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Chromosomal orientation of the lambda light chain locus: Vλ is proximal to Cλ in 22q11

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ABSTRACT

We have demonstrated that the chromosomal breakpoint at 22q11 of a Burkitt lymphoma cell line (PA682) with an 8;22 translocation interrupts the variable region of the lambda light chain locus. In these cells, all of the Cλ and some Vλ sequences translocate to the $8q^+$ chromosome whereas some Vλ sequences remain on the $22q^-$. These results indicate that the lambda light chain locus on the long arm of chromosome 22 is oriented such that Vλ is proximal to Cλ.

INTRODUCTION

The immunoglobulin lambda light chain gene has been previously mapped to chromosome 22 by somatic cell genetic techniques (1). More recently, we have regionally localized this locus to the q11 band of chromosome 22 by in situ hybridization with a probe for part of the lambda light chain constant region (2). Information regarding the molecular organization of lambda variable region genes and their germ line or chromosomal configuration has been limited by the absence of cloned probes for Vλ sequences. Our recent cloning of a cDNA probe which codes for the NEW protein of Vλ subgroup I (3) has permitted us to examine the orientation of the lambda locus in 22q11.

Recent studies have demonstrated direct involvement of immunoglobulin heavy and light chain genes in the non-random chromosomal translocations of Burkitt's lymphoma (4-8). These alterations involve the distal long arm of chromosome 8, (8q24-->qter) and the sites of immunoglobuin loci on chromosomes 14, 2 or 22 (1,5-8). They all result in transcriptional activation of the c-myc oncogene, which is moved from 8q24 to the $14q^+$ chromosome in t(8;14) Burkitt tumors and which remains on the involved chromosome 8 in the 2;8 and 8;22 variant rearrangements (6,9-11). Thus, a translocation that results in the juxtaposition of the c-myc oncogene and immunoglobulin gene sequences, regardless of whether orientation is in head-to-head or head-to-tail configuration, leads to the activation of the oncogene.

We and others have previously shown that the rearrangements of 22q11

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involved in the Burkitt tumors with the t(8;22) (q24;q11) translocation involve a breakpoint in 22q11 that is proximal to the Cλ gene cluster (2,12). Using somatic cell hybrids which retain the relevant chromosomes as well as in situ hybridization techniques, we demonstrated translocation of the Cλ locus to the $8q^+$ chromosome of the BL-2 Burkitt cell line (2,6). Thus, in BL-2, the c-myc oncogene, which stays on the involved chromosome 8, is proximal (5') to the Cλ locus, and becomes transcriptionally active as a result of the rearrangement (6). We have now studied cell line PA682, another Burkitt lymphoma cell line with the 8;22 translocation (13). We describe here in situ hybridization studies with probes for both Cλ and Vλ sequences. We show that the lambda locus is oriented with Vλ proximal to Cλ in 22q11 and that the translocation breakpoint of PA682 Burkitt lymphoma interrupts the lambda light chain locus.

MATERIALS AND METHODS

Probes

The Cλ probe for these studies was the 8.0 kb EcoRI fragment of the immunoglobulin λ light chain gene, originally a genomic clone (chr 22 λ 5) of this gene in λ Wes (6). This fragment includes the genes for two known nonallelic isotypes, Ke^-Oz^- and Ke^-Oz^+ (14). This 8.0 kb fragment of the Cλ gene was subcloned into pBR322. Two Vλ probes were used, pHλ6 and pHVλ6. pHλ6 contains both Cλ and Vλ sequences (3). This probe was isolated from a cDNA λ clone of the BL-2 cell line, a lambda producer (18). The pHλ6 probe is about 450 bp in length and contains both Cλ and Vλ exons (3). pHVλ6 is a subcloned 177 bp fragment which contains only the Vλ specific sequences from the pHλ6 probe.

Chromosome Preparation and In Situ Hybridization

Metaphase chromosome spreads were prepared from the PA682 cell line using standard techniques. These cells carry the t(8;22) translocation as previously described (13). Air dried slides were permitted to age in the cold (4°C) for at least 1 week prior to their use in the mapping studies.

In situ hybridization studies were performed using a protocol modified from several in the literature (2,8,16-17). Air-dried metaphase chromosome preparations on glass slides were used 1-3 weeks after preparation. Slides were treated with RNAse to remove any chromosomally bound RNA. The slides were washed free of RNAse and then dehydrated through an alcohol series. Chromosomal DNA was denatured by immersing the slides in 2xSSC/70% formamide at a temperature of 70°C, followed by rapid transfer through an alcohol series for dehydration.

Probe DNA was ^3H-labeled by nick translation to a specific activity of

$2x10^7$ cpm/ug according to the protocol described by Maniatis et al (15) and the DNA was separated from labeled nucleotides by chromatography on a Sephadex column. Carrier salmon sperm DNA was added to an excess of 1000x and the DNA was ethanol precipitated. DNA was resuspended in the hybridization mix which consisted of 25% formamide/2xSSC/10% dextran sulfate, pH 7.0. Probe DNA was denatured for 5 minutes at 70°C, quickly chilled in ice and was added to the slides at a concentration of 0.05 ug/ml. Coverslips were placed on the slides which were put in moist chambers and hybridized for 18 hours at 37°C. After hybridization the slides were extensively washed at 39°C to remove non-specifically bound labeled DNA, and were dehybrated through an alcohol series. Slides were dipped in liquid nuclear track emulsion (Kodak NTB-2), stored in dark boxes, and developed at appropriate intervals. Slides were banded using a modified Wright's Giemsa protocol (17) and analyzed under the microscope. Metaphase spreads were selected with good chromosome morphology and limited background grains and the location of specific grains was noted.

RESULTS AND DISCUSSION

We wished to determine the position of the constant region of the lambda light chain with respect to the translocation breakpoint. Data from four experiments using the C probe on PA682 cells are summarized in Table 1. The findings are consistent with translocation of the entire C λ gene cluster to the $8q^+$ chromosome, since there is no demonstrable hybridization of the probe to the $22q^-$ chromosome. A total of 177 metaphases from PA682 hybridized with the Cλ

Table 1. Hybridization of the Chr 22λ5, pHλ6 and pHVλ6 Probes to PA682 Cells

				Location of Grains		
Probe	Experiment No.	No. Metaphases	No Grains	$8q^+$	22	$22q^-$
Cλ	1	34	69	39/69 = 56.5%	1/69 = 1.4%	0
(chr 22 λ5)	2	33	42	18/42 = 42.9%	3/42 = 7.1%	0
	3	55	115	41/115 = 35.7%	10/115 = 8.7%	0
	4	55	137	58/137 = 42.3%	8/137 = 5.8%	0
Total		177	363	156/363 = 43%	22/363 = 6.1%	0
Cλ + Vλ (pHλ6)	5	55	113	23/113 = 20.4	11/113 = 9.7%	6/113 = 5.3%
	6	33	55	14/55 = 25.5%	6/55 = 10.9%	5/55 = 9.1%
	7	60	91	28/91 = 30.8%	2/91 = 2.2%	8/91 = 8.8%
Total		148	259	65/259 = 25.1%	19/259 = 7.3%	19/259 = 7.3%
Vλ (pHVλ6)	8	100	190	38/190 = 20%	10/190 = 5.3%	10/190 = 5.3%

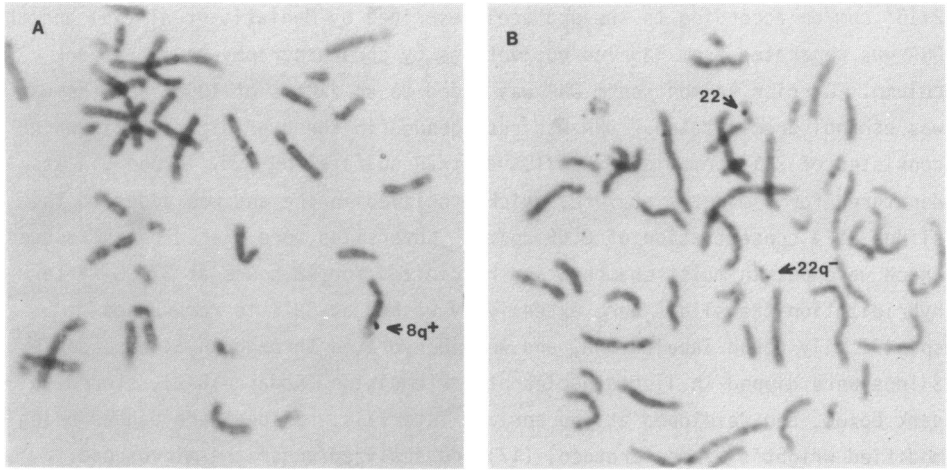

Fig. 1. Representative autoradiographs of metaphase chromosomes of PA682 cells hybridized with ^{3}H labeled pHλ6 probe having both Cλ and Vλ sequences. (a) arrow indicates hybridization to the $8q^{+}$ chromosome; (b) arrow indicates hybridization to the normal 22 and the $22q^{-}$ chromosomes.

probe were analyzed in which a total of 363 grains were located on chromosomes. Of these grains 156 (43%) were on the 8q+ chromosome and 22 (6.1%) were on the normal chromosome 22. In no experiment were grains seen on the $22q^{-}$ chromosome, suggesting an absence of C λ sequences as a result of the translocation. This result is similar to our findings with the BL-2 Burkitt lymphoma cells (2,6) and indicates that the breakpoint in 22q11 of PA682 cells is also proximal to the C λ gene cluster. Of note is the intense hybridization of the Cλ probe to the $8q^{+}$ chromosome, which was often observed as clusters of grains on the distal long arm.

In order to determine the role of Vλ in the 8;22 translocation hybridization of PA682 metaphase preparations were also carried out with the recently cloned pHλ6 probe which contains C λ and Vλ sequences. As shown in Table 1 (experiments 5, 6 and 7) and Fig. 1, this probe hybridized to both the 8q+ and the $22q^{-}$ chromosomes of PA682, as well as to the normal chromosome 22. The amount of hybridization to the normal 22 and to the $22q^{-}$ (7% of total grains) was greater than the level of hybridization to any other non-relevant chromosomal region of comparable size. The next highest site was 17q with 1.5% of all grains per copy. The hybridization to both number 22 chromosomes is therefore presumed to be light chain specific, and must indicate the presence of variable region sequences, since no grains were observed on the $22q^{-}$ using the probe containing Cλ alone. Thus, we suggest that at least some variable region sequences

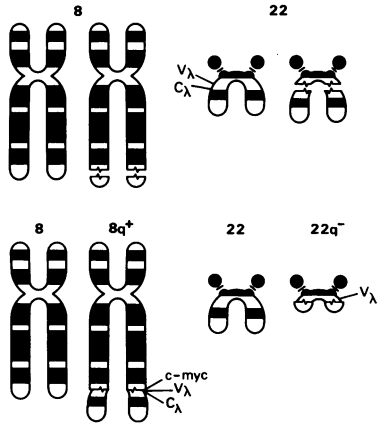

Fig. 2. Diagram of the 8;22 translocation in the PA 682 Burkitt lymphoma cell line. The Vλ genes are proximal and the Cλ gene cluster is distal in band q11 of chromosome 22. While some Vλ genes stay on the $22q^-$ chromosome, the Cλ cluster and some Vλ sequences translocate to the involved chromosome 8 ($8q^+$).

are retained on the $22q^-$ chromosome of PA682.

Finally, an additional experimental was performed using the subcloned 177 bp fragment of the pHλ6 probe (Table 1, experiment 8), called pHVλ6. This probe contains only the Vλ sequences of pHλ6. The data from this experiment, involving 100 metaphases, indicate that hybridization of the Vλ probe to the normal 22 and to the $22q^-$ chromosomes was detected with equal frequency (5% of total grains). This frequence was again greater than the hybridization signal detected on any other non-relevant chromosomal arm regardless of size, and so was taken to represent variable region specific hybridization. Interestingly, intense hybridization to the 8q+ chromosome (20% of total grains) was again observed in this experiment and presumably reflects translocation of Vλ sequences to the involved chromosome 8. The pHV 6 probe contains only Vλ sequences and does not contain any Cλ sequences. Therefore, hybridization of the pHV 6 to both derivative chromosomes, 8q+ and $22q^-$, suggests that the lambda variable region genes are interrupted as a result of the 8;22 translocation.

Taken together, these data with three different probes indicate that in the PA682 8;22 rearrangement all of the Cλ and some Vλ sequences are translocated to the 8q+ chromosome with retention of at least some Vλ sequences on the involved chromosome 22. This indicates that the lambda light chain locus on chromosome 22 is oriented with the variable region sequences proximal to the constant region with respect to the centromere (Fig. 2). This orientation is

similar to that of the kappa locus (9) but opposite to that of the heavy chain gene (5).

We have noted that all three probes showed an intense hybridization to the 8q+ chromosome. Although this could indicate increased gene copy number, there is neither cytologic nor molecular evidence for amplification of Cλ genes in these cells. We did not observe an abnormally banded region or homogeneously staining region on the $8q^+$, and there is no molecular evidence of Cλ amplification by Southern blot analysis of PA682 DNA (J. Erikson and C.M. Croce, unpublished results). It is possible that this marked hybridization may represent a position effect reflecting an increased accessibility of these sequences as a result of the translocation (6). In studies of drosophila and mice, the term "position effect" has been used to describe a change in the staining of chromosome bands as a result of structural rearrangement (18,19). In studies of 8;14 Burkitt translocations with the fluorochrome acridine orange, Hecht and McCaw demonstrated a color shift of the 8q material involved in the rearrangement (20). They suggested that the red to green shift that they observed was the result of a change from heterochromatin to euchromatin. Perhaps a similar translocation mediated alteration of chromatin could account for our finding in the PA682 Burkitt lymphoma cell line, although why it was not observed in the BL-2 or in other Burkitt tumors that we have investigated by in situ hybridization remains to be explained. In addition, these results provide evidence in a Burkitt's lymphoma with the 8;22 translocation that the breakpoint in 22q11 interrupts the lambda light chain locus, with the further indication that at least in this tumor the break is in the variable region. Thus, this 8;22 rearrangement is analogous to many of the 2;8 and 8;14 Burkitt tumor rearrangements in which the immunoglobulin chain genes are interrupted in the variable region (5,8,9). Finally, these results suggest that other chromosomal rearrangments, with breakpoints in 22q11, can be effectively studied with these techniques and these probes to determine the location and role of Vλ with respect to the translocation junction.

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