Brief Definitive Report

REGIONAL LOCATION OF T CELL RECEPTOR GENE *Tiα* ON HUMAN CHROMOSOME 14

By P. E. BARKER, HANS-DIETER ROYER, FRANK H. RUDDLE, AND ELLIS L. REINHERZ

From the Department of Biology, Yale University, New Haven, Connecticut 06511; and the Division of Tumor Immunology, Dana Farber Cancer Institute and the Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115

The human T cell receptor is a molecular complex consisting of a clonotypic, disulfide-linked heterodimer, termed Ti, and three noncovalently associated monomorphic T3 components (1). The Ti molecule that presumably serves as the binding site for nominal antigen and the major histocompatibility complex (MHC) is composed of a 49–54 kilodalton (kD) α subunit and a 43 kD β subunit (1). In contrast, the 20–25 kD T3 proteins are likely involved in signal transduction after ligand binding to Ti (1). Sequence analysis at both the protein and DNA level indicates that the Ti α and β subunits are comprised of immunoglobulin-like domains and that each contains variable, joining, and constant (V, J, C) region encoding segments that rearrange during T lineage ontogeny (1).

Recent chromosomal mapping studies (2–4) localized the human T cell receptor β chain gene (*Tiβ*) to human chromosome 7, although its regional position remains unclear. Here, we demonstrate that human chromosome region 14pter>q21 contains the human *Tiα* gene. This is of particular cytogenetic interest, since chromosomal inversions and translocations involving this region have recently been observed in human T cell malignancies (5). Furthermore, the apparent lack of genetic linkage between the *Tiα* gene and the *IgH* cluster in humans can now be explained by the great physical distance between these loci on human chromosome 14.

Materials and Methods

Cell hybrid analysis (6) and blot hybridization experiments (3, 6) followed established methods except that the electro-eluted insert was labeled by the calf thymus primer protocol (7).

Isolation of Tiα REX cDNA Clones. Based on the previously determined amino acid sequence of the Tiα REX subunit, we have synthesized an oligonucleotide of 23 bases in length with a complexity of 256 (1). This oligonucleotide pool was used to screen the previously described REX lambda gt10 cDNA library (1). Positives were isolated and inserts subcloned in pBR322 (pαREX) (8). DNA sequence analysis of pαREX indicated that it encoded a portion of the V, J, and C regions of the REX human *Tiα* subunit (~400 nucleotides long).

This work was supported by grants GM-09966, R01 AI 19807, AI 21226, and CA 34183 from the National Institutes of Health. Address reprint requests to P. Barker, Department of Biology, 1048 KBT, Yale University, New Haven, CT 06511.

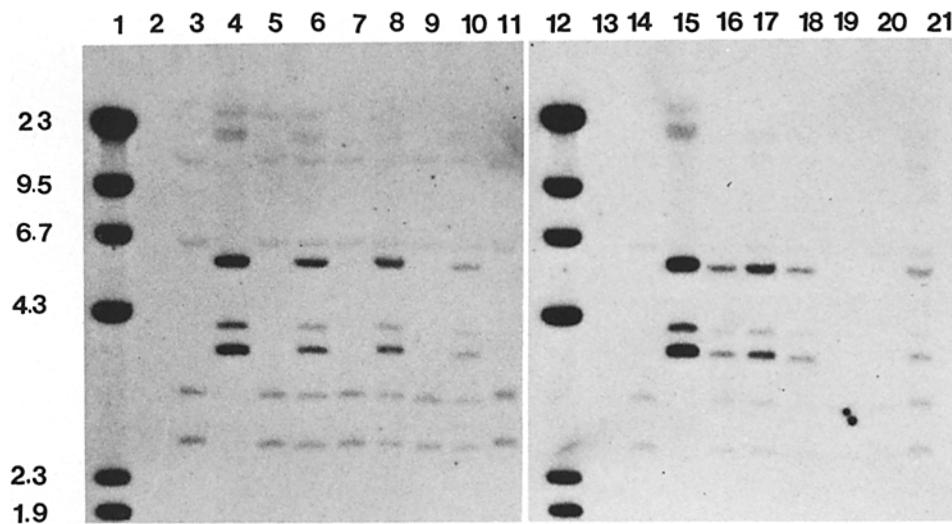

FIGURE 1. Hybridization of pαREX *Tiα*-specific insert to nitrocellulose filters containing marker, genomic control, and mouse-human somatic cell hybrid DNAs. *Bam*HI-cleaved genomic and cell hybrid DNAs were separated by gel electrophoresis, transferred to nitrocellulose filters, hybridized, and washed as reported (3, 6). (Lanes *1* and *12*) λ*Hind*III markers, (*2* and *13*) blanks, (*3* and *14*) mouse A9, (*4* and *15*) human placenta, (*5*) IIIMA9-6.1, (*6*) 5IIIMA9-6.1 SR3, (*7*) 53-87-cl.10, (*8*) 41pT2A, (*9*) WAV17, (*10*) BDA 14b25, (*11*) BDA 10a3, (*16*) FRY 4.A+ SEG, (*17*) AHA 16e, (*18*) AHA 16e6, (*19*) AHA 3d2-2, (*20*) AHA 3d2-3, (*21*) AHA 16e3. The size of marker DNA fragments is given to the left in kilobases.

Results and Discussion

Three major human *Tiα*-specific fragments were detected at molecular weights of 5.5, 4.1, and 3.8 kilobases (kb) (Fig. 1, lanes *4* and *15*). The mouse genomic signal (Fig. 1, lanes *3* and *14*) showed bands of 10.0, 6.0, 3.1, and 2.65 kb. Each cell hybrid DNA contains mouse bands; however, the human signal segregates, with some hybrids lacking (lanes *5, 7, 9, 11, 19, 20*), and some containing all three human bands. Co-segregation of the human bands indicated they originated from the same chromosome.

Table I shows the human chromosome complement of the hybrids as reported (3, 6). Each human chromosome except chromosome 14 is ruled out as the site of *Tiα* by two or more hybrid DNAs. Hybrid 5IIIMA9-6.1-SR3, containing chromosomes 3, 4, 14, and X, contains the *Tiα* signal, while hybrid IIIMA9-6.1 lacks human *Tiα* and human chromosome 14 (Fig. 1), suggesting that human chromosome 14 contains *Tiα*.

The human parent of BDA hybrids bears a translocation involving chromosomes 5 and 14 with the karyotype 46,XY,t(5;14)(5qter>5p14::14q21>14qter; 14pter>14q21::5p14>5pter). Markers *NP* at 14q13.1 (9), c-*fos* at 14q21>31 (6), and D14S1 at 14q32.1>32.2 (10), allowed identification of both translocation products (Fig. 2).

Cell hybrid BDA 10a3 contains D14S1 and c-*fos*, but not *NP* (3). Only the larger translocation product was present and this hybrid lacked *Tiα* (Fig .1, lane *11*). Further, BDA 14b25 lacks c-*fos*, D14S1, and normal chromosome 14 (by cytogenetics), but expresses *NP* (6). Thus, normal chromosome 14 and the larger translocation product were absent but the shorter translocation product was

TABLE I
Human Chromosome Content of Somatic Cell Hybrids Used in Mapping the Human Tiα Gene to Human Chromosomal Region 14pter>q21

Cell hybrid	Human chromosomes present*	Human-specific hybridization signal of cell hybrid DNA probed with pαREX insert:		
		5.5 kb	4.1 kb	3.8 kb
IIIMA9-6.1	3, 4, X	−	−	−
5IIIMA9-6.1-SR3	3, 4, 14, X	+	+	+
53-87-c1.10	7	−	−	−
41pT2A	3, 6, 14, 15, 18, 19, 22, X	+	+	+
BDA 14b25‡	1, 4, 7, 14, X	+	+	+
BDA 10a3	2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 14,§ 16, 17, 20, 22, X	−	−	−
FRY 4.A + SEG	1–4, 6–8, 10–12, 14, 15, 18, 21, 22, X	+	+	+
AHA 16e	10–14, 17–21, X	+	+	+
AHA 16e6	1–3, 10, 11, 13, 14, 16, 19, 20, X	+	+	+
AHA 3d2-2	1, 15	−	−	−
AHA 3d2-3	3, 4, 8, 11, 12, 18, 19	−	−	−
AHA 16e-3	1, 3, 10–14, 19, 21, X	+	+	+

* Cell hybrids were analyzed for human chromosome content by cytogenetic, isozyme, and DNA markers as reported (6).
‡ BDA 14b25 expressed the *NP* marker but lacked normal human chromosome 14 by cytogenetic analysis.
§ BDA 10a3 did not express *NP* but contained distal human chromosome 14 DNA markers D14S1 and *c-fos* (6).

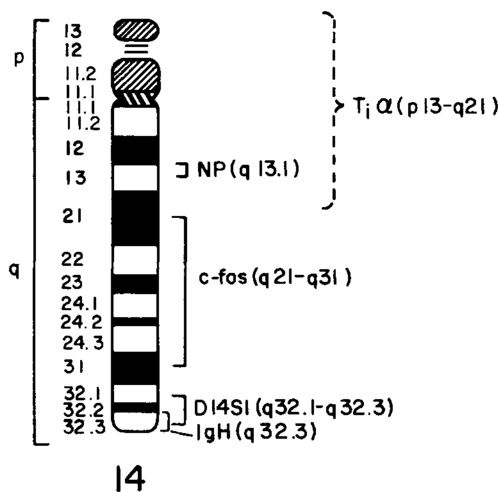

FIGURE 2. Idiogram of human chromosome 14 showing chromosomal location of markers used and the region found to contain the human *Tiα* gene. *NP*, nucleoside phosphorylase isozyme marker (23); *c-fos*, human DNA sequence with homology to the transforming gene of Finkel-Biskis-Jinkins (FBJ)-murine sarcoma virus (6); D14S1, the pAW101 restriction fragment length polymorphic DNA marker (10); *IgH*, the immunoglobulin heavy chain cluster (11). The human *Tiα* gene is mapped to the region indicated (dotted line). The gene order for these and other previously mapped-human chromosome 14 genes (9) is: [(*rDNA*-centromere-*NP*),(*Tiα*)]-[(c-*fos*,α-1-antitrypsin)-(D14S1,*IgH*)].

present; this hybrid contained human *Tiα* (Fig. 1, lane *10*). *Tiα* segregated in BDA hybrids with the shorter translocation product so we conclude that the gene lies in region 14pter>q21.

Since *Tiβ* is encoded by a chromosome 7 gene (2–4), the two Ti subunits are encoded by genes on different human chromosomes. Human *IgH* lies in region

14q32.3 (11) at great physical distance from the *Tiα* gene at 14pter>q21. Given this great intergenic distance, it is not surprising that *IgH* and *Tiα* are not genetically linked. Nevertheless, the presence of truncated *Tiα* transcripts in Epstein Barr virus–transformed B lymphoblastoid lines and μ transcripts in T cell lines (8) suggests that chromatin structure in these two regions of chromosome 14 may be altered in both differentiated cell types, permitting a common T and B recombinase to enter and interact with specific recognition sites.

Repeated observations of a chromosome translocation in human Burkitt lymphoma (12), followed by a subchromosomal mapping of c-*myc* (13) and *Igh* (14), led directly to the observation of underlying genetic events at the nucleotide level (15). Similarly, study of the chronic myelocytic leukemia (CML) translocation in the context of c-*abl* and *Igλ* led to observation of molecular genetic events specific for CML (16).

With this in mind, we note that cytogenetically fragile regions of human chromosome 14 occur in normal individuals at 14q13 and 14q24.11 (17), and in malignant lymphocytes near 14q11 and 14q32 (5). The role of *Tiα* in these conditions is unknown. Four patients with $T11^+$ T cell acute lymphoblastic leukemia (ALL) have common disease-specific chromosome translocations with 14q13 breakpoints (18), within the region that we show contains *Tiα*. Chromosome 14 aberrations in this region are also found in T cell lymphoma and T cell CLL (5). Based on our mapping results, these diseases are obvious subjects for study with regard to *Tiα*.

Human B cell–related immunoglobulin loci have been mapped (11, 14); their map location in the mouse is also known (19, 20). Among T cell genes, the human T cell receptor β chain is clearly on human chromosome 7 (2–4), although its regional position is unclear. The gene encoding the Ti α subunit is in human region 14pter>21. Analogous murine genes lie in proximal chromosome 6 (*Tiβ*) (2, 21) and 14 (*Tiα*) (22).

The assignment of a locus in one species may predict its location in others for conserved linkage groups. *Tiα* (human chromosome 14) predicts a mouse chromosome 14 assignment. In the mouse, the *Tiα-NP* synteny parallels that in human chromosome 14. The human *NP* locus at 14q13.1 (23) is well within the region containing *Tiα*, 14pter>q21. Thus, the syntenic region *Tiα-NP* may be conserved between mice and humans. Further, human c-*fos* is at 14q21>31 (6) while mouse c-*fos* is on murine chromosome 12 (24), and thus syntenic with murine *IgH* (19). Interestingly, the *Tiα-NP* syntenic region consitutes a block independent of the c-*fos*-*IgH* syntenic group. A possible manifestation of *Tiα-NP* synteny may be the T, but not B, cell immunodeficiency in children with *NP* deficiency (25).

Summary

The chromosomal location of *Tiα* was determined by hybridization of a radiolabeled cDNA for the α chain of human T cell receptor with 12 human × mouse cell hybrid DNAs cleaved with *Bam*HI. Seven hybrids contained human *Tiα*, while the remaining five lacked it. Only human chromosome 14 matched the distribution of human *Tiα* signal across the mapping panel. Hybrids segregating a chromosome 14 translocation were used to demonstrate that *Tiα* is in the region 14pter>14q21. Thus, the α and β chain genes that contribute structural components to the Ti moiety of the human T cell receptor lie on

different chromosomes. In humans, the immunoglobulin heavy chain locus and *Tiα* are in different regions of chromosome 14, with *Tiα* more proximal and the immunoglobulin heavy chain locus more distal.

We thank S. Pafka for photography; M. Reger, M. Siniscalci, and S. Gann for secretarial assistance; S. Sicignano for graphics; and A. Hughes and V. Salerno for technical assistance. In addition, we are indebted to Dr. R. Fries for comments on the manuscript and Dr. M. Kamarck for his gift of hybrids.

Received for publication 11 March 1985 and in revised form 10 May 1985.

References

1. Acuto, O., E. L. Reiherz. 1985. The human T cell receptor, structure and function. *New Engl. J. Med.* 312:1100.
2. Caccia, N., M. Kronenberg, D. Saxe, R. Haars, G. A. Bruns, J. Goverman, M. Malissen, H. Willard, Y. Yoshikai, M. Simon, L. Hood, and T. W. Mak. 1984. The T cell receptor β chain genes are located on chromosome 6 in mice and chromosome 7 in humans. *Cell.* 37:1091.
3. Barker, P. E., F. H. Ruddle, H.-D. Royer, O. Acuto, and E. L. Reinherz. 1984. Chromosomal location of human T cell receptor gene $T_i\beta$. *Science (Wash. DC).* 226:348.
4. Collins, M. K. L., P. N. Goodfellow, M. J. Dunne, N. K. Spurr, E. Solomon, and M. J. Owen. 1984. A human T-cell antigen receptor β chain gene maps to chromosome 7. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:2347.
5. Hecht, F., R. Morgan, P. K. M. Hecht, and S. D. Smith. Common regions on chromosome 14 in T cell leukemia and lymphoma. *Science (Wash. DC).* 226:1445.
6. Barker, P. E., M. Rabin, M. Watson, W. R. Breg, F. H. Ruddle, and I. M. Verma. 1984. Human *c-fos* oncogene mapped within chromosome region 14q21-q31. *Proc. Natl. Acad. Sci. USA.* 81:5826.
7. Feinberg, A. P., and B. Vogelstein. 1982. A technique for radiolabeling DNA restriction fragments to high specific activity. *Anal. Biochem.* 132:6.
8. Royer, H.-D., D. Ramarli, O. Acuto, T. Campen, and E. L. Reinherz. 1985. Genes encoding the T cell receptor Ti α and β sequences are transcribed in an ordered sequence during intrathymic ontogeny. *Proc. Natl. Acad. Sci. USA.* In press.
9. Ferguson-Smith, M. A., and D. R. Cox. 1984. Report of the committee on the genetic constitution of chromosomes 13, 14, 15, 16, and 17. *Cytogenet. Cell Genet.* 37:140.
10. Donlon, T. A., and M. Litt, S. R. Newcomb, and R. E. Magenis. 1983. Localization of the restriction length polymorphism *D14S1* (pAW-101) to chromosome 14q31.1-32.2 by *in situ* hybridization. *Am. J. Hum. Genet.* 35:1097.
11. Cox, D. W., and I. Teshima. 1984. Regional localization of the immunoglobulin heavy chain to 14q32.33 to 14 qter. *Cytogenet. Cell Genet.* 37:132.
12. Manolov, G., and Y. Manolova. 1972. Marker band in one chromosome 14 from Burkitt lymphomas. *Nature (Lond.).* 237:33.
13. Dalla Favera, R., M. Bregni, J. Erikson, D. Patterson, R. C. Gallo, and C. M. Croce. 1982. Human c-*myc onc* gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc. Natl. Acad. Sci. USA.* 79:7824.
14. Croce, C. W., M. Shaydur, J. Martinis, L. Cicurel, G. G. D'Ancoma, T. W. Dolby, and H. Koprowski. 1979. Chromosomal location of the genes for human immunoglobulin heavy chains. *Proc. Natl. Acad. Sci. USA.* 76:3416.
15. Taub, R., I. Kirsch, C. Morton, G. Lenoir, D. Swan, S. Tronick, S. Aaronson, and P. Leder. 1982. Translocation of the c-*myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmocytoma cells. *Proc. Natl. Acad.*

Sci. USA. 79:7937.

16. de Klein, A. A. Geurts van Kessel, G. Grosveld, C. R. Bertram, A. Hagemeijer, D. Bootsma, N. K. Spurr, N. Heisterkamp, J. Groffen, and J. R. Stephenson. 1982. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukemia. *Nature (Lond.).* 300:765.
17. Yunis, J. J., and A. L. Soreng. 1984. Constitutive fragile sites and cancer. *Science (Wash. DC)* 226:1199.
18. Williams, D. L., S. T. Look, S. L. Melvin, P. K. Roberson, G. Dadl, T. Flake, and S. Stass. 1984. New chromosome translocations correlate with specific immunophenotypes of childhood acute lymphoblastic leukemia. *Cell.* 36:101.
19. D'Eustachio, P., D. Pravtcheva, K. Marcu, and F. H. Ruddle. 1980. Chromosomal location of the structural gene cluster encoding murine immunoglobulin heavy chains. *J. Exp. Med.* 151:1545.
20. Swan, D., P. D'Eustachio, L. Leinwand, J. Seidman, D. Keithley, and F. H. Ruddle. 1979. Chromosomal assignment of the mouse κ light chain genes. *Proc. Natl. Acad. Sci. USA.* 76:2735.
21. Lee, M. E., P. D'Eustachio, D. Pravtcheva, F. H. Ruddle, S. M. Hedrick, and M. M. Davis. 1984. The beta chain of the murine T cell receptor is encoded on chromosome 6. *J. Exp. Med.* 160:905.
22. Kranz, D. M., H. Saito, C. M. Disteche, K. Swisshelm, D. Pravtcheva, F. H. Ruddle, H. N. Eisen, and S. Tonegawa. 1985. Chromosomal location of the murine T cell receptor α-chain gene and the T cell gamma (γ) gene. *Science (Wash. DC).* 227:941.
23. Remes, G. M., R. A. Fisher, E. Hackel, A. J. Coucinlau, and V. J. Higgins. 1982. SRO refinement for nucleoside phosphorylase by deletion mapping of chromosome 14. *Cytogenet. Cell Genet.* 37:568.
24. D'Eustachio, P. 1984. A genetic map of mouse chromosome 12 composed of polymorphic DNA fragments. *J. Exp. Med.* 160:827.
25. Giblett, E. R., A. J. Ammann, D. W. Wara, R. Sandman, L. K. Diamond. 1975. Nucleoside-phosphorylase deficiency in a child with severely defective T cell immunity and normal B cell immunity. Lancet. 1:1010.