.2, Jγ1.3, Cγ1, Jγ2.1, Jγ2.3, Cγ2 (*2–5*). The number of variable-region segments remained less certain. Two Vγ gene families, Vγ1 and Vγ2, have been extensively characterized (*3, 4*). The Vγ1 family consists of eight closely related members, only four of which are able to encode functional peptides; the Vγ2 family consists of a single gene. We recently identified two additional Vγ gene segments because these DNA segments rearranged in particular leukemic T cells (Fig. 1). Sequence analysis of these variable-region segments demonstrates that each can encode a functional protein. Because these variable-region segments do not cross-hybridize to Vγ1 or Vγ2 or to one another, we have designated these variable-region segments Vγ3 and Vγ4. Each of these variable-region sequences can join with a joining segment during T cell development (see Fig. 1).

The order of the γ-chain variable regions was elucidated by deletion analysis. Most mature human T cells contain two rearranged γ-chain genes, one on each chromosome. Earlier studies suggested that these rearrangements probably occurred with the corresponding deletion of the sequences separating the variable and joining regions in germline DNA (*2*). The variable-region content of a set of mature human T cell tumors was determined by Southern blot analysis (Fig. 1) with the four variable-region probes. For example, T cells HPB-MLT and CEM contain only Vγ1 variable-region segments and no others (Fig. 1). By contrast, T cell line HSB-2 contains a full complement of Vγ1 segments, one germline Vγ2, one rearranged Vγ2, and one rearranged Vγ2 segment.

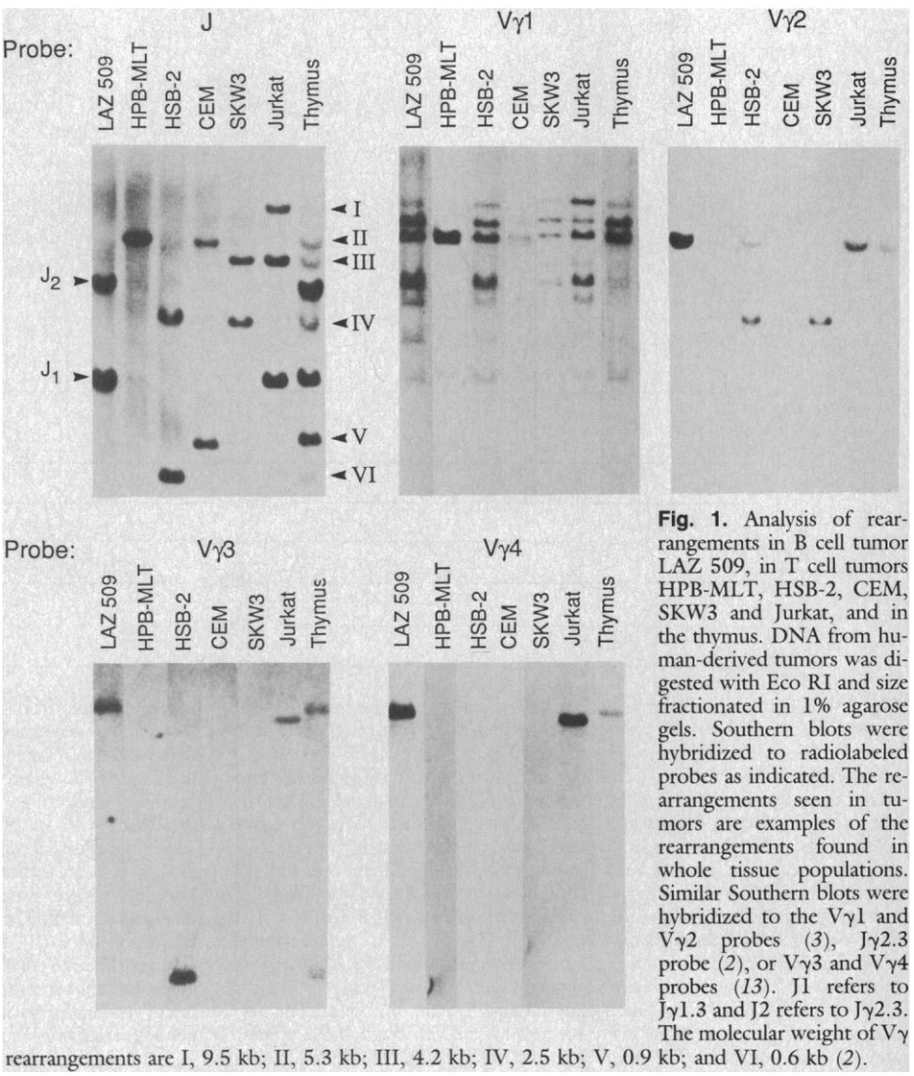

Fig. 1. Analysis of rearrangements in B cell tumor LAZ 509, in T cell tumors HPB-MLT, HSB-2, CEM, SKW3 and Jurkat, and in the thymus. DNA from human-derived tumors was digested with Eco RI and size fractionated in 1% agarose gels. Southern blots were hybridized to radiolabeled probes as indicated. The rearrangements seen in tumors are examples of the rearrangements found in whole tissue populations. Similar Southern blots were hybridized to the Vγ1 and Vγ2 probes (*3*), Jγ2.3 probe (*2*), or Vγ3 and Vγ4 probes (*13*). J1 refers to Jγ1.3 and J2 refers to Jγ2.3. The molecular weight of Vγ rearrangements are I, 9.5 kb; II, 5.3 kb; III, 4.2 kb; IV, 2.5 kb; V, 0.9 kb; and VI, 0.6 kb (*2*).

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ranged Vγ3 segment. This indicates that the Vγ1 family is the most distal with respect to JγCγ and that Vγ2 is between Vγ3 and Vγ1. Vγ4 is adjacent to Vγ3 and is closest to the Cγ segments as it is the most consistently deleted sequence.

Our deletion analysis is confirmed by physical linkage. All members of the Vγ1 family are found on a 50-kb Bam HI fragment. Lefranc *et al.* (*3*) has sequenced all of the members of the Vγ1 family and demonstrated that Vγ2 is closely linked to a member of Vγ1 family. We have found Vγ2 and Vγ3 on the same 10-kb Bam HI fragment. Vγ3 and Vγ4 lie on the same 10-kb Eco RI fragment [LAZ 509 DNA lanes hybridized to Vγ3 and Vγ4 probes (Fig. 1)]. The physical linkage data combined with our sequence analysis of the Vγ families indicate that all of the variable regions are in the same transcriptional orientation.

The physical distance between Vγ4 and Jγ1.1 could not be determined by standard restriction fragment analysis. No single restriction fragment that encoded both the variable and joining segment and that could be resolved by conventional electrophoresis was identified. In order to complete physical characterization of this locus we employed the field inversion gel electrophoretic technique (FIGE), a recent development in pulsed-field electrophoresis (*6*). FIGE allows for resolution of ultrahigh molecular

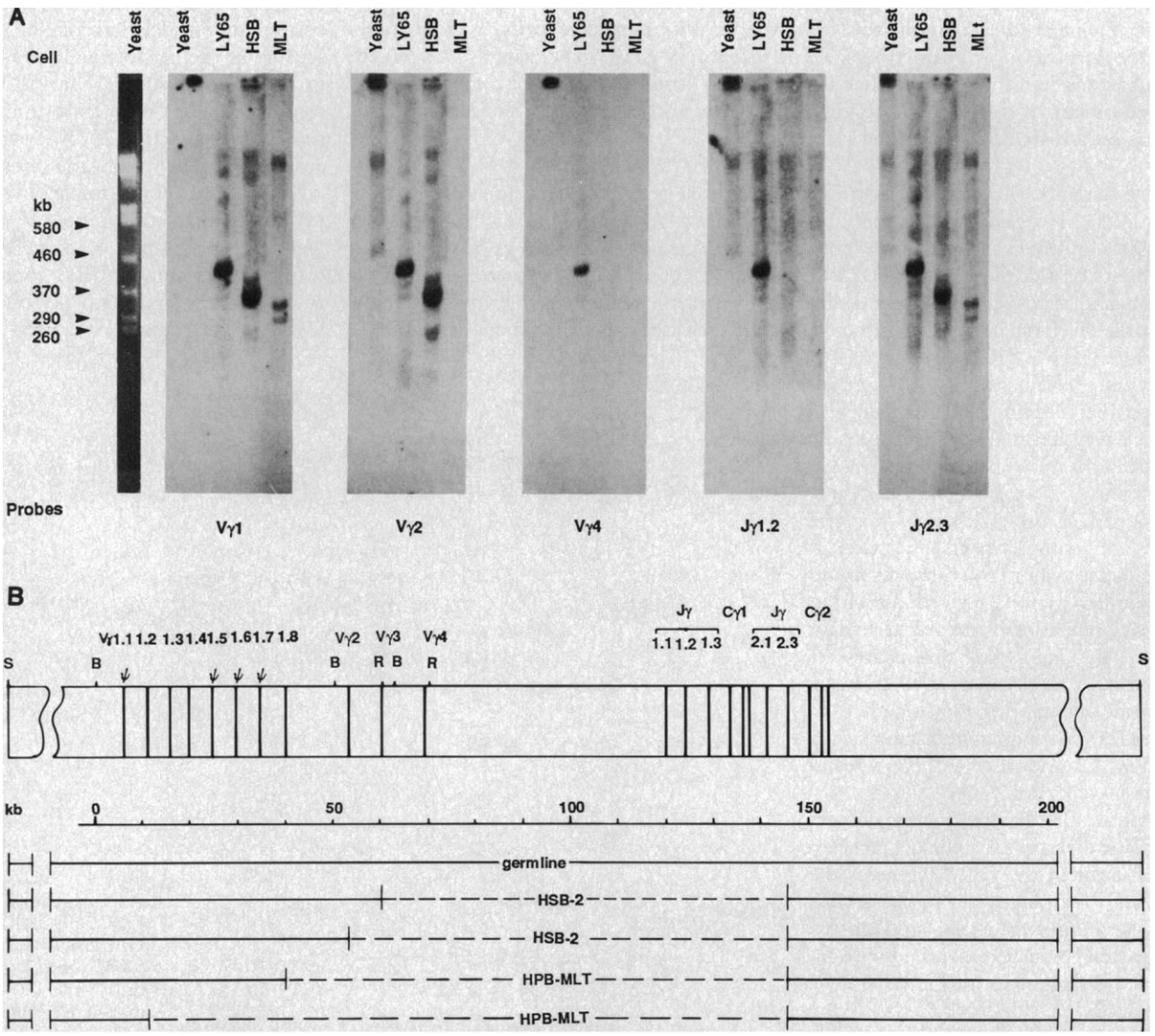

Fig. 2. (**A**) Field inversion gels used to define the map of the human TCRγ locus. DNA was prepared from nuclei embedded in agarose plugs. Cells were lysed in 75 m*M* EDTA-tris (*p*H 7.6), 20 m*M* Hepes (*p*H 7.9), 1 m*M* spermidine, and 0.5% NP-40. Nuclei were centrifuged at 150*g* and suspended in the same solution at 2×10^7 nuclei per milliliter. An equal volume of a mixture of 1.2% Sea Plaque agarose, 100 m*M* sodium EDTA, and 1 m*M* spermidine at 42°C was added, and the mixture was cast into plugs. The plugs were digested overnight at 50°C in proteinase K (1 mg/ml) and 100 m*M* sodium EDTA. The plugs were then treated with 1 m*M* phenylmethylsulfonylfluoride for 1 hour and dialyzed overnight against 0.5*M* NaCl, 10 m*M* sodium EDTA, and 10 m*M* tris-HCl (*p*H 7.6). After equilibration of plugs with restriction buffer, the plugs were digested with Sal I. Plugs were incubated in 12 units of restriction enzyme per 100 μl of plug (~5 μg of DNA) for 4 to 6 hours, and then dialyzed against running buffer. The Sal I fragments of genomic DNA were electrophoresed through 0.8% agarose and 0.5X tris-borate-EDTA (*p*H 8.3) by use of the FIGE technique (*6*), with switching times from 1 second to 41 seconds forward and 0.5 second to 10.5 seconds in reverse, and with a ramp of 4:1. The switching device was homemade and was regulated by a model 100 Radio Shack computer for which we used software we wrote for this purpose. Gels were stained with ethidium bromide (1 μg/ml), irradiated with 260 nm of radiation for 5 minutes, and denatured with 1*N* NaOH for 30 minutes. The DNA was transferred with 1*M* ammonium acetate to nylon membrane (Genescreen) and cross-linked (*14*). Probes are indicated beneath each panel. The same filter was used for all hybridizations to ensure uniformity. (**B**) The cartoon indicates the relative position of variable, joining, and constant regions; ψ indicates that the gene is a pseudogene (*3*). The relative internal distances are determined, but the position relative to the Sal I sites on either end is unknown. The deleted germline DNA in HSB and HPB-MLT chromosomes is indicated by dashed lines; germline organization is represented by the B cell Ly65, which does not rearrange this locus. Not all of the restriction sites are shown. R, Eco RI; B, Bam HI; S, Sal I.

weight DNA in a homogeneous electric field. Because the electric field is homogeneous, relative mobilities between adjacent lanes in a gel are not subject to artifactual variation. It is therefore possible to carry out analysis of the variation in molecular weight between germline and rearranged DNA.

We used the FIGE technique to characterize the Sal I fragments that encode the γ-chain locus in germline and rearranged configurations; that is, a single 425-kb Sal I fragment derived from Ly65, a B cell lymphoma (nonrearranged) DNA, hybridized to both variable and constant region probes (Fig. 2A). A smaller Sal I restriction fragment was found in digests of DNA from the T cell lines CCRF–HSB-2 and HPB-MLT; at the resolution afforded in this analysis the broad band in HSB-2 and the upper band in MLT appear to comigrate. HPB-MLT contains two rearrangements, one to Vγ1.2 and the other to another Vγ1 family member. The resolved doublet in MLT would then represent rearrangements to these family members. The HSB-2, and MLT Sal I fragments do not encode the Jγ1.2 and Vγ4 segments because they do not hybridize to these probes (Fig. 2). The germline size of the Sal I fragment is 425 kb whereas the Sal I fragment (or fragments) in HSB-2 is approximately 340 kb. The linkage between Vγ2 and Vγ3 is less than 10 kb. HSB-2 contains one chromosome with a germline Vγ2 and a rearranged Vγ3; the other chromosome contains only a rearranged Vγ2. Hence the HSB-2 Sal I fragment is an unresolved doublet, one band contributed by each chromosome. The difference in molecular weight between the Sal I fragments in HSB-2 and Ly65 is 85 kb. This segment of 85 kb represents the distance between Vγ3 and Jγ2.3. The distance between Vγ3 and Vγ4 is known, as is the complete physical map of the joining–constant-region locus. Thus, after adjustment for the 9 kb between Vγ3 and Vγ4 and the 25 kb between Jγ2.3 and Jγ1.1, the Jγ-to-Vγ4 distance is about 50 kb. Figure 2B shows the physical map of the human T cell γ locus with relevant distances.

We determined the complete molecular map of the human TCRγ locus (Fig. 2B). Rearrangement of γ-chain segments occurs by deletion of the sequences separating variable and joining segments. By measuring the sizes of the fragments bearing rearranged γ-chain genes, we can calculate the sizes of the deletions. The basis of our physical map relies on two independent lines of data: direct linkage of TCRγ genes on restriction fragments resolved by conventional agarose gel electrophoresis, and direct linkage of TCRγ genes resolved by the FIGE method. It is apparent that there are intrinsic differences between resolving restriction fragments of high molecular weight and those of conventional size. Few restriction enzymes yield large fragments. Those that have a rare distribution in mammalian DNA are sensitive to methylation. The distribution of rare cutting enzymes is also problematic. Several reports have shown that rare cutting enzymes are distributed nonrandomly in the mammalian genome (*7, 8*). It is important that our two physical approaches agree and furthermore that they confirm the deletion data. The loss of the well-characterized markers Vγ4 or Jγ1.2 with the retention of Vγ1 or Jγ2.3 is due to gene rearrangement. The most striking feature of the map is the proximity of the most 5′ Jγ to the most 3′ Vγ; this distance is about 50 kb. The overall size of the locus therefore is approximately 160 kb.

There are six human loci that encode immunoglobulin-like genes. Yancopolous and Alt (*9*) speculated that all of these loci are substrates for the same enzyme or enzymes. However, little is known about the substrate preferences of these enzymes. As this is the first complete characterization of a mammalian immunoglobulin-like locus, there is little opportunity to compare the organization to other loci. As more loci are completely described, canonical features may emerge in the organization of the germline substrates for the mammalian site-specific recombination system. Iwamoto *et al.* (*10*) linked a murine Vγ sequence to a Jγ on a single λ phage, the full organization remains to be elucidated. Most immunoglobulin-like gene loci have the arrangement V_1-V_2-V_3 . . . V_n–J-C. An exception is the TCRβ chain locus, which has one Vβ chain segment on the 3′ side of the constant-region locus (*11*), whereas most of the Vβ segments sit in tandem array, presumably on the 5′ side of the joining-region locus (*12*). However, the physical distance between the other variable and joining segments in other loci remain to be determined. Knowledge of these distances will provide further information about the complex mechanism by which two segments located at great distance in germline DNA can be joined together to form a functional gene.

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15. We thank G. Church, S. Beverley, F. Winston, and G. Tanigawa for stimulating conversations and suggestions and S. Selvaraj for technical assistance. This work was supported in part by NIH grant AI18436, by an award from the Mallinkrodt Foundation (to J.G.S.), and by NIH training grant T32-07208 (to T.Q.).

16 April 1987; accepted 26 June 1987

Cocaine Receptors on Dopamine Transporters Are Related to Self-Administration of Cocaine

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Although cocaine binds to several sites in the brain, the biochemical receptor mechanism or mechanisms associated with its dependence producing properties are unknown. It is shown here that the potencies of cocaine-like drugs in self-administration studies correlate with their potencies in inhibiting [^{3}H]mazindol binding to the dopamine transporters in the rat striatum, but not with their potencies in binding to a large number of other presynaptic and postsynaptic binding sites. Thus, the cocaine receptor related to substance abuse is proposed to be the one associated with dopamine uptake inhibition.

COCAINE IS A POWERFUL REINFORCER that has become a popular drug of abuse. It has a variety of pharmacological effects on the central nervous system (CNS), the cardiovascular system, body temperature, the sympathetic nervous system, and nerve conduction. In man, CNS effects that are related to the abuse of cocaine include feelings of well-being and eu-

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