The human minisatellite consensus at breakpoints of oncogene translocations

Anna M.Krowczynska[1,2+], Richard A.Rudders[1] and Theodore G.Krontiris[1,2*]

[1]Department of Medicine (Hematology/Oncology), New England Medical Center Hospitals, Boston, MA 02111 and [2]Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111, USA

ABSTRACT

A reexamination of human minisatellite (hypervariable) regions following the cloning and sequencing of the new minisatellite, VTR1.1, revealed that many of these structures possessed a strongly conserved copy of the *chi*-like octamer, GC[A/T]GG[A/T]GG. In oncogene translocations apparently created by aberrant VDJ recombinase activity, this VTR octamer was often found within a few bases of the breakpoint (p < 10^{-10}). Three *bcl2* rearrangements which occurred within 2 bp of one another were located precisely adjacent to this consensus; it defined the 5′ border of that oncogene's major breakpoint cluster. Several *c-myc* translocations also occurred within 2 bp of this sequence. While the appearance of a *chi*-like element in polymorphic minisatellite sequences is consistent with a role promoting either recombination or replication slippage, the existence of such elements at sites of somatic translocations suggests *chi* function in site-specific recombination, perhaps as a subsidiary recognition signal in immunoglobulin gene rearrangement. We discuss the implications of these observations for mechanisms by which oncogene translocations and minisatellite sequences are generated.

INTRODUCTION

Highly polymorphic, tandemly-repetitive sequences, designated hypervariable minisatellites or variable tandem repeats (VTRs), are dispersed throughout the genomes of higher vertebrates (1–5). The genesis and polymorphic variation of these structures seem intuitively to arise in errors of DNA replication or reiterative recombination events. Indeed, a G-rich consensus (GGGCAGGAXG; ref. 1) has previously been defined within the repeat units of some minisatellites which shares a degree of homology with the procaryotic activator of recombination, *chi*

(GCTGGTGG, ref. 6). Presumably, a *chi*-like component of VTR repeat units would generate tandemly-repetitive sequences either by promoting recombination at some stage in the mitotic or meiotic cell cycle or by promoting replication errors through slippage.

During the characterization of the new human minisatellite, VTR1.1, we noted the presence of a better *chi* homologue, GC[A/T]GG[A/T]GG, within a subgroup of minisatellites identified through VTR1.1. We present evidence here that the new consensus is also associated with site-specific recombination within the constant region genes of the human immunoglobulin heavy chain locus. Furthermore, examination of oncogene translocation breakpoints has implicated the same octamer in somatic recombination.

METHODS

VTR1.1 Cloning and Sequencing

Leukocyte DNA was digested to completion with HaeIII; 3–9 kb fragments from a 10–40% (w/v) sucrose gradient were treated with EcoRI methylase and, following ligation to EcoRI linkers, inserted into the EcoRI site of λgtWES [BRL, Inc, Gaithersburg, MD]. Because the higher molecular weight region of HaeIII-digested DNA represented such a small fraction of the total digest, DNA from 10 individuals was pooled to obtain the requisite amount for cloning. DNA was packaged *in vitro* using Gigapack Plus [Stratagene Cloning Systems, La Jolla, CA] and plated onto *E. coli* strain LE392. The resulting library (36,500 recombinant phage) was screened by *in situ* plaque hybridization using VTR4.1 (7). The EcoRI-BglII fragment of one positive phage, 1.1, containing VTR.1.1 was subcloned into pBS+ [Stratagene Cloning Systems, La Jolla, CA] and propagated in *E. coli* strain JM109. DNA sequencing of the EcoRI-BglII fragment of VTR1.1 was by the dideoxynucleotide chain termination method using a Sequenase DNA sequencing kit [US Bioch Corp, Cleveland, OH]. To sequence internal subunits, a set of clones differing in size by 300 bp was made utilizing the

unidirectional deletion technique described by Ozkaynak and Putney (8). DNA sequence comparisons were performed using the Microgenie sequence analysis program [Beckman, Inc, Palo Alto, CA] and the Genetic Sequence Data Bank (GenBank). Southern blots were performed as previously described (7).
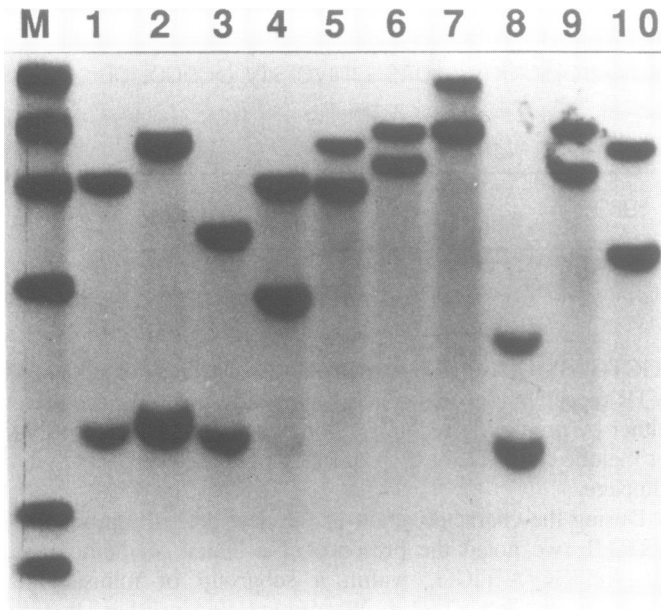

Figure 1: Polymorphism of VTR1.1. Leukocyte DNA of ten unrelated caucasians was digested to completion with HaeIII and subjected to Southern blotting. An EcoRI-BglII fragment containing VTR1.1 (see Figure 2) was used as probe. Among the twenty alleles sampled in this group, at least eleven distinct bands are resolved. The HaeIII genomic fragments recognized by the VTR1.1 probe contain several polymorphisms; hence, in addition to the variation in size, there is often inversion of expected band intensity differencies (lanes 2, 4–10), with larger bands not displaying the more intense hybridization usually demonstrated at a VTR locus. (Similar behavior is exhibited by VTR4.1 [ref. 7].) Subsequent work has shown that the VTR1.1 polymorphism is best isolated and resolved by AluI digestion (J. Weitzel and T. G. K., unpublished) with little change in the rate of heterozygosity. (M) HindIII-digested λ DNA marker: from the bottom, 2.0, 2.3, 4.4, 6.6, 9.4 and 23 kb.

RESULTS

Isolation and Characterization of the Minisatellite, VTR1.1

Screening the composite library of large HaeIII fragments with the minisatellite probe, VTR4.1, resulted in the isolation of a 4.5 kb HaeIII fragment which recognized a highly polymorphic region of the human genome (Figure 1). Southern blotting of DNA from thirty-five unrelated caucasians revealed no homozygotes; a minimum of twenty-five distinct fragments were present in this small sample. DNA sequence of the clone identified a tandemly-repetitive segment with a 70 bp repeat unit (Figure 2).

VTR Consensus Refinement Strengthens the *chi* Homology

A GenBank search employing the 70 bp repeat unit of VTR1.1 resulted in three interesting matches. All were within transcribed genes that had not been characterized previously as minisatellites. Yet, at least a portion of each of these genes possessed tandemly-repetitive sequences which showed varying degrees of homology to VTR1.1. The first, the hinge region of the immunoglobulin $C\gamma3$ gene, consisted of a tandem repeat in which the short hinge exon and flanking introns, a total of 392 nucleotides, were repeated four times (9). At the junction of each repeat pair, homology with VTR1.1 was detected—for example, the 12/15 match shown in the top two lines of Figure 3a. The two additional matches were involucrin (INVO; ref. 10) and salivary proline-rich protein (PRP; ref. 11). Both of these genes encode proteins with tandemly-repetitive amino-acid subunits; portions of the underlying VTR-like repeats homologous to VTR1.1 and $C\gamma3$ are shown in the third through fifth lines of Figure 3a. (The DNA sequence of many internal subunits of these latter two genes have diverged, presumably the result of selection for coding information on a preexisting tandem repeat.) The most strongly conserved region among these four loci was, however, the *chi*-like octamer consensus, GC[A/T]GG[A/T]GG. This unexpected result prompted our reexamination of an additional seventeen published VTR sequences. We determined that 8 of the latter 17 (and therefore 12 of 21 total) possessed the same core sequence, GC[A/T]GG[A/T]GG. Several VTRs, including VTR1.1, $C\gamma3$, myoglobin (12) and 33.6 (1), bore two copies of the sequence within the minisatellite repeat unit. For myoglobin
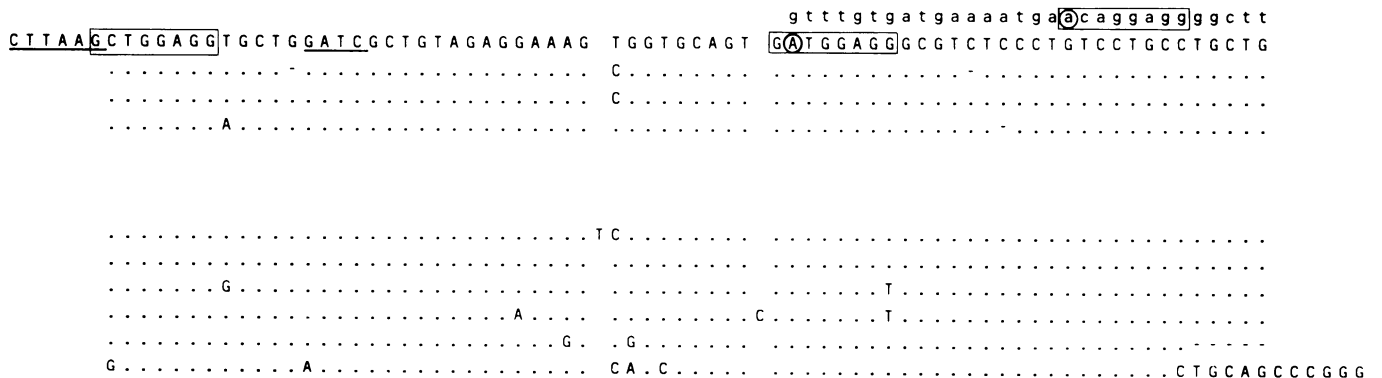

Figure 2: DNA Sequence of VTR1.1. DNA sequence of the 70 bp repeat units of VTR1.1, four from the 5′ end and six from the 3′ end, are depicted in upper case. The remaining 25 internal units are omitted. Departures from the sequence of the first repeat are noted; otherwise, the common bases are indicated by dots. Dashes represent base deletions. Restrictions enyzme sites immediately bordering and within the repeat units are underlined. Flanking sequence, except for restriction enzyme sites, is given in lower case. For this and succeeding Figures, the region of *chi* consensus is boxed; base mismatches to this consensus are circled. The first box contains the 8/8 *chi* consensus match of VTR1.1 [VTR1.1(a) in Figure 3]. Another internal 7/8 match [VTR1.1(b) in Figure 3] and flanking 7/8 match [VTR1.1(c) in Figure 3] are also indicated.

the second (7/8) copy of our consensus was contained within the previously reported GGGCAGGAXG consensus (enclosed in parentheses in Figure 3a). Two VTRs (VTR1.1 and IgH(J) [13]; Fig. 3a) demonstrated the consensus sequence just *outside* the VTR boundary (9 and 14 bp outside, respectively). Thus, a large number of minisatellites possessed an octamer with a more striking resemblance to *chi* than the original GGGCAGGAXG consensus.

The VTR Consensus Is Present at the Crossover in the Cγ3 Gene

The first indication that the *chi*-like octamer of VTRs might be associated with recombination outside hypervariable regions came from further analysis of Cγ3. The duplication of the hinge exons in this gene was initiated by a recombination between the constant region gene, Cγ1, and the constant region pseudogene, Cγψ (9). When we examined sequence data of the relevant regions from both parents and the Cγ3 recombinant (9), we observed a 7/8

match of the consensus in Cγ3 and the Cγψ parent. The position of the match was at precisely the GA dinucleotide where the crossover occurred (Figure 3b).

The VTR Consensus and Oncogene Translocations

We wished to determine if the VTR consensus appeared in somatic recombination events, particularly those resulting in oncogene translocations. Accordingly, we examined published DNA sequences from twenty-five breakpoints of the aberrantly-rearranged oncogenes, *c-myc*, *bcl1*, *bcl2*, and *tcl2*. To prevent potential selection bias, analyses were performed on consecutively acquired sequences from the literature which represented the bulk of data available. No sequence was rejected for comparison. Five rearrangements, involving *c-myc* and *bcl2*, contained perfect copies of the VTR consensus within 8 bp of the breakpoint (Figure 4 and Table 1.) The probability of this observation as a chance occurrence was 3×10^{-6} (see Appendix). Another five rearrangements (Table 1), involving *c-myc* and the putative

Figure 3: Consensus Element in VTR Subunits and the Cγ3 Recombinant. Panel A: *VTRs*. A portion of the individual subunits of 12 VTRs is displayed. Again, the *chi* consensus is boxed; palindromes are underlined. Additional consensus elements for three VTRs [1.1(a,b,c), Cγ3 (a, b [panel b]), and PRP(a,b)] are also included. The minisatellite consensus and bacterial *chi* are listed below the VTR subunit sequences. Additional hypervariable region sequences from INVO, IGH(J), myoglobin (MYOG), retinoblastoma (Rb-1, ref. 35), and the minisatellites 33.1, 33.5, 33.6, 33.11 and 33.15 are listed. The MYOG sequence enclosed in parentheses represents the original GGGCAGGAXG consensus. Panel B: *The Cγ1-Cγψ Recombination*. Sequences immediately flanking the crossover are given. Upper case, Cγψ (C$_H$ pseudogene) parent; lower case, Cγ1 parent. Strands complementary to those provided in ref. 9 are shown for ease of identifying chi. *The dashed rectangle marks crossover point.*
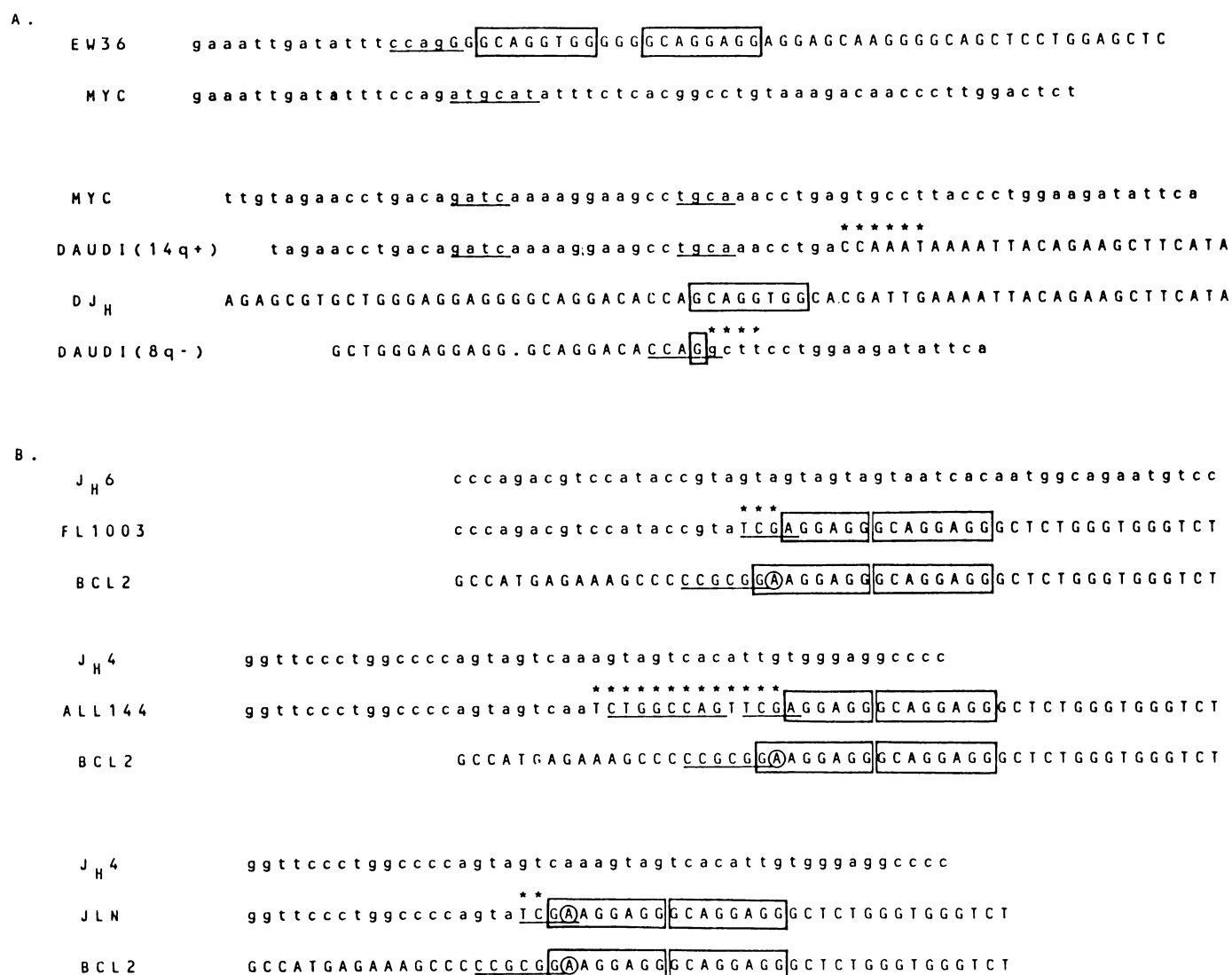
A.

EW36   gaaattgatatttccagGG|GCAGGTGG|GGG|GCAGGAGG|AGGAGCAAGGGGCAGCTCCTGGAGCTC

MYC   gaaattgatatttccagatgcatatttctcacggcctgtaaagacaacccttggactct

MYC   ttgtagaacctgacagatcaaaaggaagcctgcaaacctgagtgccttaccctggaagatattca
                                                              ******
DAUDI(14q+) tagaacctgacagatcaaaag,gaagcctgcaaacctgaCCAAATAAAATTACAGAAGCTTCATA

DJ_H   AGAGCGTGCTGGGAGGAGGGGCAGGACACCA|GCAGGTGG|CACGATTGAAAATTACAGAAGCTTCATA
                                                        ****
DAUDI(8q-)   GCTGGGAGGAGG.GCAGGACACCA|G|gcttcctggaagatattca

B.

J_H6   cccagacgtccataccgtagtagtagtagtaatcacaatggcagaatgtcc
                                                         ***
FL1003   cccagacgtccataccgta|TCG|AGGAGG|G|CAGGAGG|GCTCTGGGTGGGTCT

BCL2   GCCATGAGAAAGCCCCCGCG|G(A)|AGGAGG|G|CAGGAGG|GCTCTGGGTGGGTCT

J_H4   ggttccctggccccagtagtcaaagtagtcacattgtgggaggcccc
                                                    ****
ALL144   ggttccctggccccagtagtcaaT|CTGGCCAGTTCG|AGGAGG|G|CAGGAGG|GCTCTGGGTGGGTCT

BCL2   GCCATGAGAAAGCCCCCGCG|G(A)AGGAGG|G|CAGGAGG|GCTCTGGGTGGGTCT

J_H4   ggttccctggccccagtagtcaaagtagtcacattgtgggaggcccc
                                              **
JLN   ggttccctggccccagta|TC|G(A)AGGAGG|G|CAGGAGG|GCTCTGGGTGGGTCT

BCL2   GCCATGAGAAAGCCCCCGCG|G(A)AGGAGG|G|CAGGAGG|GCTCTGGGTGGGTCT

Figure 4: Consensus Element at Breakpoints of *tc-myc* and *tbcl2* Oncogene Translocations. Panel A: *c-myc*. Sequences are provided from the EW36 (16) and Daudi (34) breakpoints. Oncogene sequence is in lower case; chromosome 14, upper case. No chromosome 14 germline sequence was provided for EW36. Note the disappearance of the consensus element in the reciprocal products of the Daudi translocation. Panel B: *bcl2*. FL1003 (25), ALL144 (26) and JLN (27) breakpoints are shown. Oncogene parent in upper case; J_H sequences in lower case. Asterisks mark inserted bases ('N' nucleotides) which are not derived from either parent.

oncogenes, *bcl1* (14) and *tcl2* (15), had a 7/8 match within 12 bp of the breakpoint (p = 2×10^{-5}).

A pattern emerged for the appearance of the consensus at translocation breakpoints. three of the four *c-myc*/DJ_H rearrangements we examined contained a VTR consensus (Table 1). (The EW36 breakpoint, while described as a switch recombination, occurred 25 bp 5' to switch-region-homologous nucleotides and involved a portion of the *c-myc* gene not otherwise seen in switch translocations [see below; ref. 16]). Rearrangements of the other three oncogenes associated with the VTR consensus or near-consensus also occurred exclusively into D and J segments (Table 1). However, none of the *c-myc* rearrangements into switch or constant gene regions, including BL22 (17), CA46 (18), Manca (19), ST486 (20), Raji (21), JD39 (22), AW-Ramos (23) and BL37 (24), contained the consensus.

The octamer was tandemly-repeated in rearrangements of *c-myc* (EW36, Figure 4a) and *bcl2*. In the case of *bcl2*, the chromosome 18 breakpoints for three independent tumors, FL1003 (rearranging into J_H6; ref. 25); ALL144 (J_H4;

ref. 26) and JLN (J_H4; ref. 27) were 2 bp apart: precisely adjacent to a tandem repeat of the VTR consensus sequence in the *bcl2* parent (Fig. 4b and Table 1). The FL1003 and ALL144 breakpoints occurred at the identical base (Fig. 4b). In fact, this consensus doublet was the 5' border of a 'major breakpoint cluster' for *bcl2*; another three rearrangements occurred within 103 bp of it (28). Two independent rearrangements of *tcl2*, 8511 and LALW-2, occurred just upstream from the same D_δ, ACTGGGGGATACGC (15), which had been fused to other D-segments prior to the oncogene rearrangements. In each instance, the fusion altered the sequence to the near-consensus, GCTGGGGGATACGC (Table 1).

All but two of the breakpoints (and the Cγψ-Cγl crossover) occurred on the same (5') side of the consensus, which always appeared on one parental strand. Interestingly, these two features have been ascribed to *chi*-mediated recombination (6). Unlike *chi*-mediated recombination, however, homology of parental sequences surrounding the breakpoints we examined was minimal. An additional property of rearrangements associated

Table 1: The VTR Consensus in Breakpoints of Aberrantly-Rearranged Oncogenes

ONCOGENE (TRANSL)	TUMOR/ CELL LINE	RECIPIENT	CONSENSUS SEQUENCE	DISTANCE FROM BREAKPOINT	CONSENSUS SEQUENCE ORIGIN	REFERENCE
c-myc t(8,14)	Daudi	DJ_H	GCAGGTGG	0 bp	ch 14	34
	EW36	'$S\mu,\gamma$'	GCAGGTGG	2 bp	ch 14	16
			GCAGGAGG	12 bp		
	SKW-3	J_α	GCAGGAAG	2 bp	ch 14	31
			GCTGGTGG	42 bp	ch 14	
bcl2 t(14,18)	FL1003	J_H6	GAAGGAGG	0 bp	ch 18	25
			GCAGGAGG	9 bp		
	ALL144	J_H4	GAAGGAGG	0 bp	ch 18	26
			GCAGGAGG	9 bp		
	JLN	J_H4	GAAGGAGG	2 bp	ch 18	27
			GCAGGAGG	11 bp		
	FL7832	J_H4	GAAGGAGG	53 bp	ch 18	25
			GCAGGAGG	62 bp		
	SU-DHL-6	J_H6	GAAGGAGG	85 bp	ch 18	28
			GCAGGAGG	94 bp		
	FL1144	J_H6	GAAGGAGG	103 bp	ch 18	25
			GCAGGAGG	112 bp		
bcl1 t(11,14)	CLL1386	J_H4	TCAGGAGG	12 bp	ch 11	14
	CLL271	J_H4	TCAGGAGG	7 bp	ch 11	14
tcl2 t(11,14)	8511	$DDJ\delta$	GAAGGAGG	10 bp	ch 14	15
			GCTGGGGG	24 bp	ch 14	
	LALW-2	$DJ\delta$	GCTGGGGG	9 bp	ch 14	15

Perfect matches of the VTR consensus are underlined. The remainder are 7/8 matches.

with the VTR consensus was the frequent appearance of short palindromes at the breakpoint (Fig. 4) and, less often, on the 3' side of the consensus.

DISCUSSION

The observations just described link two ostensibly distinct processes and compartments of recombination, namely, the generation of human hypervariable regions and the translocation of oncogenes. The statistical case for this linkage is impressive: Five 8/8 matches and five 7/8 matches of the new VTR consensus we have defined appeared within 12 bp of the oncogene/immunoglobulin gene breakpoints in twenty-five lymphocyte-derived tumors. If we exclude all second and subsequent positive rearrangements encountered for each oncogene (for example, counting only one of the six bcl2 breakpoints listed in Table 1), the probability of these appearances as a chance event is still only 7×10^{-10}. (See Appendix.) Therefore, some speculation on a functional role for this VTR octamer seems justified.

There are two broad possibilities: (1) The *chi*-like octamer influences the probability of a nearby rearrangement; or (2) it alters the regulation of oncogene transcription once rearrangement occurs. This latter possibility is consistent with the observation that the consensus we defined is a 6/8, 7/8 and 8/8 match for several different factor binding sites (μE3, μE5 and $\varkappa$E2, respectively) present in immunoglobulin gene enhancers (29). Thus, certain regions nearby oncogenes may be favored translocation sites if they are already capable of binding tissue-specific transcriptional regulatory factors.

The alternative explanation is that this consensus, which looks like *chi* and was defined in a context redolent with recombination, may be acting as *chi*. Although function is the primary constraint on oncogene rearrangement, small, high-probability sites for breakpoints may arise within large, functional targets because of the recombinogenic potential of the consensus.

The *bcl2* rearrangements ostensibly support either proposition. Most are clustered within approximately 100 bp of the VTR consensus doublet at the noncoding terminus of exon 2 (28). Yet rearrangement within the preceding, 350-kb-long intron, which has been observed once, yields the same malignant phenotype (30). The large difference between these two targets may reflect a highly selectable phenotype of the *bcl2* recombinant resulting from exon 2 rearrangement, namely, the repositioning of elements

responsive to Ig gene transcriptional controls. However, an attractive, alternative hypothesis is the existence of a consensus-defined recombinational hotspot in exon 2.

We favor this latter hypothesis because of the proximity of the VTR consensus to the breakpoints and because of the apparent specificity of the VTR octamer for D/J rearrangements. *c-myc* translocations in B-lymphocytes fall into two principal categories: those joining the *c-myc* first intron and subsequent exons to the switch region of a constant gene; and those joining either the upstream or downstream region of *c-myc* to D/J segments (31). This latter group of rearrangements, because the breakpoints contain extra ('N') nucleotides and D- or J-segment boundaries with characteristic deletions, has been ascribed to the aberrant action of the VDJ recombinase (31). As additional evidence for this hypothesis, heptamer/nonamer nucleotides in the *c-myc* sequences near the breakpoints have been described. In follicular lymphomas bearing *bcl2* rearrangements, all breakpoints involve J segments and demonstrate N nucleotides. Thus, a similar mechanism implicating the VDJ recombinase in these tumors has been proposed (25).

Our findings indicate that the VTR consensus may be as important as the VDJ recombinase signal sequences in this second group of rearrangements. This could be because the *chi*-like octamer normally plays a role in accelerating VDJ recombination (6,32), even acting as an enhancer (33). An 8/8 *chi* octamer initiates a 22 base sequence perfectly conserved between the murine 7183 and human III V_H gene families, leading to the suggestion that *chi*, and other, sequences conserved in these families may 'target' V_H recombination (36). Alternatively, an octamer-specific recombinase may exist which interacts with or supplants the VDJ recombinase to effect a translocation. Since D/J-type translocations are relatively infrequent for *c-myc*, the VTR consensus would influence only a small number of these rearrangements. For oncogenes like *bcl2*, however, GC[A/T]GG[A/T]GG may be a major determinant of breakpoint location.

The observations presented herein, when considered in light of recent studies on the genealogy of VTR alleles at the HRAS1 and apoB loci, suggest that the mechanism by which minisatellites arise and the mechanism by which polymorphism is subsequently generated may be subtly, or even drastically, different. The conservation of GC[A/T]GG[A/T]GG within minisatellite repeat units, as well as its appearance in the Cγ3 recombination and in somatic rearrangements representing oncogene translocations, support strongly the hypothesis that this sequence is implicated in site-specific recombination. Therefore, one might expect that the polymorphic variation of minisatellites would occur by *chi*-mediated, unequal crossing-over. However, we have obtained compelling genetic evidence from population studies of the HRAS1 VTR (37; A. Kasperczyk, N. DiMartino and T. G. K., manuscript submitted) that new VTR alleles arise by a mechanism distinct from *single*, unequal crossovers, since randomization of markers upstream of the VTR does not occur following new allele generation. Equally compelling is structural evidence from DNA sequencing of the apoB VTR (38), which reveals short insertions or deletions in a group of alleles related in size—a result more consistent with replication errors (or double crossovers) than simple recombination. We propose, therefore, that the genesis of VTRs is recombinationally-mediated, requiring—at the minimum—the presence of *chi* signals at sites where VTRs eventually occur. We also suggest that subsequent polymorphic diversification of a VTR locus then procedes more commonly

by replication errors or double crossovers than by single, unequal crossovers.

APPENDIX

The probability of observing the octamer sequence *r* times in *n* observations is given by the appropriate term of the binomial expansion, where the probability of one observation within *bp* base pairs of the breakpoint is $([8 + \{2 \times bp\}] \times [3 + 3 + 2 + 3 + 3 + 2 + 3 + 3])/4^8$ for one mismatch at any position. In computing the probabilities discussed in the text, we included only the first *bcl2* rearrangement (FL1003) and the first *bcl1* rearrangement (CLL1386) we analyzed. This is because the clustering of breakpoints for these tumors, particularly *bcl2*, introduces a significant non-random component into the computation. Although we propose (see text) that this cluster results from the presence of the *chi*-like consensus doublet, we exclude second and subsequent breakpoints near the same consensus to avoid the bias of clustering which results from causes not compatible with our hypothesis. Therefore, finding *three* 8/8 matches within 8 bp of the breakpoint in $(25 - 6 = 19)$ tumors is given by $(19!/3!16!)([24 \times 4]/4^8)^3(1-\{[24 \text{ X4}]/4^8\})^{16}$, or 3×10^{-6}. Similarly, finding seven occurrences of a 7/8 match or better within 12 bp of the breakpoint in 19 tumors would be expected to occur by chance with a probability of 7×10^{-10}.

ACKNOWLEDGMENTS

REFERENCES

1. Jeffreys, A.J., Wilson, V., Thein, S.L. (1985) *Nature* 314, 67–73.
2. Wetton, J.H., Carter, R.E., Parkin, D.T., Walters, D. (1987) *Nature* 327, 147–149.
3. Jeffreys, A.J., Wilson, V., Kelly, R., Taylor, B.A., Bulfield, G. (1987) *Nucl. Acids Res.* 15, 2823–2836.
4. Kominami, R., Kohnosuke, M., Muramatsu, M. (1988) *Nucl. Acids Res.* 16, 1197.
5. Georges, M., Lequarre, A.-S., Castelli, M., Hanset, R., Vassart, G. (1988) *Cytogenet Cell Genet.* 47, 127–131.
6. Smith, G.R. (1983) *Cell* 34, 709–710.
7. Colb, M., Yang-Feng, T., Francke, U., Mermer, B., Parkinson, D., Krontiris, T.G. (1986) *Nucl. Acid Res.* 14, 7929–7937.
8. Ozkaynak, E., Putney, S.D. (1987) *BioTechniques* 5, 770–773.
9. Takahashi, N., Ueda, S., Obata, M., Nikaido, T., Nakai, S. Honjo, T. (1982) *Cell* 29, 671–679.
10. Eckert, R.L., Green, H. (1986) *Cell* 46, 583–589.
11. Azen, E., Lyons, K.M., McGonigal, T., Barrett, N.L., Clements L.S., Maeda, N., Vanin E.F., Carlson, D.M., Smithies, O. (1984) *Proc. natn. Acad. Sci. USA* 81, 5561–5565.
12. Weller, P., Jeffreys, A.J., Wilson, V., Blanchetot, A. (1984) *Embo J* 3, 439–446.
13. Silva, A.J., Johnson, J.P., White, R.L. (1987) *Nucl. Acid Res.* 15, 3845–3857.
14. Tsujimoto, Y., Jaffe, I., Cossman, J., Gorham, J., Nowell, P.C., Croce, C.M. (1985) *Nature* 315, 340–343.
15. Boehm, T., Buluwela, L., Williams, D., White, L., Rabbitts, T.H. (1988) *Embo J* 7, 2011–2017.
16. Haluska, F.G., Tsujimoto, Y., Croce, C.M. (1988) *Nucl. Acid Res.* 16, 2077–2085.

17. Moulding, C., Rapoport, A., Goldman, P., Battey, J., Lenoir G.M., Leder, P. (1985) *Nucl. Acid Res.* 13, 2141–2152.
18. Showe, L.C., Ballantine, M., Nishibura, K., Erikson, J., Kayi H., Croce, C.M. (1985) *Mol. Cell Biol.* 5, 501–509.
19. Saito, H., Hayday, A.C., Winman, K., Hayward, W.S., Tonegawa, S. (1983) *Proc natn. Acad. Sci. USA* 80, 7476–7480.
20. Gellmann, E.P., Psallidopoulos, M.C., Papas, T.S., Dalla Favera, R. (1983) *Nature* 306, 799–803.21.Dyson, P.J., Rabbitts, T.H. (1985) *Proc. natn. Acad. Sci. USA* 82, 1984–1988.
22. Dalla Favera, R., Martinotti, S., Gallo, R.C. (1983) *Science* 219, 963–967.
23. Winman, K.G., Clarkson, B., Hayday, A.C., Saito, H., Tonegawa, S., Hayward, W.S. (1984) *Proc. natn. Acad. Sci. USA* 81, 6798–6802.
24. Hollis, G.F., Mitchell, K.F., Battey, J., Potter, H., Taub, R., Lenoir, G. M., Leder, P. (1984) *Nature* 307, 752–755.
25. Tsujimoto, Y., Gorham, J., Cossman, J., Jaffe, E., Croce, C.M. (1985) *Science* 229, 1390–1393.
26. Gauwerky, C.E., Haluska, F.G., Tsujimoto, Y., Nowell, P.C., Croce, C. M. (1988) *Proc. natn. Acad. Sci. USA* 85, 8548–8552.
27. Cleary, M.L., Sklar, J. (1985) *Proc. natn. Acad. Sci. USA* 82, 7439–7443.
28. Bakhshi, A., Wright, J.J., Graninger, W., Seto, M., Owens, J., Cossman, J., Jensen, J.P., Goldman, P., Korsmeyer, S.J. (1987) *Proc. natn. Acad. Sci. USA* 84, 2396–2400.
29. Murre, C., McCaw, P.S., Baltimore, D. (1989) *Cell* 56, 777–783.
30. Cleary, M.L., Smith, S.D., Sklar, J. (1986) *Cell* 47, 19–28.
31. Showe, L.C., Croce, C.M. (1987) *Ann. Rev. Immunol.* 5, 253–277.
32. Kenter, A.L., Birshtein, B.K. (1981) *Nature* 293, 402–404.
33. Yancopoulos, G.D., Alt, F.W. (1986) *Ann. Rev. Immunol.* 4, 339–368.
34. Haluska, F.G., Tsujimoto, Y., Croce, C.M. (1987) *Proc. natn. Acad. Sci. USA* 84, 6835–6839.
35. Wiggs, J., Nordenskjgold, M., Yandell, D., Rapaport, J., Grondin, V., Janson, M., et al. (1988) *New Engl. J. Med.* 318, 151–157.
36. Tutter, A., Riblet, R. (1989) *Proc. natn. Acad. Sci. USA* 86, 7460–7464.
37. Kasperczyk, A., Mermer, B. A., Parkinson, D. R., Lonergan, J. A., Krontiris, T. G. (1989) *Am. J. Hum. Genet.* 45, 689–696.
38. Ludwig, E. H., Friedl, W., McCarthy, B. J. (1989) *Am. J. Hum. Genet.* 45, 458–464.