We have studied a 21-year-old female with severe chromosomal breakage, immunodeficiency, and growth failure (case 1). Examination of her chromosomes in cell cultures stimulated with T-cell mitogens [PHA, phorbol ester (TPA), and interleukin 2 (IL-2)] revealed a number of cells with abnormalities involving chromosomes 7 and 14. These included isochromosome 7q and translocations involving breakpoints at 7p13, 7q35, and 14q11 (Table 1, Fig. 3A). We have also studied a patient with ataxia telangiectasia (case 2) in whom we found increased chromosome breakage and various abnormalities of chromosomes 7 and 14 in lymphocyte cultures similarly stimulated, including an inverted 7 with breakpoints at p13 and q35, a 7;7 reciprocal translocation with breakpoints at 7p13 and 7q35, a t(7;14) translocation with breakpoints at 7p13 and 14q11, and an isochromosome 14q (Table 1 and Fig. 3B).

Taken together, these observations on normal individuals and on patients with increased chromosomal fragility suggest that the terminal segment of 7q, as well as sites on 7p and 14q, are "hot spots" for chromosomal rearrangements in nonneoplastic cells in vivo. Because the data cited above are derived from cytogenetic studies of lymphocyte cultures stimulated with T-cell mitogens, it is tempting to speculate that T lymphocytes are particularly fragile at these chromosomal sites. This view is supported by the results of one study in which such rearrangements could not be found in bone marrow cells (*11*). If this is indeed a T-cell phenomenon, perhaps it reflects structural instability generated by somatic rearrangements taking place in genes for T-cell receptor subunits during T-cell differentiation, at least for the 14q11 (α-subunit) (*6*) and 7q35 (β-subunit) locations. Further molecular studies are needed to determine whether these two genes are in fact involved in these translocations, both in normal and in neoplastic T cells.

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Genes for β Chain of Human T-Cell Antigen Receptor Map to Regions of Chromosomal Rearrangement in T Cells

Abstract. *The T-cell antigen receptor is a cell-surface molecule that participates in the immune response. In the present experiments the genes encoding the β chain of the T-cell receptor were found to reside on the long arm of human chromosome 7 at or near band q32. Related sequences were found on the short arm of chromosome 7 in bands p15–21 in some experiments. Chromosomal rearrangements in T-cells from normal individuals and patients with ataxia telangiectasia have previously been observed at and near these map assignments for the β-chain genes.*

The organization of the genes for the β subunit of the T-cell receptor was recently revealed through identification of appropriate surface proteins (*1*) and cloning of T cell–specific complementary DNA's (cDNA's) (*2*). The molecular events by which rearrangement of the genes for the T-cell receptor β chain results in an active receptor resemble those that occur among immunoglobulin genes (*3*). We therefore investigated the possibility that this locus might be involved in such specific translocations as occur in chromosomes from patients with Burkitt's lymphoma (*4*). As a first approach to this inquiry we chose to investigate the precise chromosomal location of the β-chain gene locus.

The gene for the β chain of the murine T-cell receptor was previously assigned, by somatic cell hybridization, to chromosome 6 (*5, 6*); by chromosome hybridization in situ, the gene was shown to be at band B (*6*). The gene for the human β chain was assigned by somatic cell hybridization to chromosome 7 (*6–8*); however, the regional assignment is controversial. Chromosome hybridization in

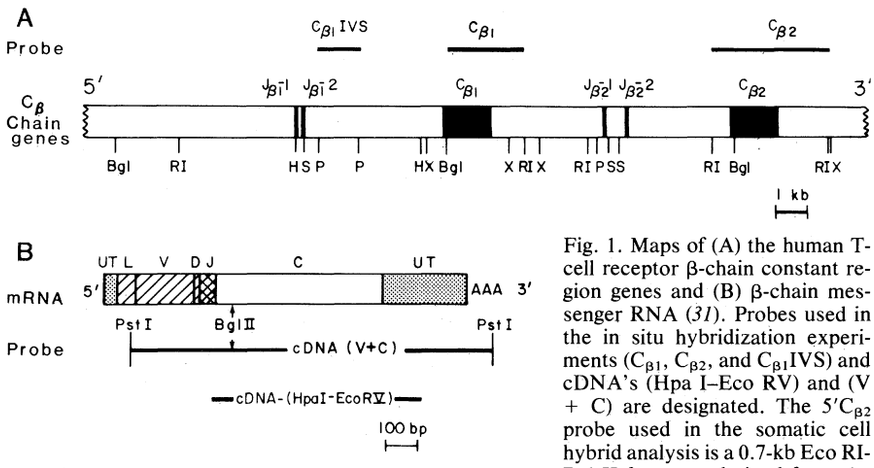

Fig. 1. Maps of (A) the human T-cell receptor β-chain constant region genes and (B) β-chain messenger RNA (*31*). Probes used in the in situ hybridization experiments ($C_{\beta 1}$, $C_{\beta 2}$, and $C_{\beta 1}$IVS) and cDNA's (Hpa I–Eco RV) and (V + C) are designated. The 5′$C_{\beta 2}$ probe used in the somatic cell hybrid analysis is a 0.7-kb Eco RI-Bgl II fragment derived from the $C_{\beta 2}$ probe. Restriction enzyme sites indicated are: Bgl II (Bgl), Eco RI (RI), Hind III (H), Sma I (S), Pvu II (P), and Xba I (X). Other symbols are: C (constant), IVS (intervening sequence), J (joining), UT (untranslated), L (leader), V (variable), D (diversity), and AAA (poly A).

situ indicated that the β-chain gene was located on the short arm at bands 7p13–21 (*6*), whereas analysis of a hybrid cell line containing a translocated chromosome 7 indicated that the gene was on the long arm in the region 7q22–qter (*7*).

We have analyzed DNA extracted from several somatic cell hybrids, including one cell line in which the segregation of a rearranged chromosome 7 has resulted in the deletion of sequences from the distal end of chromosome 7 including 7q22–qter. Samples of DNA (*9*) were digested with Sst I and hybridized with the 5′$C_{\beta 2}$ probe (Fig. 1A). Bands corresponding to fragments of 6.3 and 5.6 kb appeared in lanes containing a normal human chromosome 7 (Fig. 2A, lane 2), as well as a lane containing total human genomic DNA (Fig. 2A, lane 4). However, no such bands appeared in a lane containing DNA derived from either a hybrid cell line with a human chromosome 7 with a terminal deletion of bands 7q22–qter (Fig. 2B) and no intact human chromosome 7 (Fig. 2A, lane 1) or mouse DNA (Fig. 2A, lane 3). This finding confirmed that the β-chain gene resides on the long arm of chromosome 7.

We also hybridized various clones of genomic and cDNA from the locus of the β-chain gene ($C_{\beta 2}$) to metaphase chromosomes from both human fibroblast cell lines and peripheral blood lymphocytes (PBL) stimulated with phytohemagglutinin to map precisely the location of the β-chain genes and investigate the prior mapping discrepancy (*6, 7*). After hybridization to the $C_{\beta 2}$ probe (*18*), 23 percent of 230 metaphase chromosomes from the phytohemagglutinin (PHA)-stimulated PBL from individual B (*19*) showed a major peak of silver grain accumulation at the 7q32–qter region (Figs. 3 and 4). The $C_{\beta 2}$ probe was also hybridized to chromosomes derived from fibroblast cell lines GM3240 and GM3733 (*20*), which have deletions of the distal end of one chromosome 7, del(7)(pter → q34:). On the normal and deleted chromosome 7 in these cells, grains accumulated in the 7q32–qter and 7q32–34 regions, respectively. Thus the gene for the β-chain is located at bands 7q32–34.

We then hybridized four different probes for the β-chain gene to metaphase chromosomes from PBL of two different individuals and from one normal human fibroblast cell line, TC133 (Fig. 4). In every experiment the major accumulation of silver grains occurred in the region 7q32–qter, confirming that this region is the location of the β-chain genes in non–T cells as well as in T cells.

In several experiments, a secondary

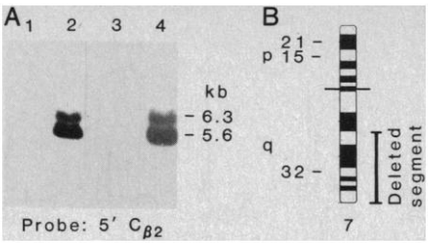

Fig. 2. (A) Autoradiograph from Southern blot hybridization of the 5′$C_{\beta 2}$ probe (Fig. 1A) to DNA from mouse × human somatic cell hybrids. DNA's from: 1, a somatic cell hybrid lacking the terminal part of the long arm of chromosome 7 (JSR); 2, a somatic cell hybrid with only a complete human chromosome 7 (DUA); 3, a parental mouse cell line; and 4, a parental human cell line (WI-38) digested with Sst I and fractionated on an agarose gel. Hybridization is indicated when the distal portion of human chromosome 7 is retained. (B) Idiogram of a normal human chromosome 7 (*17*) demonstrating the region q22–qter which is deleted in the hybrid cell line, JSR. The regions to which hybridization of the β-chain probes have been observed in our studies in situ (7p15–21 and 7q32) are indicated to the left of the idiogram.

peak of hybridization was detected on the short arm of chromosome 7 at bands p15–21. For example, when the $C_{\beta 2}$ probe was hybridized to chromosomes from individual B, 621 silver grains were located on or beside chromosomes, 53 (8.5 percent) grains were located in the 7q32–qter region, and 16 (2.6 percent) grains were located at 7p15–21. If we considered only the grains on chromosome 7, 52 percent were scored in bands q32-qter and 15.7 percent in the region including 7p15–21 (Fig. 4). Similar secondary peaks of hybridization on 7p were observed when the $C_{\beta 1}$IVS and $C_{\beta 1}$ probes were hybridized to chromosomes from individual B (16.9 and 20 percent of all grains on chromosome 7). Surprisingly, neither the $C_{\beta 2}$ probe nor the $C_{\beta 1}$ probe caused an accumulation of grains over the 7p15–21 region on chromosomes from individual A. Thus, the fraction of grains accumulating on 7p differed significantly when chromosomes from different individuals were used for the experiments. It is interesting that Caccia *et al.* (*6*) reported using a cDNA-derived C-region probe in similar experiments to map the β-chain locus to 7p13–21; however, a small cluster of grains can be seen on 7q32–qter in their histogram (*6*). We suggest that the differences in our results may reflect differences in the mixed populations of T cells used in these analyses, that the major peak of hybridization at 7q32 may represent the germline location of the β-chain genes, and that the weaker signal on the short arm of chromosome 7 may indicate either another region of homology to β-chain probes or potentially a region involved in some form of somatic recombination in T-cells. Because there is no evidence for an additional gene locus from Southern blots of genomic DNA's from normal individuals or T-cell leukemias hybridized with the $C_{\beta 1}$ or $C_{\beta 2}$, we believe it unlikely that the 7p signal represents another germline locus for the β chain or a population polymorphism.

The T-cell receptor β-chain locus appears to be involved in ataxia telangiec-

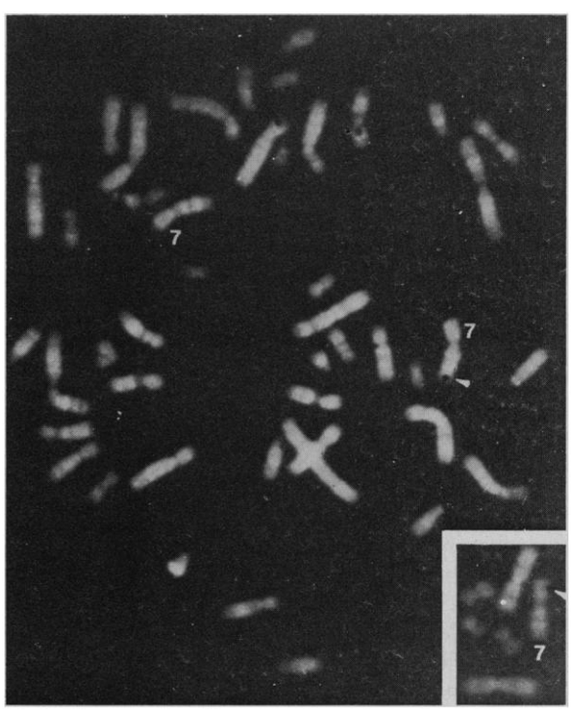

Fig. 3. Hybridization of metaphase chromosome spread from a normal human male (individual A) to the $C_{\beta 1}$IVS probe. The chromosomes were prepared from PHA-stimulated PBL cultures and stained with quinacrine mustard dihydrochloride after hybridization and autoradiography. They were photographed under a combination of incident ultraviolet and transmitted visible light for simultaneous observation of silver grains and identification of chromosomes. A grain can be seen on the long arm of chromosome 7 in the region 7q32-qter. The inset shows that a chromosome 7 from individual A also hybridized with the $C_{\beta 1}$IVS probe on which a silver grain can be seen on the short arm in bands p15–21.

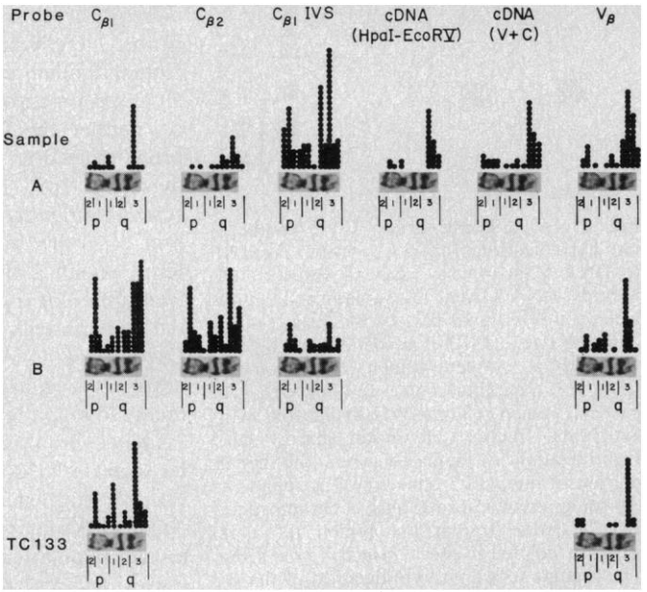

Fig. 4. Distribution of silver grains on chromosome 7's derived from PHA-stimulated PBL from two karyotypically normal males (A and B) and from TC133, a karyotypically normal human fibroblast cell line. The chromosomes were hybridized with probes from the β-chain gene of the human T-cell receptor. Probes were made by subcloning the restriction fragments indicated in Fig. 1 into pBR322 or pBR327. The variable region probe (V_β) is a 4.4-kb genomic Eco RI fragment encoding an entire variable region sequence (*32*). Significant hybridization is seen on the long arm of chromosome 7 in bands 7q32–qter for all samples with all probes and also on the short arm of chromosome 7 in bands p15–21 for sample A with the $C_{\beta 1}$IVS probe and for sample B with the $C_{\beta 2}$, $C_{\beta 1}$, and $C_{\beta 1}$IVS probes. The significance of the peak of hybridization on the short arm of chromosome 7 in TC133 with the $C_{\beta 1}$ probe is unclear in view of the peak seen on the proximal portion of the long arm in band 7ql.

tasia (AT), an autosomal recessive disorder characterized by oculocutaneous telangiectasia, progressive cerebellar ataxia, immunodeficiency, a predisposition to the development of neoplasia, and an increased incidence of chromosomal breakage and rearrangement (*23*). Patients with AT have a high frequency of somatic chromosomal rearrangements involving chromosome 7 (*24, 25*). The specific breakpoints on chromosome 7, whether they are associated with a translocation with a heterologous chromosome (most often chromosome 14) or with its homolog, or with a pericentric inversion, are clustered at bands 7q32–35 and bands 7p13–15. Several authors have noted the similarity of occasional structural rearrangements in short-term PBL cultures of presumed normal patients (*26*), including one individual carrying adult T-cell leukemia–associated antigens, and AT patients. These rearrangements involve translocations between 7p13–22 or 7q32–35 regions and chromosome 14q11.

The locus of the T-cell β-chain gene would appear to be a good site for chromosome rearrangement because it is a region of high transcriptional activity in T cells. Translocations of the β-chain locus may be analogous to those of the immunoglobulin locus in both Burkitt lymphoma (*27*), chronic lymphocytic leukemia (*28*), and follicular lymphoma (*29*). Further analogies may be made to the α- and β-globin gene loci in erythroleukemic cells that appear also to be the sites for specific translocations (*30*). Future studies may further define the nature of the secondary site of hybridization of the T-cell receptor gene probes and should provide insight into the translocations of chromosome 7 in patients with AT and normal individuals.

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9. Human-mouse somatic cell hybrids were karyotyped and analyzed for chromosome-specific enzyme markers as described (*10–12*). Selected cell lines were investigated from the following hybrid sets: JSR [JoSt × RAG (*11*)], DUA [DUV × A9 (*13*)], WIL [WI-38 × LTP (*10*)], REW [WI-38 × RAG (*14*)], and JWR [Jo-Va × RAG (*15*)]. Nine cell hybrids from these independent hybrid sets involving four unrelated human parental cells and three different mouse enzyme deficient cell lines were used. The cell hybrid from the JSR series, JSR-17S, contains a der(7) chromosome from a t(7;9)(q22;q24) and no normal intact chromosome 7. Analysis of the human T-cell antigen receptor β chain in the hybrids was performed on the same cell passage in which the hybrids were examined for human chromosome constitution and human chromosome-specific enzyme markers. DNA was isolated from human, mouse, and hybrid cell lines (*16*) and Southern blot analysis performed as described (*16*).
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18. For our in situ hybridization studies we prepared metaphase chromosome spreads from PHA-stimulated PBL from two normal human males (designated A and B) according to standard cytogenetic procedures (*19*) and from three human fibroblast cell lines cultured and harvested by routine procedures (*20*): a 46,XY fibroblast cell line (established by S. A. Latt), TC133, and two fibroblast cell lines from the Institute for Medical Research in Camden, N.J., GM3240 and GM3733, with de novo deletions of the distal end of one chromosome 7, 46,XY, del(7)(pter → q34:). The hybridization was performed according to a modification of the procedure of Harper and Saunders (*21*) as described (*22*) with the use of multiple tritium-labeled genomic and cDNA clones from the β-chain locus of the human T-cell receptor including $C_{\beta 2}$, $C_{\beta 1}$, $C_{\beta 1}$IVS, cDNA (Hpa I–Eco RV), cDNA (V+C), and V_β (Fig. 1, A and B).
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Template-Directed Synthesis of Novel, Nucleic Acid–Like Structures

Abstract. *In studying the origins of life, it is important to examine reactions of substrate mixtures that could plausibly have accumulated on the primitive earth. Nucleoside diphosphates would probably have been synthesized along with the standard nucleotides under prebiotic conditions. For these reasons, the template-directed reactions of activated derivatives of these diphosphates, alone or mixed with activated nucleotides, were investigated. An activated derivative of deoxyguanosine 3′,5′-diphosphate condensed efficiently on a polycytidylate template to give oligonucleotide analogues in which each 3′,5′-phosphodiester bond was replaced by a pyrophosphate linkage. Oligomers were formed even in the absence of a template, but much more slowly. Template-directed condensation occurred also with an analogous deoxyadenosine derivative on polyuridylic acid and with an analogous acycloguanosine derivative on polycytidylic acid.*

All naturally occurring nucleic acids have in common a backbone formed by joining the 3′- and 5′-OH groups of successive nucleosides via a phosphodiester bond. However, a double-stranded structure analogous to the Watson-Crick helix is sometimes stable even when the covalently linked backbone of one strand is interrupted or modified. Thus polycytidylic acid [poly(C)] (*1*) will form stable, double-helical complexes with a variety of monomeric derivatives of guanine, including guanosine and guanosine 5′-phosphate (*2*), while long, 2′,5′–linked oligoguanylic acids [oligo(G)'s] can be formed on poly(C) and form a double helix with the template (*3*).

These observations raise two general questions. Is the energy of base-pairing between G and C (or A and U), plus energy obtained by stacking base pairs on top of each other, sufficient to guarantee the stability of a variety of DNA-like double helices with one or both backbones very different from those familiar in biology? If so, can such oligonucleotide analogues be formed by template-directed synthesis?

As a first step toward answering these questions we have prepared and studied the template-directed reactions of the activated nucleotide analogues **1**, **2**, and **3** (Fig. 1) that can condense to form oligomers in which the phosphate group of the oligonucleotides is replaced by a pyrophosphate group. We chose these compounds because we have already shown that the formation of a pyrophosphate from a nucleotide and a nucleoside 5′-phosphoimidazolide in aqueous solution is a general and efficient reaction (*4*).

Phosphodiesterase I (snake venom phosphodiesterase), 2′-deoxyadenosine 3′,5′-diphosphate and 2′-deoxyguanosine 3′,5′-diphosphate were purchased from Pharmacia P-L. The 9-(1,3-dihydroxy-2-propoxy)methylguanine($\bar{G}$) (*5*) was converted to the diphosphate with phosphoryl chloride by a modification of a published procedure (*6*), and its identity was confirmed by comparison in several chromatographic systems with an authentic sample (*5*). The diimidazolides (**1**, **2**, and **3**) were prepared by a standard procedure that has been used for the synthesis of 5′-phosphoimidazolides of nucleotides (*7*).

Prior to analysis by high-performance liquid chromatography (HPLC) on RPC-5 (*7*), samples of the reaction mixtures were incubated at *p*H 4 (room temperature) overnight to hydrolyze phosphoimidazolides. If the overnight hydrolysis with acid was omitted, a complex family of products, many of which contained one or more phosphoimidazolide groups, was obtained. Individual oligomers were collected from the RPC-5 column, desalted by dialysis, and degraded at room temperature with phosphodiesterase I (Pharmacia; 0.01 unit per 0.01 optical density unit of nucleotide, in 0.1*M* tris-HCl containing 0.01*M* $CaCl_2$, *p*H 8.5). The pdGp (*1*) obtained by phosphodiesterase digestion of isolated oligomers was identified by co-chromatography with authentic material on RPC-5.

Compound **1** condensed rapidly on a poly(C) template to form a homologous series of product oligomers with chain lengths up to about 20 (Fig. 2A). The ultraviolet absorption of guanine in the

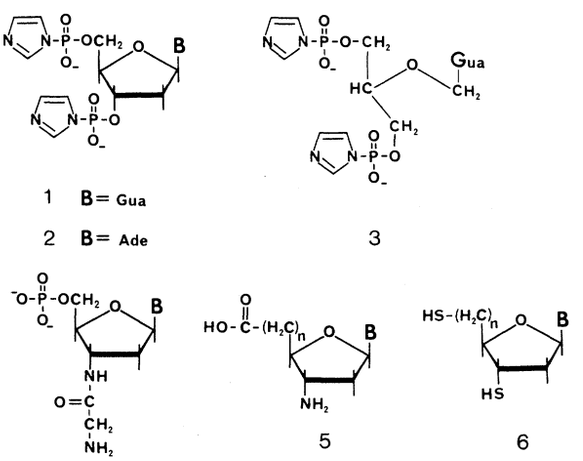

Fig. 1. The chemical names of the derivatives used are the following: **1**, 2′-deoxyguanosine-3′,5′-diphosphoimidazolide; **2**, 2′-deoxyadenosine-3′,5′-diphosphoimidazolide; **3**, 9-(1,3-dihydroxy-2-propoxy)-methylguanine-1,3-diphosphoimidazolide; **4**, 3′-glycylamido-2′,3′-dideoxynucleoside 5′-phosphate; **5**, homologues of 3′-amino-2′,3′-dideoxyuronic acids; **6**, homologues of 3′,5′-dithio-2′,3′,5′-trideoxypentafuranosylnucleosides. For derivatives **4**, **5**, and **6**, B is a nucleoside base.