[CANCER RESEARCH 52, 6083–6087, November 1, 1992]

Advances in Brief

The *RCK* Gene Associated with t(11;14) Translocation Is Distinct from the *MLL/ALL-1* Gene with t(4;11) and t(11;19) Translocations[1]

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Abstract

We previously demonstrated that the 11q23 breakpoint region, designated the *RCK* locus, of the RC-K8 B-lymphoma cell line with t(11;14)(q23;q32) is centromeric to *PBGD*, while breakpoints of infantile leukemia cell lines with t(11;19)(q23;p13) are detectable by pulsed-field gel electrophoresis with the *CD3D* probe. In the present study, using a probe within 1.0 kilobase of the t(11;14) breakpoint, we isolated a partial complementary DNA clone for the putative *RCK* gene, which detects a 7.5-kilobase mRNA. Sequence analysis predicted a novel protein of 472 amino acids which demonstrated sequence homology to a translation initiation factor/helicase family. We also isolated a phage clone from the *CD3D/G* yeast artificial chromosome clone (yB22B2) which detects 11- and 12-kilobase mRNAs, most likely for the *MLL/ALL-1* gene associated t(4;11)(q21;q23) and t(11;19)(q23;p13) translocations. By pulsed-field gel electrophoresis after *Not*I digestion, this recombinant clone is on a 96-kilobase fragment, while *RCK* and *PBGD* probes are on a more telomeric 690-kilobase *Not*I fragment. These results, altogether, suggested that two different genes, *RCK* and *MLL/ALL-1*, are associated with 11q23 translocations of hematopoietic tumors.

Introduction

A wide variety of hematopoietic malignancies, including acute leukemia, myeloproliferative disorder, and malignant lymphoma, are associated with chromosomal translocations at chromosome 11 band q23 (11q23), although the donor chromosomes are variable. Considering the variety of the cytogenetic events, it would be interesting to know whether the same gene is associated with some or all of these translocations. A candidate gene, designated mixed lineage leukemia (*MLL*) or acute lymphocytic leukemia-1 (*ALL-1*), has been reported by two groups of investigators (1, 2) to be associated with t(4;11)-(q21;q23) and t(11;19)(q23;p13) translocations observed in tumors which often exhibit biphenotypic characteristics. In our previous study, we cloned the breakpoint of t(11;14)(q23;q32) in B-cell lymphoma, the RC-K8 cell line, and named the locus *RCK* (3, 4). By PFGE,[3] *RCK* was shown to be centromeric to *PBGD*, while the breakpoints of t(11;19)(q23;p13) were detected by the *CD3D* gene probe which is centromeric to *RCK* (5, 6). In this study, we isolated a partial cDNA for the putative *RCK* gene and also a phage clone from the *CD3* YAC clone, which probably detects transcripts of the *MLL/ALL-1* gene. By PFGE, we studied the long range mapping from *CD3* genes to the *PBGD* gene on 11q23 in order to see the relationship between *RCK* and *MLL/ALL-1*.

Materials and Methods

Cell Lines and YAC. Karpas 422, RS4;11, KOCL-45, KOCL-48, and KOCL-58 cell lines have t(4;11) translocations, while KOPN-1, KOCL-33, and KOCL-44 cell lines have t(11;19) translocations. RC-K8 has a t(11;14) translocation. The culture and characterization of these lines have been described (5). The YAC clone, yB22B2, containing *CD3* (7) was generously provided by Dr. M. O. Diaz at Chicago University and Dr. P. Domer at Washington University on request to Dr. M. Olson at Washington University.

Isolation of a Partial cDNA Clone for RCK. Recombinant clones (6×10^5) of a cDNA library prepared from a human small cell lung cancer cell line, SCLC-SA, in λgt10 vector (8) were screened with the conserved *Bss*HII/*Bss*HII 0.6-kilobase fragment (probe c) which is within 1.0 kilobase of the t(11;14) breakpoint (Fig. 1*A*). Hybridization was performed in a buffer containing 40% formamide, 100 μg/ml of salmon sperm DNA, 10% dextran sulfate, and 5 × standard sodium-citrate buffer (1 × concentration is 0.15 M NaCl-0.015 M sodium citrate) at 42°C. The filters were washed in a final stringency of 0.1 × standard saline-citrate-0.1% sodium dodecyl sulfate at 56°C. Positive phage plaques were purified by standard procedure (8) and used for further analysis.

Construction of Genomic Library from a YAC Clone yB22B2. A genomic library was constructed using the λDASH II vector (Stratagene, La Jolla, CA). High-molecular-weight DNA extracted from yB22B2 was digested to completion with *Bam*HI and size fractionated on low melting point agarose, and DNA ranging from 9 to 23 kilobases was purified. After ligation into λDASH II and packaging, 3×10^4

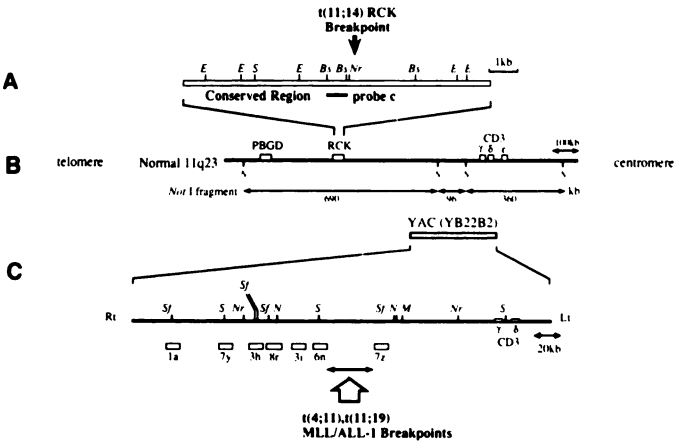

Fig. 1. Restriction map of 11q23 region. (*A*) Germline chromosome 11q23 near the *RCK* breakpoint region. *Solid line*, conserved region (probe *c*); *arrow*, t(11;14) *RCK* breakpoint. (*B*) Long-range map of the 11q23 region. The gene order, cen-*CD3-RCK-PBGD*, has been reported by us (5). (*C*) yB22B2 YAC clone was mapped with rare-cutting endonucleases. *Open arrow*, breakpoints of t(4;11) and t(11;19) translocation. Phage clones isolated from the YAC clone are mapped (▭). *E*, *Eco*RI; rare-cutting enzymes, *Bs*, *Bss*HII; *M*, *Mlu*I; *N*, *Not*I; *Nr*, *Nru*I; *S*, *Sal*I; *Sf*, *Sfi*I. *kb*, kilobases.

Received 5/28/92; accepted 9/17/92.

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[1] This work was supported in part by a Grant-in-Aid for the Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare; a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan; and a grant from the Cancer Research Institute, Inc., New York.

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[3] The abbreviations used are: PFGE, pulsed-field gel electrophoresis; cDNA, complementary DNA; YAC, yeast artificial chromosome.

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A

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1    GCGACTTCGGCGGCGCCACGAGAGCGGGCAGCGGAGGAGATTGACGTGAGTGAATTCAGA
61   TATAACTCAAGCTTGTTAGAGGGCTTTTTAAAAATAAAAAGTTGATTCCGTGCAAGAGAG
121  CAAGTTACTGCTGCTTACCGTTCAGAGACTTACAGGTGCTTGCCTGCATTGCAATAAAGG
181  ACTCATTTATTGAGCAAGACTTATATTTATCTCTTCATTTTGGAGAGCCTAATAAACTGT
241  TATTACAGTTTCTCTACTGACTTTCAAAAGTTTTGAAGTTTGAAAGACCTTTGCAATTAA
301  AACAGCATGAGCACGGCCAGAACAGAGAACCCTGTTATAATGGGTCTGTCCAGTCAAAAT
1                                        M  G  L  S  S  Q  N

361  GGTCAGCTGAGAGGCCCTGTGAAACCCACTGGTGGCCCTGGAGGAGGGGGCACACAGACA
8    G  Q  L  R  G  P  V  K  P  T  G  G  P  G  G  G  G  T  Q  T

421  CAGCAACAGATGAACCAGCTGAAAAACACCAACACAATCAATAATGGCACTCAGCAGCAA
28   Q  Q  Q  M  N  Q  L  K  N  T  N  T  I  N  N  G  T  Q  Q  Q

481  GCACAGAGTATGACCACCACTATTAAACCTGGTGATGACTGGAAAAAGACTTTAAAACTC
48   A  Q  S  M  T  T  T  I  K  P  G  D  D  W  K  K  T  L  K  L

541  CCTCCAAAGGATCTAAGAATCAAAACTTCGGATGTGACCTCCACAAAAGGAAATGAGTTT
68   P  P  K  D  L  R  I  K  T  S  D  V  T  S  T  K  G  N  E  F

601  GAAGATTACTGTTTGAAACGGGAGTTACTGATGGGAATTTTTGAAATGGGCTGGGAAAAG
88   E  D  Y  C  L  K  R  E  L  L  M  G  I  F  E  M  G  W  E  K

661  CCATCTCCTATTCAGGAGGAGAGCATTCCCATTGCTTTATCTGGTAGGGATATCTTAGCT
108  P  S  P  I  Q  E  E  S  I  P  I  A  L  S  G  R  D  I  L  A...

721  AGAGCAAAAAATGGAACAGGCAAGAGCGGTGCCTACCTCATTCCCTTACTTGAACGGCTA
128  R  A  K  N  G  T  G  K  S  G  A  Y  L  I  P  L  L  E  R  L

781  GACCTGAAGAAGGACAATATACAAGCAATGGTGATTGTTCCCACTAGAGAACTTGCTCTA
148  D  L  K  K  D  N  I  Q  A  M  V  I  V  P  T  R  E  L  A  L

841  CAGGTCAGTCAAATTTGCATCCAGGTCAGCAAACACATGGGAGGGGCCAAAGTGATGGCA
168  Q  V  S  Q  I  C  I  Q  V  S  K  H  M  G  G  A  K  V  M  A

901  ACCACAGGAGGAACCAATTTACGAGATGACATAATGAGGCTTGATGATACAGTGCACGTG
188  T  T  G  G  T  N  L  R  D  D  I  M  R  L  D  D  T  V  H  V

961  GTGATTGCTACCCCTGGGAGAATCCTGGATCTTATTAAGAAAGGAGTAGCAAAGGTTGAT
208  V  I  A  T  P  G  R  I  L  D  L  I  K  K  G  V  A  K  V  D

1021 CATGTCCAGATGATAGTATTGGATGAGGCAGATAAGTTGCTGTCACAGGATTTTGTGCAG
228  H  V  Q  M  I  V  L  D  E  A  D  K  L  L  S  Q  D  F  V  Q

1081 ATAATGGAGGATATTATTCTCACGCTACCTAAAAACAGGCAGATTTTACTATATTCCGCT
248  I  M  E  D  I  I  L  T  L  P  K  N  R  Q  I  L  L  Y  S  A

1141 ACTTTCCCTCTTAGTGTACAGAAGTTCATGAATTCCCATTTGCAGAAACCCTATGAGATT
268  T  F  P  L  S  V  Q  K  F  M  N  S  H  L  Q  K  P  Y  E  I

1201 AACCTGATGGAGGAACTAACTCTGAAGGGAGTAACCCAGTACTACGCATATGTAACTGAG
288  N  L  M  E  E  L  T  L  K  G  V  T  Q  Y  Y  A  Y  V  T  E

1261 CGCCAAAAAGTACACTGCCTCAACACACTTTTCTCCAGGCTTCAGATAAACCAGTCGATC
308  R  Q  K  V  H  C  L  N  T  L  F  S  R  L  Q  I  N  Q  S  I

1321 ATTTTCTGTAACTCCTCTCAGCGAGTTGAATTGCTAGCCAAGAAGATTTCTCAACTGGGT
328  I  F  C  N  S  S  Q  R  V  E  L  L  A  K  K  I  S  Q  L  G

1381 TATTCTTGCTTCTATATTCATGCTAAAATGAGGCAGGAACATCGAAATCGTGTATTTCAT
348  Y  S  C  F  Y  I  H  A  K  M  R  Q  E  H  R  N  R  V  F  H

1441 GATTTCCGAAATGGCTTATGCCGCAATCTTGTTTGCACTGATCTGTTTACCCGAGGTATT
368  D  F  R  N  G  L  C  R  N  L  V  C  T  D  L  F  T  R  G  I

1501 GATATACAAGCTGTGAATGTGGTAATAAACTTTGATTTCCCAAAGCTGGCAGAGACCTAT
388  D  I  Q  A  V  N  V  V  I  N  F  D  F  P  K  L  A  E  T  Y

1561 CTCCATCGTATTGGAAGATCAGGTCGCTTTGGTCATCTTGGCTTAGCCATCAACTTGATC
408  L  H  R  I  G  R  S  G  R  F  G  H  L  G  L  A  I  N  L  I

1621 ACATATGATGATCGCTTCAACCTGAAAAGTATTGAGGAGCAGCTGGGAACAGAAATTAAA
428  T  Y  D  D  R  F  N  L  K  S  I  E  E  Q  L  G  T  E  I  K

1681 CCTATTCCGAGCAACATTGATAAGAGCCTGTATGTGGCAGAATACCACAGCGAGCCTGTA
448  P  I  P  S  N  I  D  K  S  L  Y  V  A  E  Y  H  S  E  P  V

1741 GAAGATGAGAAACCTTAACAAGCATGCTTTGACAAATTACAAAAGGCTCGTTTGGATCTG
468  E  D  E  K  P  *

1801 TGACACATCGTTTTGGGGGGAATGCTCTTCTCTTTGTGGGTTTTTCATCTTTTATTTTGG
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1921 GAAAAGAAGGAATATACCTTTTTTGTTCCACTTGTTTGCACTGTGTGCTGACTGAACATT
1981 AGTTGCACTAACTGCTGGTTTTTAAAAAATGTTTTCTGGGGAAAGGGGACAAGGAAGGAA
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4141 CGAGATAAGAGGG
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B

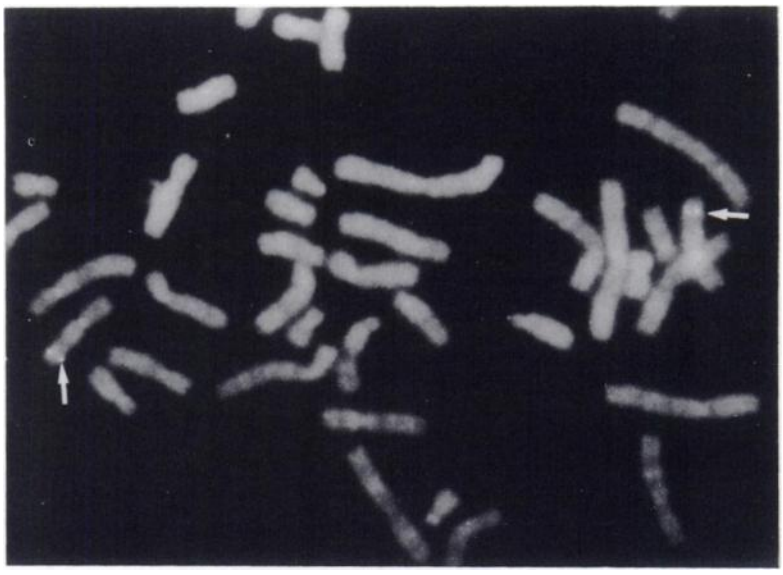

Fig. 2. Primary structure of *RCK* cDNA with the deduced amino acid sequence and *in situ* hybridization of *RCK* cDNA to R banding metaphase peripheral lymphocyte. (*A*) Nucleotide sequence of RCK cDNA shown with the deduced amino acid sequence. The 39-base pairs at the 5′ end found in conserved region c (Fig. 1*A*) is *double underlined. Wavy line,* an in-frame stop codon. Two possible initiation codons are *single underlined.* The second initiation codon, ATG, is in good agreement with Kozak's rule (11) and the deduced amino acid is shown under the nucleotide sequence. Motif I (AxAxxGTGK) and motif II (LDEAD) of the translation initiation facator/helicase family (12, 13) are marked by *dotted underlines.* (*B*) *RCK* cDNA clone, SA-30 (1.8-kilobase fragment at 5′ end of cDNA), used as a probe for *in situ* hybridization. *White arrows*, positive fluorescence at chromosome 11 band q23.

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recombinant phages were screened with radiolabeled human placental DNA. A total of 41 phages were randomly selected and were classified into 10 independent clones by restriction map analysis. Seven of them were further analyzed by PFGE.

PFGE, Northern Blot, and Southern Blot Analyses. These analyses were conducted as described previously (5, 9).

Fluorescence *in Situ* Hybridization. Biotin-labeled probe was prepared by nick-translation using Bio-16-dUTP (Boehringer Mannheim, Tokyo, Japan). *In situ* hybridization was performed using the method described previously (10).

DNA Sequencing and Homology Search. Nucleotide sequence was determined by the dideoxy chain termination method using a Sequenase kit (United States Biochemicals, Cleveland, OH). DNA and amino acid sequences were analyzed using the GENETYX program (Software Development Co., Tokyo, Japan). Homology search was done with the use of the data base of LASL-GDB Rel. 70 and EMBL-GDB Rel. 29.

Results and Discussion

***RCK* Gene Is Associated with t(11;14)(q23;q32) Chromosome Translocation.** The nucleotide sequence across the breakpoint on 11q23 of the t(11;14) translocation in RC-K8 cells is GC rich [69%] and 9 potential SP-1 binding sequences [GGGCGG] existed, suggesting that this region is a promoter region of a housekeeping gene. Probe c [*Bss*HII 0.6-kilobase fragment (Fig. 1*A*)], containing the region conserved in various species, was used to screen a cDNA library from a small cell lung cancer cell line, SCLC-SA. Five overlapping cDNA clones with the same restriction map at the 5′ end were obtained. Repeated cDNA walking resulted in overlapping clones covering 4.2 kilobases (Fig. 2*A*).

Sequence analysis of the cDNA demonstrated that the initiation codon at the 340 nucleotide surrounded by Kozak's consensus sequence (11) was followed by a long open reading frame encoding a 472-amino acid protein with a relative molecular mass of 53,216 Da (Fig. 2*A*). A homology search of the entire RCK amino acid sequence revealed a sequence similar to that of mouse eIF-4A (38.4% identity in 407 amino acids) or *Drosophila* ME31B (70.0% identity in 426 amino acids) of a translation initiation factor/helicase family (12, 13). In this regard, it is noted that one translation initiation factor, eIF-4E, exhibits transforming activity in NIH3T3 cells when it is overexpressed by retroviral expression construct (14). Comparisons of the nucleotide sequence revealed that the first 39-base pair sequence of the 5′ end of the cDNA was in the conserved region c (Figs. 1*A* and 2*A*). The orientation of the sequence is directed to the centromere, indicating that the promoter region and the possible first exon of 39 base pairs of the *RCK* gene are decapitated from its coding region and that the coding region on der (11) is likely to be under the influence of the immunoglobulin heavy chain gene. Cloning analysis of der (11) confirmed that the region juxtaposed to *RCK* was a part of immunoglobulin genes (data not shown).

Localization of the *RCK* gene at 11q23 was demonstrated by *in situ* hybridization using a cDNA clone, SA-30. As shown in Fig. 2*B*, it hybridized to chromosome 11 band q23 of 20-metaphase peripheral blood lymphocytes, confirming that the transcriptional unit is from this region. Northern blot analysis with cDNA probe revealed a 7.5-kilobase mRNA (Fig. 3*A*) and it was found that the expression was ubiquitous in various tissues (data not shown). Although *RCK* is at the t(11;14) breakpoint region, the pattern of *RCK* expression in RC-K8 and other cell lines as well as in normal peripheral blood lymphocytes are not detectably altered (Fig. 3*A*). The mechanism of the *RCK* gene leading to malignant transformation by this translocation needs to be studied.

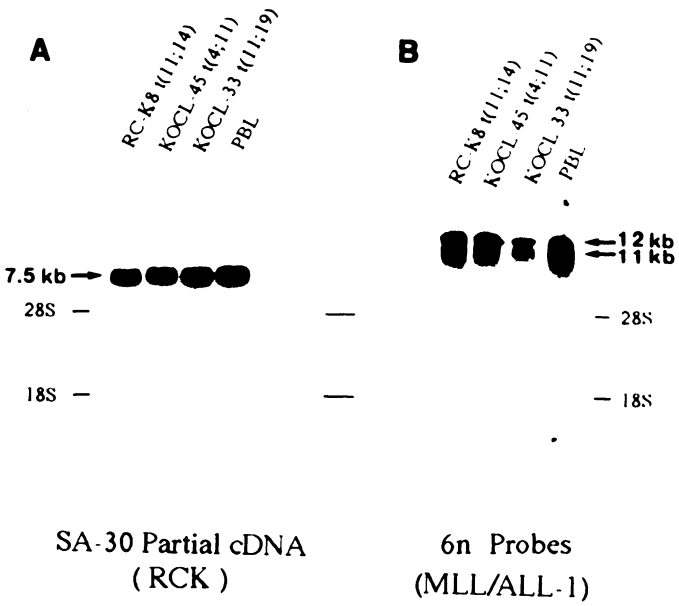

Fig. 3. Northern blot analysis of total cellular RNA from various human cell lines with *RCK* and *MLL/ALL-1* probes. *RC-K8*, B-lymphoma cell line with t(11;14); *KOCL-45*, infantile leukemia cell line with t(4;11); *KOCL-33*, infantile leukemia cell line with t(11;19); *PBL*, peripheral blood lymphocytes. The filter was hybridized with the *RCK* cDNA probe SA-30 (*A*), or with a mixture of reiterated sequence-free probes from 6n (*B*). The specific hybridized bands are denoted by *arrows*. mRNA sizes in kilobases (*kb*) are also shown.

Probes Isolated from yB22B2 YAC Clone. We showed previously that the breakpoints of t(11;19) are detectable by PFGE with the *CD3D* probe (5). Thus, we attempted to isolate phage clones, which can detect rearrangements and transcriptional units, from a YAC clone with a 300-kilobase insert containing *CD3D/G*, but not *CD3E* (Fig. 1*B*). A genomic library was constructed with yB22B2 DNA after *Bam*HI complete digestion and screened with radiolabeled total human DNA. Seven independent clones, 1a, 7y, 3h, 8r, 3i, 6n, and 7z, were mapped on yB22B2 with the reiterated sequence-free probe of each clone (Fig. 1*C*). The restriction map of the yB22B2 insert constructed by us was almost identical to those reported by other investigators (1, 2) except for some enzyme sites. *Not*I and *Sal*I double digestion enabled us to map 8r, 3i, 6n, and 7z to the 96-kilobase *Not*I fragment (Fig. 1*C*).

The unique probes from 8r, 3i, and 6n detected rearranged bands in all t(4;11) cell lines except Karpas 422 and in all three t(11;19) cell lines by *Not*I and *Nru*I digestion, respectively (a part of the results with 6n is shown in Fig. 4, *A* and *C*). On the other hand, the unique probe from 7z also detected rearranged bands with different sizes from those detected by 8r, 3i, and 6n unique probes (Fig. 4, *B* and *D*). These data indicated that some of the breakpoints of t(4;11) and t(11;19) lines are between 6n and 7z clones.

An attempt was then made to detect transcriptional units. A mixture of reiterated sequence-free probes from 6n detected 12- and 11-kilobase mRNA signals, the size and the pattern of which are similar to those of the *MLL/ALL-1* gene which has been reported by two groups of investigators (1, 2). The expression in cell lines with t(4;11) and t(11;19) is not significantly different from RC-K8 or peripheral blood lymphocytes, which is in agreement with the previous reports by van der Poel *et al.* (1) and Cimino *et al.* (2).

***RCK* and *MLL/ALL-1* Genes.** The unique probes from 1a, 7y, and 3h hybridized to a 690-kilobase *Not*I germline fragment and to the rearranged band in RC-K8 on the same filter, while these probes did not detect any rearranged band in t(4;11) and

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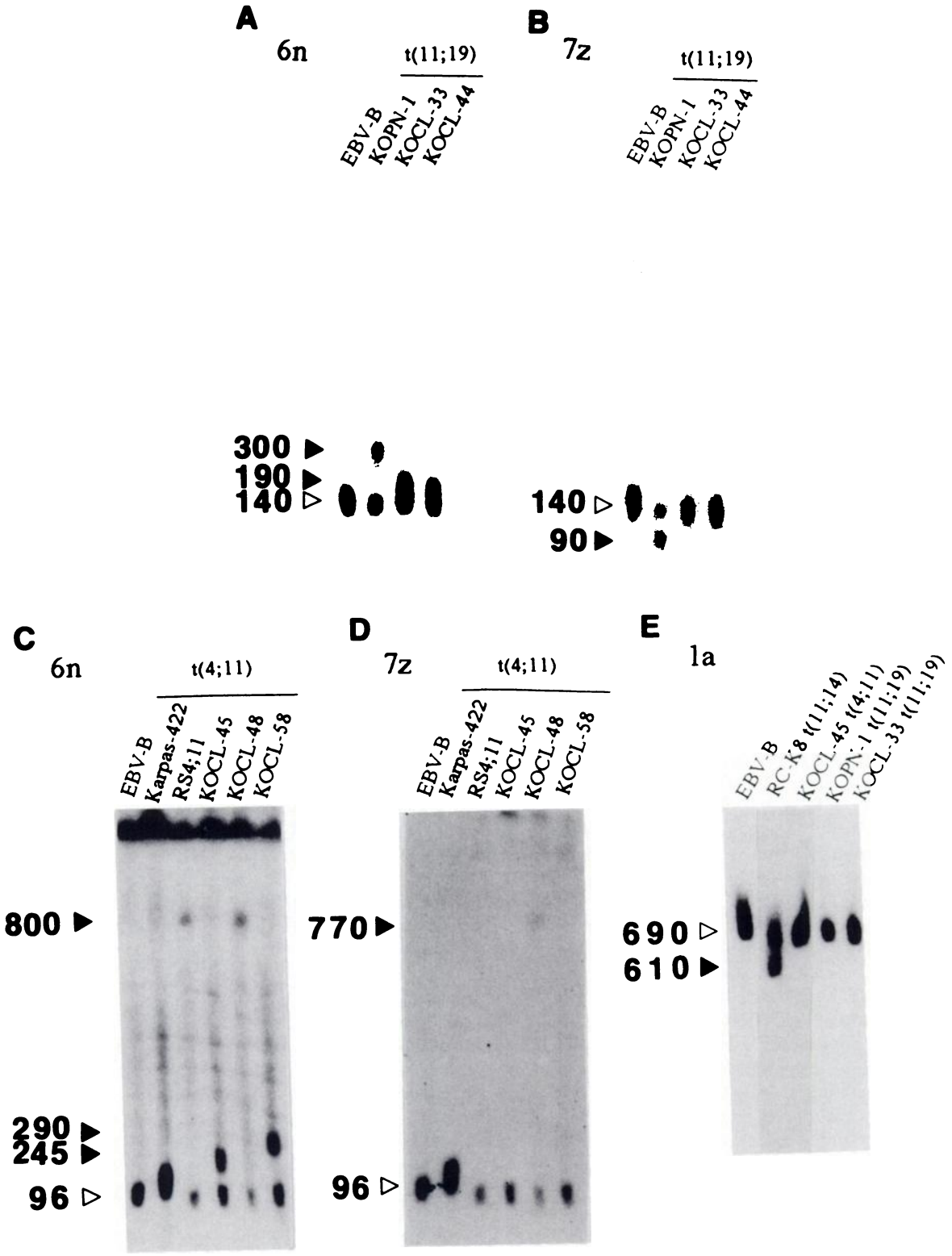

Fig. 4. PFGE analysis of cell lines with 11q23 abnormalities using 6n, 7z, and 1a probes. The probe for A and C filters is a reiterated sequence-free 0.7-kilobase *Xba*I/*Bam*HI fragment of centromeric end of 6n. The probe for B and D filters is a reiterated sequence-free *Hin*dIII/*Pst*I 1.0-kilobase fragment from 7z. The probe for E is a reiterated sequence-free *Hin*dIII/*Xba*I 1.0-kilobase fragment from 1a. *A* and *B* are the same filter, while *C* and *D* are the same filter. The enzyme used is *Nru*I for *A* and *B*, and *Not*I for *C, D*, and *E. EBV-B*, Epstein-Barr virus-transformed B-cells with normal karyotype. See "Materials and Methods" for the other cell lines. DNA fragment sizes in kilobases are shown on the *left* of each filter. *Open triangle*, germ-line band; *closed triangle*, rearranged band.

t(11;19) cell lines by *Not*I digestion (Fig. 4*E*). The observation that 1a, 7y, and 3h probes derived from yB22B2 hybridized to the same *Not*I fragment detected by *RCK* probe indicated that the 690- kilobase *Not*I fragment adjoins to the 96-kilobase *Not*I fragment where t(4;11) and t(11;19) breakpoints are located (Fig. 1*B*). Although the *Not*I fragments adjoin each other, the size of transcriptional units near the breakpoints of *RCK* and *MLL/ALL-1* is different, suggesting that the gene involved for t(11;14) is different from the one for t(4;11) and t(11;19) translocations.

The present study showed that a novel gene, *RCK*, is found at the breakpoint region of t(11;14) and is a candidate gene to be

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involved in this translocation. Although *MLL/ALL-1* cDNA has not been cloned yet, the transcriptional units detected by van der Poel *et al.* (1) and Cimino *et al.* (2) are most likely to be derived from the candidate gene associated with t(4;11) and t(11;19). Thus, at least two different genes, *RCK* and *MLL/ALL-1*, are involved in the translocations at 11q23 region. Further study on these genes as well as the genes on the donor chromosomes will bring new aspects in understanding hematopoietic malignancies with 11q23 translocations.

Acknowledgments

The authors are grateful to Drs. M. O. Diaz at the University of Chicago and P. Domer and M. Olson at Washington University for providing the yB22B2 YAC clone. The critical discussions and helpful suggestions of Drs. T. Imai at the Cancer Institute (Tokyo, Japan) and E. Soeda at Riken (Tsukuba, Japan) were acknowledged. We also thank C. Nakamura, K. Nishida, and K. Okumura for their technical assistance.

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The *RCK* Gene Associated with t(11;14) Translocation Is Distinct from the *MLL/ALL-1* Gene with t(4;11) and t(11;19) Translocations

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Cancer Res 1992;52:6083-6087.

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