CONSISTENT BREAKAGE BETWEEN CONSENSUS RECOMBINASE HEPTAMERS OF CHROMOSOME 9 DNA IN A RECURRENT CHROMOSOMAL TRANSLOCATION OF HUMAN T CELL LEUKEMIA

By BENJAMIN TYCKO,* THOMAS C. REYNOLDS,* STEPHEN D. SMITH,‡ AND JEFFREY SKLAR*

*From the *Laboratory of Experimental Oncology, Department of Pathology, and the ‡Department of Pediatrics, Stanford University, Stanford, California 94305*

Chromosomal translocations constitute important diagnostic markers in many different forms of neoplasia and are coming under increased scrutiny for their possible role in the development and progression of a variety of human tumors. The molecular events leading to chromosomal translocation are at present unknown. However, in principle, chromosomal translocations may be conceptualized as arising by two general types of mechanisms. Breakage and rejoining of DNA could take place at random sites by illegitimate, interchromosomal recombination. On the other hand, certain sites in DNA may be predisposed to recombine in certain cells or under certain conditions.

Because translocations in lymphoid tumors often show a chromosomal breakpoint located within a normally rearranging antigen receptor gene, it has been suggested that the lymphocyte recombinase might catalyze chromosomal breakage and rejoining (1). This model predicts the presence of heptamer/nonamer-like recombinase recognition sequences in the DNA adjoining the breakpoints of both participating chromosomes, although only one of the breakpoints lies within a normally rearranging locus. However, in previously analyzed cases of translocations involving Ig (2–8), TCR-α (9, 10), -δ (11, 12), and -β (13, 14) loci, sequences with convincing similarity to the heptamer/nonamer consensus sequence have only sporadically been found adjoining the breakpoint of the participating non-antigen receptor locus. Even when heptamer/nonamer-like sequences have been found near translocation breakpoints, the significance of these sequences has been difficult to evaluate because most studies have been limited to descriptions of breakpoints in single cases. Furthermore, previous studies of breakpoint sequences have generally not examined the reciprocal product breakpoints, thereby leaving the exact site of initial chromosomal breakage ambiguous. With these considerations in mind, we have performed sequence analyses of chromosomal breakpoints of both recombination products in several examples of the recurrent translocation t(7;9)(q34;q32), recently described in human T cell neoplasms (13).

This work was supported by a contract from the National Foundation for Cancer Research and grant CA-34233 from the National Institutes of Health. B. Tycko is the recipient of a National Research Service Award from NIH. J. Sklar is the recipient of a Research Career Development Award from NIH.

Materials and Methods

Cell Lines and Tissues. The T-ALL cell lines SUP-T3 and SUP-T5, showing the t(7;9) (q34;q32), were maintained in culture as described (15). Both show concordance with the original tumor biopsy samples from which they were derived, as assessed by karyotyping and by Southern blot analysis using βTCR J-region probes. The third case, BT, is not represented by a cell line. The source of DNA from this case was a tumor biopsy sample from the involved bone marrow of a patient with T-ALL.

DNA Probes. The origins of DNA fragment hybridization probes are shown in Fig. 1. The R.5 probe is a 0.5-kb Rsa I fragment derived from chromosome 9 DNA and located 100 bp 5′ of the der(9) SupT3 breakpoint. The A.5 probe is a 0.5-kb Ava II/Hind III fragment derived from chromosome 9 DNA located 3 kb 3′ of the der(7) breakpoint. Both probes were subcloned from the appropriate phage clones into Puc 18 or Puc 19 vectors. The Cβ1 probe, used in the initial phase of cloning, consists of a 3.5-kb Hind III fragment containing constant region sequence of the Cβ1 segment of the TCR-β gene which strongly cross-hybridizes to the Cβ2 segment (16).

Phage Cloning. Construction and screening of phage libraries was carried out by standard methods (17). The der(9) SUP-T3 clone was obtained by ligating Bam HI-digested SUP-T3 DNA, size-selected to encompass each of two TCR-β gene rearrangements detected on Southern blots, into λEMBL3A arms and screening the resulting phage libraries with the Cβ1 probe. Two clones were obtained, each corresponding to one of the two TCR-β gene rearrangements. Both clones were restriction mapped and then sequenced in the region 5′ to and including the Jβ2 cluster. One clone proved to represent a normal, potentially productive, βTCR rearrangement. The second, λder(9)SUP-T3, showed chromosome 9 DNA juxtaposed 5′ to Jβ2.3 (13). To obtain germline chromosome 9 DNA spanning the translocation breakpoint region, probe R.5, derived from this clone, was used to screen a library of size-selected human sperm DNA fragments ligated into the Eco RI site of phage λ607. This screening produced a phage clone λGL9 from which the probe A.5 was isolated. This probe in turn was used to screen a library of size-selected DNA fragments from SUP-T3 ligated into the Hind III site of phage λ590 to obtain λder(7)SUP-T3, which contained the breakpoint region of the reciprocal product of the translocation. The der(9) and der(7) breakpoint regions of the two additional cases were isolated in a similar fashion from λ590 size-selected genomic libraries of leukemia DNA, yielding phage clones λder(9)SUP-T5, λder(7)SUP-T5, λder(9)BT, and λder(7)BT. Restriction mapping of all phage clones was carried out after subcloning of phage inserts into Puc 18 or Puc 19 plasmid vectors.

DNA Sequencing. Appropriate DNA restriction fragments were subcloned into M13 phage vectors and their sequence determined by the dideoxy chain-termination method (18). In some cases specific synthetic oligonucleotides were used as sequencing primers.

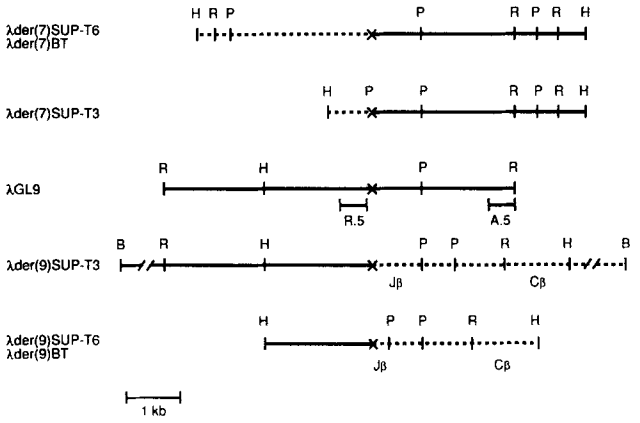

FIGURE 1. Restriction maps of DNA spanning the breakpoints of both products of the t(7;9) (q34;q32) and the germline region of chromosome 9. Chromosome 9 DNA is depicted as a solid line; chromosome 7 DNA is a dashed line. The positions of translocation breakpoints are indicated by *X*. *E, H,* and *P* designate the restriction enzymes Eco RI, Hind III, and Pst I, respectively.

Results and Discussion

Analysis of the Chromosomal Breakpoints in the t(7;9) Translocation of the SUP-T3 Cell Line. The t(7;9) (q34;q32) chromosomal translocation is carried by the established T-ALL cell line SUP-T3 (13), the first cell line or tumor found to contain this translocation. Since chromosomal band 7q34 is the cytologic locus of the TCR-β gene, we reasoned that the translocation breakpoint might be amenable to molecular cloning by screening an appropriate genomic phage library with a TCR-β probe. Starting from this assumption, we cloned DNA spanning the breakpoint in the der(9) translocation product from this cell line. A DNA probe (probe R.5) subcloned from this DNA was confirmed to derive from chromosome 9 by hybridization to Southern blots of DNA from a panel of CHO/human hybrid cell lines containing isolated human chromosomes (13). Probe R.5 was also used to isolate from human sperm a DNA fragment that contained the region of the germline chromosome 9 split by the translocation. A second probe (probe A.5) generated from the 3′ end of the germline chromosome 9 DNA fragment was used in turn to isolate from SUP-T3 cells a third DNA fragment, which contained the breakpoint region of the reciprocal, or der(7) product of the translocation. Comparison of the positions of restriction sites in these three DNA fragments showed that the SUP-T3 translocation is probably balanced with respect to chromosome 9 DNA (i.e., shows no evidence of deletions), although not with respect to chromosome 7 DNA, from which deletion of some DNA had evidently occurred (Fig. 1).

To establish the DNA sequence surrounding the exact site of breakage on chromosomes 7 and 9, the relevant regions of the der(9), der(7), and germline chromosome 9 DNA clones were sequenced. Alignment of these sequences (Fig. 2) confirmed that the translocation is balanced with respect to chromosome 9, but not with respect to chromosome 7. In the der(9) product chromosome 9 DNA is juxtaposed 5′ to Jβ2.3 while in the der(7) product chromosome 9 DNA is juxtaposed 3′ to sequence identifiable as TCR-β variable and diversity gene segments in the form of a Vβ-Dβ1.1 joint. The Vβ gene shows strong sequence similarities with published Vβ cDNA sequences (19, 20). Chromosome 7 DNA which originally lay between the Dβ1.1 segment and Jβ2.3 has been deleted. The translocation therefore suggests an abortive attempt at Dβ1.1-Jβ2.3 joining in which the Dβ1.1 segment was mistakenly joined to chromosome 9 sequence, giving rise to the der(7), while Jβ2.3 was mistakenly joined to chromosome 9 sequence, giving rise to the der(9). The Vβ-Dβ joint found upstream of the der(7) breakpoint may have been formed later, after the translocation event but before outgrowth of the tumor clone represented in the SUP-T3 cell line.

The germline chromosome 9 sequence which has been interrupted by the SUP-T3 translocation, as identified by sequence alignment (Fig. 2), shows two palindromic heptamer sequences, CAC(A/T)GTG, in opposite orientations, separated by 7 bp. Breakage has occurred between these two sequences; the 5′ heptamer is absent from the der(7) product, while the 3′ heptamer is absent from the der(9) product. The chromosome 9 heptamer sequences perfectly match the canonical heptamer of the conserved heptamer/nonamer recombination signal that flanks all known rearranging antigen receptor gene segments (21). Evidence derived from both enzymatic in vitro (22, 23) and functional in vivo (24) studies indicates that the lymphocyte recombinase apparatus specifically recognizes this sequence motif, and cuts DNA at the

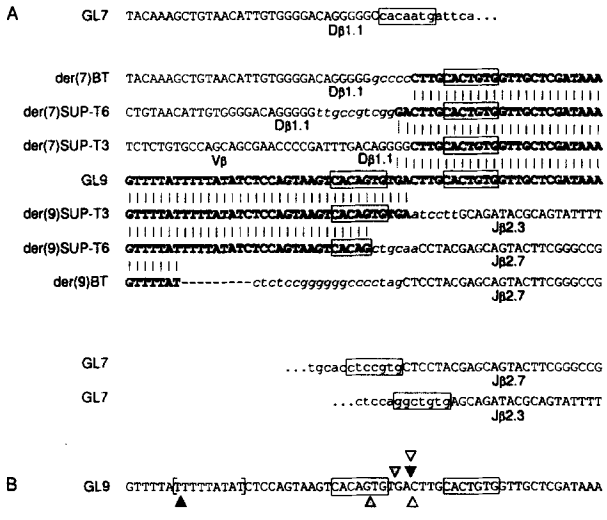

FIGURE 2. Nucleotide sequences of DNA spanning the breakpoints of both products of the t(7;9) (q34; q32) and the corresponding germline region of chromosome 9. (*A*) Breakpoint sequences of the three cases aligned with germline sequences of chromosome 9 and Dβ and Jβ regions of chromosome 7. The sequence derived from chromosome 9 is in bold type, while that derived from chromosome 7 is in standard type. The heptamer consensus sequences are boxed. N-insertions are in italics. Vertical bars denoting alignment of sequence have been placed where there are perfect and uninterrupted matches. Only the relevant portion of each sequence is shown; additional sequence was obtained showing continued agreement in appropriate comparisons to 500 bp 5′ to the breakpoint and 250 bp 3′ to the breakpoint. (*B*) Germline chromosome 9 sequence displayed to show the positions of divergence from this sequence in the der(9) and der(7) products of each case. Upper arrows, der(7); lower arrows, der(9). SUP-T3 (▽), SUP-T6 (▼), BT (▼). A potential nonamer-like sequence located 12 bp upstream of the 5′ heptamer has been bracketed.

boundaries of the heptamer sequences. Therefore, the structure of the SUP-T3 translocation is consistent with a translocation mechanism in which the lymphocyte recombinase has recognized both the "normal" or physiologic TCR-β recombination signals at chromosome 7q34 and the "ectopic" heptamer signals at chromosome 9q32. Formation of the der(9) product would result from utilization of signals flanking Jβ2.3 and the 3′ heptamer at chromosome 9q32, while formation of the der(7) product could be enhanced by utilization of the signals flanking Dβ1.1 and the 5′ heptamer of 9q32. The opposite relative orientations of the two chromosome 9 heptamers is consistent with this model. The presence or absence of nonamer-like sequences is of unclear importance, due to the poor conservation of these sequences within antigen receptor genes. However, an A/T-rich 9-bp sequence is present in chromosome 9 DNA at the predicted location 12 bp 5′ to the 5′ heptamer. In any event, observations in other systems imply that a heptamer alone is sufficient for recombinase recognition (25, 26).

Analyses of the Chromosomal Breakpoints in Two Additional Examples of the t(7;9) Translocation. If the chromosome 9 paired heptamers are in fact specifically recognized by the lymphocyte recombinase in the translocation mechanism, additional examples of the t(7;9) (q34;q32) would be expected to show this same chromosome 9 breakpoint. To investigate this hypothesis, we analyzed two additional cases of T-ALL which,

on our initial screening of 22 cases, were found to show rearranged bands on Southern blots hybridized with the chromosome 9 breakpoint probe R.5 (13). By a strategy analogous to that used in cloning the SUP-T3 translocation products, we isolated the breakpoint regions of both products from these two additional cases. The restriction maps of DNA from the resulting phage clones, λder(9)SUP-T6, λder(7)SUP-T6, λder(9)BT, and λder(7)BT, show agreement with the maps of the SUP-T3 clones in the regions consisting of chromosome 9 DNA (Fig. 1). DNA sequence in the vicinities of the breakpoint in the four clones was analyzed, yielding results displayed in Fig. 2. As predicted by the recombinase-mediated breakage model, examination of the two reciprocal products in each case indicates that there are variable N-insertions and deletions, of the type characteristic of normal D-J joints, at the breakpoints of all three cases. The N-insertions found at normal D-J joints are usually G/C rich (21). This feature is also observed at the translocation breakpoints of at least two of the three translocation cases.

The only feature of the t(7;9) (q34;q32) breakpoints that is perhaps not a common characteristic of normal D-J joints is the presence in the der(9) product from one case (BT) of a deletion of 28 bp from 5′ chromosome 9 DNA. This deletion is somewhat longer than those usually found in normal gene rearrangements and may have resulted from a relative inefficiency of the recombinase-mediated re-ligation of the translocation intermediates, allowing slightly more time for action by the putative recombinase-associated exonuclease. Nevertheless, the der(7) product of the BT translocation does not show a large deletion, nor do either of the translocation products of the other two cases.

Most importantly, by comparing the sequences of the der(7) products with that of the germline chromosome 9, all three cases show chromosome 9 breakpoints between the two consensus heptamers, within 2 bp of each other. However, due to the junctional modifications (removal and/or addition of nucleotides) which are typically associated with recombinase activity, it is not possible to exactly localize the initial cleavage site. The sequence alignments (Fig. 2 *A*) place outside boundaries on the region of initial endonucleolytic cleavage in each case. The actual cleavage site could have occurred anywhere between the endpoints of the chromosome 9 sequences in the two reciprocal products in each case, followed by deletion of chromosome 9 sequences from the free ends before joining to chromosome 7 DNA.

The events inferred to underlie the translocation process differ somewhat from normal antigen receptor gene rearrangement in several respects, for instance by including two chromosomes (rather than just one), four free DNA ends (rather than just two), and at least three endolytic cleavages (one in chromosome 9 and two in chromosome 7, as opposed to just two cleavages during normal intrachromosomal gene rearrangement). No direct evidence demonstrates that all of the cleavages and ligations presumed to occur during translocation necessarily proceeded in a closely coupled, concerted fashion, as they apparently do in normal gene rearrangements. However, the repeated appearance of the same or similar sites in the translocation products implies that all of the involved DNA regions are held in some type of physical complex, probably associated with the recombinase enzyme, during the translocation process.

The lymphocyte recombinase normally produces joints whose structure is most consistent with an initial double-stranded endonucleolytic attack; that is, direct repeats

characteristic of staggered single-stranded breakage are not found when the sequence of the D-J joint is compared with that of the physiological reciprocal product containing the fused heptamers (21, 27). In this regard the recombinase would appear to differ from other prokaryotic and eukaryotic enzymes mediating general and site-specific recombination, which produce short sequence duplications at sites of recombination (28–30). Similar to sites of D-J joining, our sequence data show no duplicated sequence (direct repeats) on comparison of der(9) and der(7) translocation products in any of the three t(7;9) (q34;q32) cases studied by us.

Heptamer-like Sequences Are Variably Present at Breakpoints in Other Chromosomal Translocations. Partial homologies to consensus heptamer sequences, usually less complete than the homologies described here, have been found previously near breakpoints in the chromosomal translocations of lymphoid neoplasms (2, 7, 9–14). Croce and co-workers, as well as other investigators, have suggested the possible role of such homologies in recombinase-mediated translocations involving antigen receptor genes on one chromosome participating in the translocation (1, 2). However, besides the limited homology of these sequences to canonical heptamer sequences, in most cases previously analyzed, only one of the two translocation products was sequenced, leaving the actual position of the breakpoint target site unclear.

In at least two specific common lymphoid tumor translocations there would appear to be contradictory evidence as to the role of recombinase-mediated breakage. Several laboratories have analyzed breakpoints in the t(14;18) of B cell lymphoma (3, 4), a type of translocation suggested by Croce and co-workers to represent an example of recombinase-mediated breakage (1), and have found no well-conserved heptamer-like sequences in the 150-bp major breakpoint cluster region (*mbr*). Indeed, the wide scatter of breakpoints (3–5, 7, 31, 32) observed in this particular type of translocation would seem, *a priori*, incompatible with strong recombinase-mediated targeting. Furthermore, in the analysis of the der(14) and der(18) products in one case, Bakhshi et al. found a trinucleotide direct repeat at the breakpoints of both products (3). On this basis they proposed that recombinogenic random staggered breaks determine the positions of the chromosome 18 breakpoints and that breakage within the *mbr* is simply selected for based on the resulting activation or deregulation of the *bcl-2* gene. Some support for this alternative model is also provided by a study of reciprocal products in t(12;15) translocations of murine plasmacytomas (33). One of four c-*myc* breakpoints in these translocations showed a duplication of target sequences, as did a single t(8;22) translocation in a Burkitt's lymphoma (8). Recently, Boehm et al. (12) have analyzed the breakpoints of four independent cases of the t(11;14) (p13;q11) in T-ALL. They found breakage adjoining a heptamer-like sequence on chromosome 11 in only one case. However, the breakpoints of the reciprocal translocation products were not sequenced. Overall, the number of translocation cases that have been analyzed in terms of both translocation products is probably insufficient to allow a definite conclusion regarding the generality of a recombinase-mediated mechanism.

Of the two alternative hypotheses for the mechanism of chromosomal translocation in lymphoid neoplasms, recombinase-mediated translocation versus random breakage, our data bearing on the t(7;9) (q34;q32) translocation strongly favor the former. All three independent examples of the t(7;9) (q34;32) translocation show a junction with chromosome 9 DNA 5′ to a TCR-β J region in the der(9) product

and 3′ to a D region in the der(7) product, consistent with translocation having occurred by aberrant attempts at D-J joining. We find that all three cases possess breakpoints within a narrowly defined region in chromosome 9 DNA between two closely spaced perfect consensus heptamers. There are no directly repeated target sequences which might suggest staggered breakage. Variable junctional deletions and N-insertions are present, characteristic of recombinase activity. All of these features provide strong circumstantial evidence for the existence of a t(7;9) (q34;q32) translocation mechanism involving targeted recombinase-mediated breakage on both chromosomes.

However, while consistent chromosomal breakage between consensus heptamer sequences in the t(7;9) (q34;q32) of T-ALL suggests a mechanism for chromosomal recombination, it does not explain why cells carrying this translocation are leukemic. What factors govern the presumptive growth advantage of these cells, such as structural or regulatory alteration of genes closely linked to translocation breakpoints, remain to be determined.

Summary

Chromosomal translocations in lymphoid tumors frequently result from recombination between a normally rearranging antigen receptor gene and a normally nonrearranging second locus. The possibility that the lymphocyte recombinase apparatus plays a role in determining the position of breakage at the second locus has been a matter of controversy because of the inconsistent presence of heptamer-like recognition sequences adjoining breakpoints at this site. To further investigate this issue, sites of DNA recombination were analyzed in both the der(9) and der(7) products of t(7;9) (q34;q32), a recurrent translocation of human acute lymphoblastic leukemias (T-ALL). In each of three separate cases, the translocation has divided the TCR-β locus, juxtaposing chromosome 9 DNA 5′ to a J-region in the der(9) product and 3′ to a D-region in the der(7) product, with variably sized N-insertions and small deletions detectable at the junctions. All three cases contain breakpoints in chromosome 9 DNA tightly clustered between two closely spaced, and oppositely oriented heptamer sequences, CAC(A/T)GTG, which perfectly match the consensus heptamer sequence recognized by the lymphocyte recombinase apparatus in normal antigen receptor gene rearrangement. In no case was there evidence of directly duplicated sequences in the two reciprocal products, as is often associated with recombination involving random staggered breakage of DNA. Taken together, these results support a mechanism for this particular translocation proceeding by recombinase-mediated breakage of both participating chromosomes.

Received for publication 16 August 1988 and in revised form 17 October 1988.

References

1. Showe, L. C., and C. M. Croce. 1987. The role of chromosomal translocations in B- and T-cell neoplasia. *Annu. Rev. Immunol.* 5:253.
2. Finger, L. R., R. C. Harvey, R. C. A. Moore, L. C. Showe, and C. M. Croce. 1986. A common mechanism of chromosomal translocation in T- and B-cell neoplasia. *Science (Wash. DC).* 234:982.
3. Bakhshi, A., J. J. Wright, W. Graninger, M. Seto, J. Owens, J. Cossman, J. P. Jensen, P. Goldman, and S. J. Korsmeyer. 1987. Mechanism of the t(14;18) chromosomal trans-

location: structural analysis of both derivative 14 and 18 reciprocal partners. *Proc. Natl. Acad. Sci. USA.* 84:2396.

4. Cleary, M. L., and J. Sklar. 1985. Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint cluster region near a transcriptionally active locus on chromosome 18. *Proc. Natl. Acad. Sci. USA.* 82:7439.
5. Bakhshi, A., J. P. Jensen, P. Goldman, J. J. Wright, D. W. McBride, A. L. Epstein, and S. J. Korsmeyer. 1985. Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around J_H on chromosome 14 and near a transcriptional unit on 18. *Cell.* 41:899.
6. Showe, L. C., R. C. A. Moore, J. Erikson, and C. M. Croce. 1987. *myc* oncogene involved in a t(8;22) chromosome translocation is not altered in its putative regulatory regions. *Proc. Natl. Acad. Sci. USA.* 84:2824.
7. Tsujimoto, Y., J. Gorham, J. Cossman, E. Jaffe, and C. M. Croce. 1985. The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joing. *Science (Wash. DC).* 229:1390.
8. Denny, C. T., G. F. Hollis, I. T. Magrath, and I. R. Kirsch. 1985. Burkitt lymphoma cell line carrying a variant translocation creates new DNA at the breakpoint and violates the hierarchy of immunoglobulin gene rearrangement. *Mol. Cell. Biol.* 5:3199.
9. Bernard, O., C. J. Larsen, A. Hampe, M. Manchauffe, R. Berger, and D. Mathieu-Mahul. 1988. Molecular mechanisms of a t(8;14) (q24;q11) translocation juxtaposing *c-myc* and TcR-α genes in a T-cell leukemia: involvement of a Vα internal heptamer. *Oncogene.* 2:195.
10. Baer, R., A. Foster, and T. H. Rabbitts. 1987. The mechanism of chromosome 14 inversion in a human T-cell lymphoma. *Cell.* 50:97.
11. Boehm, T., R. Baer, I. Lavenir, A. Forster, J. J. Waters, E. Nacheva, and T. H. Rabbitts. 1988. The mechanism of chromosomal translocation t(11;14) involving the T-cell receptor Cδ locus on human chromosome 14q11 and a transcribed region of chromosome 11p15. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:385.
12. Boehm, T., L. Buluwela, D. Williams, L. White, and T. H. Rabbitts. 1988. A cluster of chromosome 11p13 translocations found via distinct D-D and D-D-J rearrangements of the human T-cell receptor δ chain gene. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:2011.
13. Reynolds, T. C., S. D. Smith, and J. Sklar. 1987. Analysis of DNA surrounding the breakpoints of chromosomal translocation involving the β T cell receptor gene in human lymphoblastic neoplasms. *Cell.* 50:107.
14. Russo, G., M. Isobe, L. Pegoraro, J. Finan, P. C. Nowell, and C. M. Croce. 1988. Molecular analysis of a t(7;14) (q35;q32) chromosome translocation in a T-cell leukemia of a patient with ataxia telangectasia. *Cell.* 53:137.
15. Smith, S. D., M. Shatsky, P. S. Cohen, R. Warnke, M. P. Link, and B. E. Glader. 1984. Monoclonal antibody and enzymatic profiles of human malignant T-lymphoid cells and derived cell lines. *Cancer Res.* 44:5657.
16. Weiss, L. M., E. Hu, G. S. Wood, C. Moulds, M. L. Cleary, R. Warnke, and J. Sklar. 1985. Clonal rearrangements of T-cell receptor genes in mycosis fungoides and dermatopathic lymphadenopathy. *N. Engl. J. Med.* 313:539.
17. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 545 pp.
18. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-termination inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463.
19. Ikuta, K., O. Toshihiko, A. Shimizu, and T. Honjo. 1985. Low frequency of somatic mutation in β-chain variable region genes of human T-cell receptors. *Proc. Natl. Acad. Sci. USA.* 82:7701.
20. Tillinghast, J. P., M. A. Behlker, and D. Y. Loh. 1986. Structure and diversity of the

human T-cell receptor β-chain variable region genes. *Science (Wash. DC).* 233:879.
21. Alt, F. W., K. Blackwell, and G. D. Yancopoulos. 1987. Development of the primary antibody repertoire. *Science (Wash. DC).* 238:1079.
22. Hope, T. J., R. J. Aguilera, M. E. Minie, and H. Sakano. 1986. Endonucleolytic activity that cleaves immunoglobulin recombination sequences. *Science (Wash. DC).* 231:1141.
23. Aquilera, R. J., A. Shizuo, K. Okazaki, and H. Sakano. 1987. A pre-B cell nuclear protein that specifically interacts with the immunoglobulin V-J recombination sequences. *Cell.* 51:909.
24. Yancopoulos, G. D., T. K. Blackwell, H. Suh, L. Hood, and F. W. Alt. 1986. Introduced T cell receptor variable region gene segments recombine in pre-B cells: evidence that B and T cells use a common recombinase. *Cell.* 44:251.
25. Siminovitch, K. A., A. Bakhshi, P. Goldman, and S. J. Korsmeyer. 1985. A uniform deleting element mediates the loss of κ genes in human B cells. *Nature (Lond.).* 316:260.
26. Kleinfield, R., R. R. Hardy, D. Talinton, J. Dangl, L. A. Herzenberg, and M. Weigert. 1986. Recombination between an expressed immunoglobulin heavy chain gene and a germline variable gene segment in Ly 1 B-cell lymphoma. *Nature (Lond.).* 32:843.
27. Okazaki, K., D. D. Davis, and H. Sakano. 1987. T-cell receptor β gene sequences in the circular DNA of thymocyte nuclei: direct evidence for intramolecular DNA deletion in V-D-J joining. *Cell.* 49:477.
28. Cox, M. M., and I. R. Lehman. 1987. Enzymes of general recombination. *Annu. Rev. Biochem.* 56:229.
29. Sadowski, P. 1986. Site-specific recombinases: changing partners and doing the twist. *J. Bacteriol.* 165:341.
30. Shoemaker, C., and D. Baltimore. 1980. Structure of a cloned circular moloney murine leukemia virus DNA molecule containing an inverted segment: implications for retrovirus integration. *Proc. Natl. Acad. Sci. USA.* 77:3932–3936.
31. Cleary, M. L., N. Galli, and J. Sklar. 1986. Detection of a second t(14;18) breakpoint cluster region in human follicular lymphomas. *J. Exp. Med.* 164:315.
32. Weiss, L. M., R. A. Warnke, J. Sklar, and M. L. Cleary. 1987. Molecular analysis of the t(14;18) chromosomal translocation in malignant lymphomas. *N. Engl. J. Med.* 317:1185.
33. Gerondakis, S., S. Cory, and J. M. Adams. 1984. Translocation of the *myc* cellular oncogene to the immunoglobulin heavy chain locus in murine plasmacytomas is an imprecise reciprocal exchange. *Cell.* 36:973.