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Cloning, expression and localization of an RNA helicase gene from a human lymphoid cell line with chromosomal breakpoint 11q23.3

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ABSTRACT

A gene encoding a putative human RNA helicase, p54, has been cloned and mapped to the band q23.3 of chromosome 11. The predicted amino acid sequence shares a striking homology (75% identical) with the female germline-specific RNA helicase *ME31B* gene of *Drosophila*. Unlike *ME31B*, however, the new gene expresses an abundant transcript in a large number of adult tissues and its 5′ non-coding region was found split in a t(11;14)(q23.3;q32.3) cell line from a diffuse large B-cell lymphoma.

INTRODUCTION

Members of the rapidly expanding superfamily of RNA helicases are involved in specialized RNA functions and contain ten motifs that have been evolutionarily conserved (1,2). Three of these motifs have been found to be the A and B sites for ATP binding (2,3) and a helicase domain for RNA unwinding activity (4). Variations in gene structure, cellular localization and function makes it possible to divide the superfamily into three main groups of proteins. One group functions as translation initiation factors, represented by eIF-4AI/II in the mouse (5) and *TIF1*/*TIF2* in yeast (6). The second group includes proteins involved in pre-mRNA splicing (e.g., PRP5 and PRP28) [7,8], pre-rRNA splicing (SPB4) [9], and mitochondrial RNA splicing (MSS116) [10] in yeast. The third group of RNA helicases includes genes abundantly or specifically expressed in germline cells (*vasa* in male and female germline of *Drosophila* [11], *ME31B* in female germ cells of *Drosophila* [12], and PL10 in male germline of mouse [13]). In this work, we describe a human p54 gene with structural features very closely resembling the oocyte-specific *ME31B* gene of *Drosophila*, but having widespread and abundant transcriptional expression in human adult tissues. The gene maps to band q23.3 of chromosome 11, since it was disrupted at the 11q23.3 breakpoint of a balanced reciprocal chromosomal translocation t(11;14)(q23.3;q32.3) found in a human RC-K8 lymphoma cell line.

MATERIALS AND METHODS

Genomic DNA cloning

A Chinese hamster-human somatic cell hybrid DNA, containing as a single human chromosome the derivative 14q+ (14) of the t(11;14)(q23.3;q32.3) RC-K8 cell line (15), was used to construct a genomic library as previously described (16). Positive clones were obtained by screening 5×10^5 recombinants with a $C\gamma3$ cDNA probe (17), which is known to cross hybridize to all $C\gamma$ genes (18). A second genomic library constructed from EBV-transformed B-lymphocytes was screened to obtain the normal chromosome 11 clone, using a chromosome 11-specific p11HH0.5 probe (Figure 1).

Isolation of Poly(A)$^+$ RNA

Total human cellular RNA was extracted with guanidium thiocyanate (19). Oligo(dT)-cellulose chromatography (Collaborative Research, Inc.) was used to obtain polyadenylated RNA.

Northern and Southern blots

Northern and Southern blot analysis were basically performed as previously described (16), except that Northern hybridization was done at 45°C, and Southern hybridization at 42°C in 50% formamide, 5×SSC, 1×Denhardt's solution and 1% SDS.

Isolation of p54 cDNA

Both oligo(dT) and random-primed cDNA libraries were constructed from the human RC-K8 cell line. For each cDNA library, five micrograms of poly(A)$^+$ mRNA was used as template, using the ZAP-cDNA synthesis kit (Stratagene) for the oligo(dT)-primed library and the You-prime cDNA synthesis kit (Pharmacia) for random hexamer-primed library. Double strand cDNAs were cloned into lambda ZAP II vector (Stratagene). From the random-primed library, clones RC-rp1 and RC-rp2 were obtained, which contain the open reading frame of the normal p54 gene. With the oligo(dT) cDNA library, we obtained clone RC-dT, which represents a fusion product of a segment of the 5′ non-translated region of the p54 gene with the membrane domains of an immunoglobulin $\gamma2$ heavy chain gene.

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Sequencing analysis of p54 cDNA

Purified cDNA clones were sequenced by the dideoxy chain termination method (20) after subcloning in a pBluescript Vector (Stratagene), using the US Biochemical sequencing kit. The deduced protein sequences were compared to entries in A-GeneSeq 2.0, PIR 30 and Swiss-Prot 20 data bases, using the program FASTDB (21) in the IntelliGenetics Suite 5.4 Software. Protein molecular weight was derived with the help of the PC/GENE program AACOMP.

In vitro transcription and translation of p54

cDNA clones RC-rp1 and RC-rp2, containing the coding region of p54 gene were cloned into the vector pBluescript SK− (Stratagene). *In vitro* transcription and translation was accomplished following the protocol of the Translation *In Vitro* Kit (Promega). The protein products were analyzed by SDS-PA-GE electrophoresis (19).

Somatic cell hybrids

Chinese hamster-human somatic cell hybrids used in Southern blot were derived having as a single human chromosome (i) a normal human chromosome 11, (ii) the 11q− or the 14q+ derivative of a t(11;14)(q23.3;q32.3) RC-K8 cell line, (iii) the 4q− or the 11q+ derivative of a t(4;11)(q21;q23.3) from a patient with acute lymphocytic leukemia, (iv) the Xq+ or the 11q− derivative of an inv ins (X;11)(q24;q23.3q21) from a patient with acute myelomonocytic leukemia (14).

RESULTS

Genomic cloning of the t(11;14)(q23.3;q32.3) breakpoint

We cloned the 11q23.3 breakpoint of an RC-K8 diffuse B-cell lymphoma cell line with a translocation t(11;14)(q23.3;q32.3) [15] that showed rearrangement of an immunoglobulin γ heavy chain constant region gene (Cγ). Cloning was accomplished using a Cγ cDNA probe (17) to screen a somatic cell hybrid genomic library containing the human 14q+ derivative chromosome of the translocation t(11;14). A Cγ-positive clone λ14q+4 was isolated (Figure 1) and the 5′ end of the clone, consisting of a single copy 1.8 kb *Eco* RI-*Bam* HI DNA segment (p14RB1.8), was used as probe for chromosome walking. A new partially overlapping clone, λ14q+1O, was identified and found to include a second Cγ-positive region, as well as a chromosome 11-specific DNA segment. The normal counterpart of chromosome 11 (clone λN11) was obtained by using a chromosome 11-specific 0.5 kb *Hind* III DNA segment (p11HH0.5 probe) from λ14q+10, to screen a normal human B-lymphocyte genomic library. The translocation breakpoint in the chromosome 14q+ was identified by comparison of the restriction enzyme patterns of the λ14q+10 and λN11 clones (Figure 1) and by sequence analysis (data not shown).

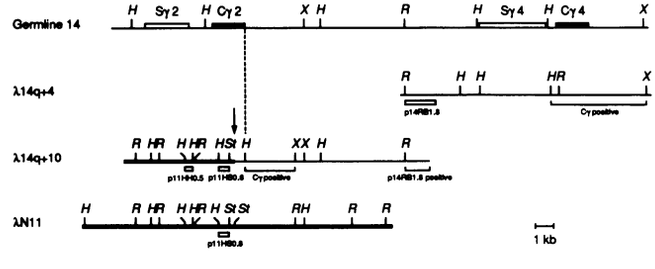

Figure 1. Restriction maps of genomic DNA from somatic cell hybrids containing the 14q+ chromosome of RC-K8 cells (14) and normal chromosome 11 from EBV-transformed B-lymphoid cells. The top line shows germline Cγ2 and Cγ4 DNA from a normal chromosome 14 (33). The switch and exon regions of Cγ2 and Cγ4 are indicated by open and solid boxes, respectively. The second and third lines are clones λ14q+4 and λ14q+10 isolated from chromosome 14q+. Clone λN11C illustrates a normal chromosome 11. Thin lines represent regions from chromosome 14, while thick lines represent those from chromosome 11. The breakpoint in the 14q+ is indicated with an arrow. H, *Hind* III; R, *Eco* RI; X, *Xho* I; St, *Stu* I. See text for details.

Isolation, sequencing and in vitro translation of cDNA encoding p54

Chromosome 11-specific DNA segments of genomic clones λ14q+10 and λN11 were tested on Zooblots containing DNA from gibbon, deer, cat, rabbit, hamster and mouse. Only a 0.6 kb *Hind* III-*Stu* I DNA segment (p11HS0.6), which was adjacent to the breakpoint (Figure 1), was found to be evolutionarily conserved (data not shown). p11HS0.6 was then used as probe in Northern blots and detected a normal 6.7 kb transcript in several human B-lymphoid cell lines. The RC-K8 cell line showed the 6.7 kb and an additional 1.8 kb fusion message (Figure 2A).

To clone the cDNA encoding the normal 6.7 kb mRNA, a random-primed cDNA library was constructed from the RC-K8 cell line. Using the genomic p11HSO.6 probe, clones RC-rp1 and RC-rp2 were isolated (Figure 3A). Clone RC-rp1 was sequenced as shown in figure 3B. The most 5′ methionine triplet (ATG) was found at 334−336, six base pairs from an in-frame stop. The sequence surrounding this triplet, C-A-G-C-ATG-A,

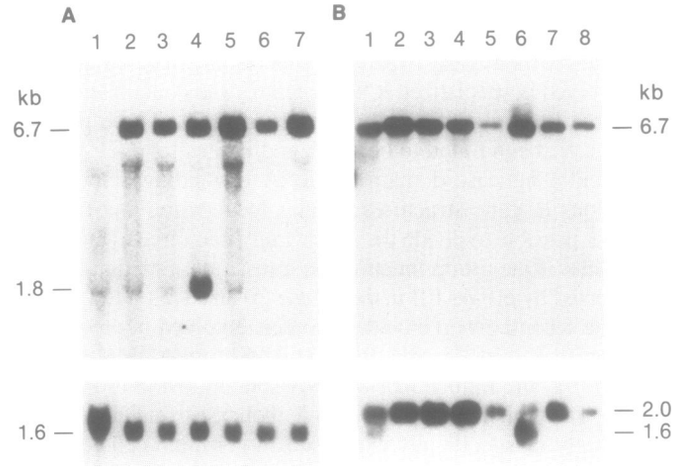

Figure 2. Northern blot analysis of p54 mRNA from various human tissues. **A** Each lane in the filter contained 5 μg of partially purified poly(A)$^+$ RNA from hematologic cell lines, hybridized with a p11HS0.6 genomic probe. Lane 1, H9 (late T-lymphoblasts) [34]; lane 2, LA369 (EBV immortalized B cells); lane 3, CL75, t(4;11)(q21;q32)-positive pre-B acute lymphoblastic leukemia; lane 4, RC-K8 t(11;14)(q23;q32)-positive B-cell diffuse lymphoma B-cell line; lane 5, Reh (pre-B cell line) [35]; lane 6, K562 (erythroleukemia cell line) [36]; and lane 7, HPB-Null (pre-B cell line). All cells showed a 6.7 kb transcript, except for an additional 1.8 kb transcript found in RC-K8 cells. On the bottom of the figure, the same filter is shown re-hybridized with an HPRT cDNA probe (37), as internal control. **B** Hybridization of pES1.9 cDNA probe (Figure 3A) to Human Multiple Tissue Northern Blot (Clontech Laboratories, Inc.). Lanes 1−8 contain 2 μg of poly(A)$^+$ RNA from each of heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas, respectively. The same filter was re-hybridized with a β-actin cDNA probe (38) as internal control (bottom).

is very close to the reported consensus sequence for optimal initiation of translation, C-A/G-C-C-ATG-G/A (22,23). The open reading frame extends from nucleotide 334 to 1782 and predicts a polypeptide of 483 amino acids, with a calculated molecular weight of 54,416 (p54). To confirm the protein size, the RNA transcript derived from cDNA RC-rp1 and RC-rp2 was translated *in vitro*. The electrophoretic mobility of the translated protein product showed an approximate size of 54 kd (Figure 4).

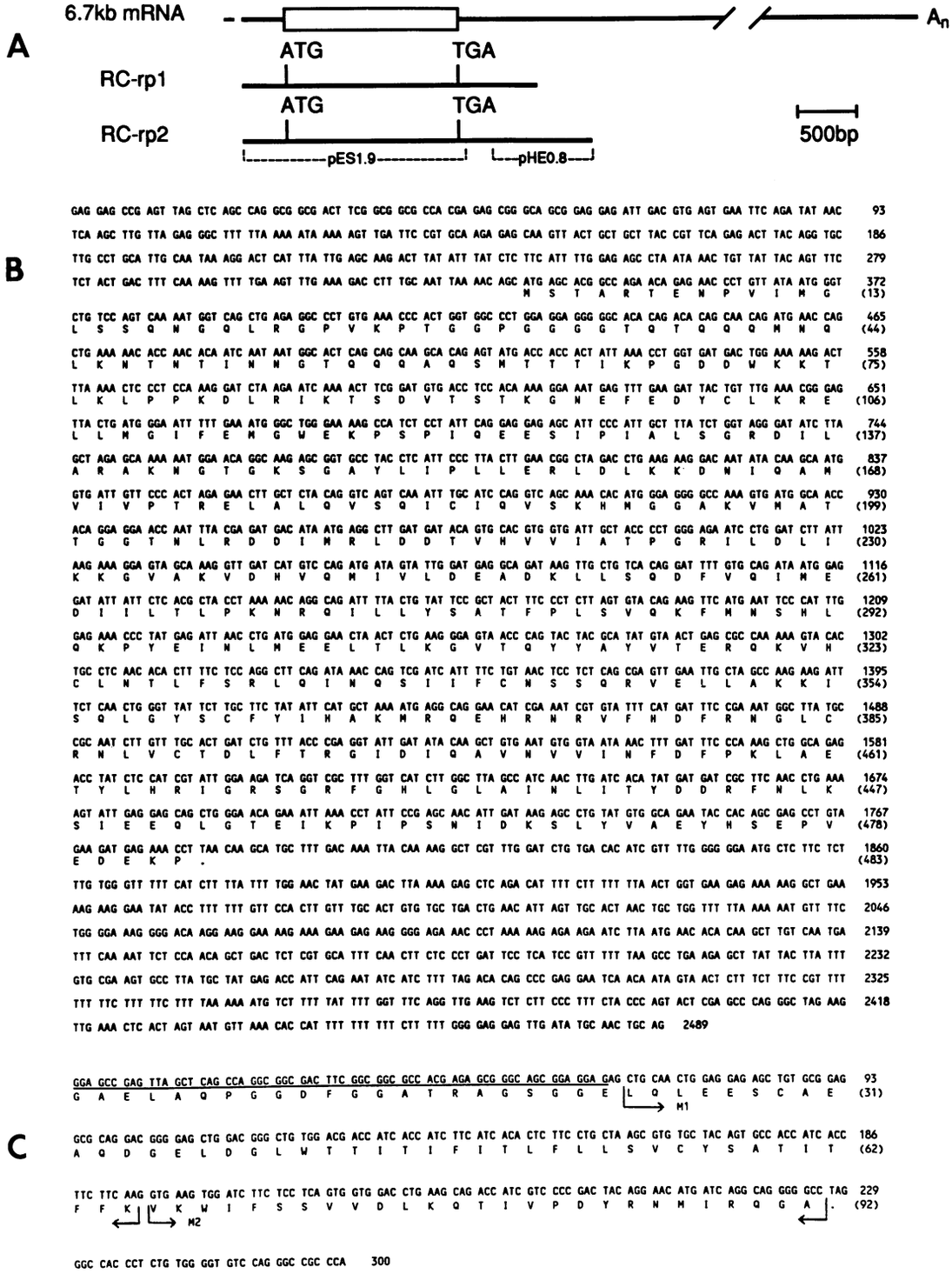

Figure 3. **A** Diagrammatic representation of p54 mRNA and cDNA clones obtained from an RC-K8 random-primed cDNA library (clones RC-rp1 and RC-rp2). **B** Nucleotide sequence and predicted translation product of p54 cDNA clone RC-rp1. The amino acid sequence of p54 is shown in the one letter code below the corresponding codons. **C** Nucleotide sequence and possible translation product of cDNA clone RC-dT. It represents a product of a fusion transcript between a normally untranslated region of the p54 gene and a truncated Igγ2 gene consisting of membrane exons M1 and M2. Underlined segment represents 64 bases from p54 (Figure 2B). See text for additional details.

p54 protein belongs to the ATP-dependent RNA helicase superfamily

The deduced polypeptide of 483 amino acids (Figure 3B) was compared to all the protein entries of IntelliGenetics databases and found to have the highest degree of homology with the oocyte-specific *ME31B* gene of *Drosophila*, showing in the 386 amino acid conserved region a 75% amino acid identity and 89% similarity, when conservative amino acid substitutions are included (Figure 5). The second closest homology found was with the better known murine eIF-4AI and yeast *TIF1* translation initiation factors (each having 38% amino acid identity or 59% similarity in the conserved RNA helicase domain). The p54 amino acid sequence contains the 10 motifs that characterize the well known members of the RNA helicase superfamily. They include the A site for ATP binding or Hodgman motif I, A/G-X-X-G-X-G-K-T/S, at amino acid position 140–147 (3); the DEAD box, also known as motif II or B site for ATP binding, M-X-V-L-D-E-A-D-X-X-L, at position 242–252 (2); the RNA helicase motif Y-X-H-R-I-G-R, at 418–424 (4); and seven other motifs of no known function (1). Among members of the DEAD box containing RNA helicases, 37 amino acids have been found conserved (2). p54 differs in two of the 37 amino acids, one of which represents a conservative substitution (Figure 5).

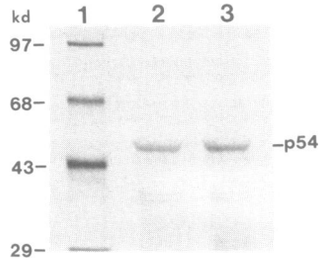

Figure 4. *In vitro* translation of p54 cDNA. Lane 1, [^{14}C]-labeled protein molecular weight standards (BRL). Lanes 2 and 3, [^{35}S] methionine-labeled *in vitro* translated RC-rp1 and RC-rp2 p54 cDNAs. Proteins were analyzed by 10% SDS-PAGE and the gel was exposed to X-ray film.

Transcription of p54 gene in various human tissues

Messenger RNAs from different human adult tissues were analyzed by Northern blot hybridization, using a 1.9 kb cDNA probe (pES1.9, Figure 3A) that includes both the 5′ untranslated region and the coding region of p54 (basepairs 1–1911). All tissues tested showed abundant expression of the 6.7 kb transcript. They included heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas (Figure 2B). The same size transcripts were also abundantly expressed in B-lymphoid and erythroleukemic cell lines, using the 5′ genomic p11HS0.6 probe of which only a small segment is transcribed. With this probe, however, such transcript was at best barely detectable in a mature T-lymphocyte cell line (Figure 2A).

Cloning and sequencing of a cDNA clone derived from the 1.8 kb fusion mRNA

By screening an oligo(dT)-primed RC-K8 cDNA library with the 5′ p11HS0.6 p54 genomic probe, a single 1.7 kb cDNA clone was isolated (RC-dT). DNA sequence analysis showed that the RC-dT cDNA clone resulted from a fusion of a 64 bp segment from the 5′ untranslated region of the p54 gene with a truncated Igγ sequence, containing the membrane exons M1 and M2 of a presumptive Igγ2 gene (Figure 3C). Although no start codon

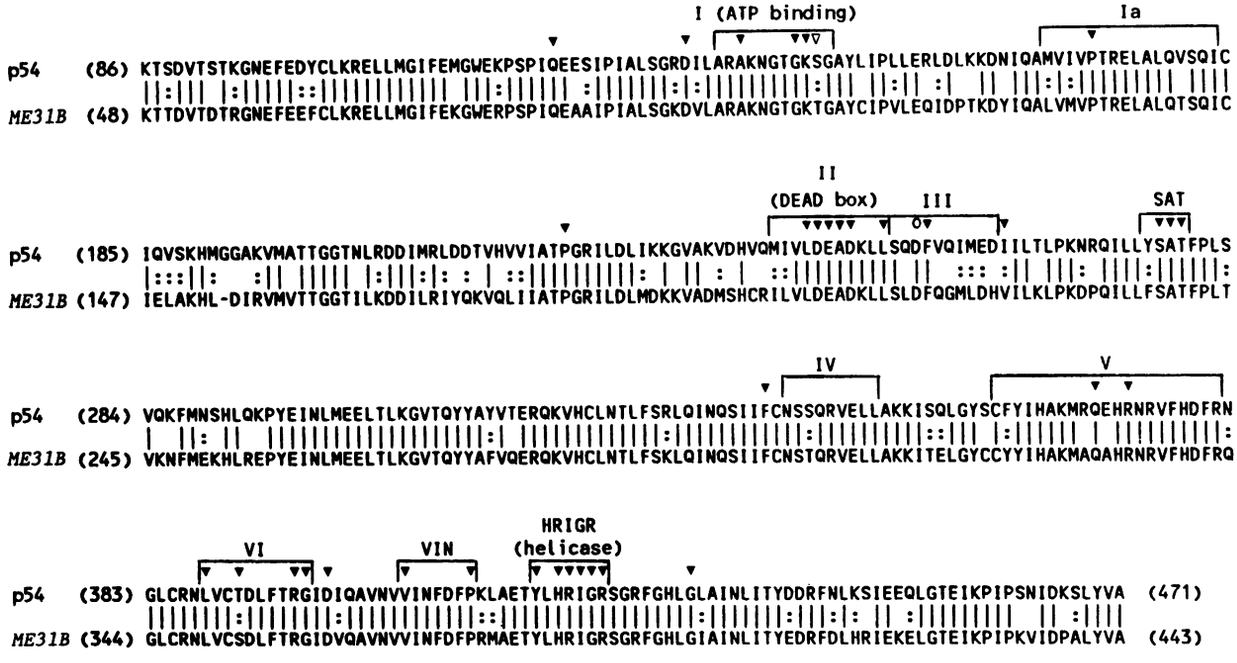

Figure 5. Similarity of the human p54 to the *Drosophila ME31B* gene. The deduced amino acid sequences of p54 and *ME31B* are aligned. Identical residues are connected by vertical bars and conservative substitutions by two dots. Conserved groups are P,S,A,T,G; I,L,V,M; K,R,H; D,E,N,Q; and F,Y,W. Ten highly conserved motifs are boxed. They include Hodgman motif I, or A site of ATP binding motif; Hodgman motif II, DEAD box or B site of ATP binding motif; and the HRIGR or RNA helicase motif (1,13). Thirty-seven amino acids are known to be conserved in all members of the RNA helicase superfamily (2). p54 shows 35 identical (▼), one conservative substitution (△), and one variant (○) amino acids.

(ATG) was found by sequence analysis of RC-dT (Figure 3C), a potential ATG codon was found in-frame, 69 bp upstream in the genomic sequence (data not shown).

Chromosome localization, orientation and rearrangement of the p54 gene

To map the p54 gene locus, we examined the relationship of the 3′ and 5′ segments of the p54 gene to the 11q23.3 breakpoint of t(11;14)(q23.3;q32.3). Southern blots of DNA from somatic cell hybrids containing either the 11q− or 14q+ derivative chromosomes of the t(11;14) were tested. The 3′ pHE0.8 probe, obtained from RC-rp2 cDNA clone, gave a positive signal on the derivative 11q− and no signal on the derivative 14q+ chromosome (Figure 6A). On the contrary, when the 5′ p11HS0.6 probe of p54 gene was used, the derivative 11q− chromosome was negative and the derivative 14q+ chromosome showed a positive, but displaced band (Figure 6B). This data, combined with the results of sequence analysis described above, indicates that the 5′ untranslated region of the p54 gene has translocated from the telomeric segment of chromosome 11 to the 14q+ chromosome and, therefore, the 5′ end of the gene has a telomeric orientation (for schematic representation, see Figure 6C). Interestingly, in somatic cell hybrids with the derivative chromosomes of a t(4;11)(q21;q23.3) acute lymphocytic leukemia, and in somatic cell hybrids with the derivative chromosomes of an inv ins (X;11)(q24;q23.3q21) acute myelomonocytic leukemia, the breakpoint was found to be more centromeric than the one observed in the t(11;14) breakpoint (Figure 6).

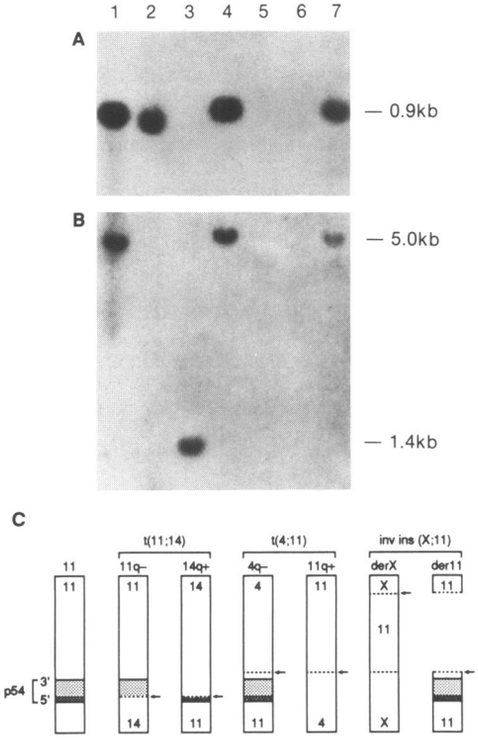

Figure 6. A and **B** Southern blots of somatic cell hybrids containing a single normal or derivative chromosome from three different translocations found in human hematologic malignancies (14). DNA samples were digested with *Hind* III from hybrids containing a normal chromosome 11 (lane 1); 11q− derivative (lane 2) and 14q+ derivative (lane 3) of a t(11;14)(q23.3;q32.3) from RC-K8 lymphoma cells (15); a 4q− derivative (lane 4) and 11q+ derivative (lane 5) of a t(4;11)(q21;q23.3) from a patient with acute lymphocytic leukemia; and a Xq+ derivative (lane 6) and deletion 11q21q23.3 (lane 7) of an inv ins (X;11)(q24;q23.3q21) from a patient with acute myelomonocytic leukemia. The figures show the results of hybridization using a pHE0.8 probe representing the 3′ untranslated region of p54 cDNA (A), and a p11HSO.6 probe representing a 5′ region of the p54 gene (B) **C** Diagram of chromosomes illustrating the orientation of the p54 gene and its relationship with the t(11;14), t(4;11) and inv ins (X;11) breakpoints. Top and bottom of each chromosome scheme indicate centromeric and telomeric direction, respectively.

DISCUSSION

In this work, we have cloned a new human RNA helicase gene at the breakpoint of a t(11;14)(q23.3;q32.3) from an RC-K8 diffuse B-cell lymphoma cell line showing Ig by Southern blot analysis. We initiated this work in an attempt to clone the gene(s) that may be involved in the 11q23 chromosome breakpoint region, commonly found rearranged in various types of hematologic malignancies (14,24). Because the heavy chain immunoglobulin genes map to band 14q32.3 and one of these genes is often found rearranged with another gene/oncogene in B-cell malignancies (24), cloning of the t(11;14)(q23.3;q32.3) breakpoint was facilitated by screening a genomic library, constructed from a somatic cell hybrid that contains as a single human chromosome the derivative 14q+ chromosome of t(11;14) from RC-K8 cells, and using an Ig heavy chain gene $C\gamma$ as probe. A single copy chromosome 11-specific probe (p11HS0.6), found close to the breakpoint (Figure 1), was used for Northern analysis and revealed a normal 6.7 kb transcript in several human hematologic cell lines. In addition, a normal 6.7 kb and an abnormal 1.8 kb transcript was found in the RC-K8 cells (Figure 2A).

The predicted protein product of the 6.7 kb transcript, cloned from a cDNA library of RC-K8 cells, was found to represent a new member of the ATP-dependent RNA helicase superfamily. It consists of 483 amino acids, with a molecular weight of approximately 54 kd (Figures 3 and 4). The amino acid sequence includes the RNA helicase motif and the A and B sites of ATP binding motifs, characteristic of the RNA helicase superfamily genes (2−4) [Figure 5]. Almost all the genes of this superfamily have been reported in yeast, mouse and *Drosophila* and can be broadly divided into three main groups: translation initiation factors, splicing factors and a lesser known group of RNA helicases found to be preferentially or exclusively expressed during gametogenesis (5−13).

p54 has good amino acid sequence homology with the murine eIF-4AI and yeast *TIF*1 proteins (38% identity), known as prototypes of translation initiation factors. This compares with a 62−65% amino acid identity observed between the *TIF*1/*TIF*2 and eIF4AI/II, which are proteins known to recognize mRNA cap structure, bind mRNA to ribosomes and catalyze RNA unwinding (25,26). A more striking homology was found between p54 and the recently reported *Drosophila* oocyte-specific *ME31B* gene. These two genes show, in the conserved RNA helicase domain, a 75% amino acid identity (Fig. 5). This is a remarkable degree of evolutionary conservation. The two genes primarily differ in their amino terminus where p54 has 85 and *ME31B*, 47 amino acids that have no similarity among themselves and are in excess to the amino acid sequence of the prototype eIF-4AI and *TIF*1 RNA helicases. Since the p54 gene appears to be the human cognate of the *Drosophila ME31B* gene, it is interesting that *ME31B* has a restricted transcriptional expression

to oocytes, while p54 shows abundant expression in most adult tissues tested (Fig. 2). At present, there is no clear explanation for this discrepancy. Nevertheless, there are known examples of genes in *Drosophila* for which the maternally supplied product is sufficient to take the organism through adulthood (27). For instance, the gene for cyclic nucleotide phosphodiesterase is widely expressed in mammals (28), while in *Drosophila*, the maternally supplied gene product is sufficient for the enzyme needs of the adult organism (27). Recently, the *ste* 13 yeast cognate of the *ME31B* has been cloned from *Schizosaccharomyces Pombe* (ref 29; C. Shimoda, personal communication). Such gene is essential for yeast entry into meiosis and it has been possible to rescue sterile *ste* 13 mutants by expressing the cDNA of *ME31B* in *S. Pombe*. It would be of interest to examine whether the p54 cDNA clone could also rescue *ste* 13 mutants.

In humans, the only RNA helicase previously cloned is the p68 gene, mapped to band region q23-25 of chromosome 17 (30). It represents a nuclear protein possibly involved in early nucleolar assembly and has an extra SV40 large T epitope domain in its C-terminus (31,32). p54 and p68 only share a 27% identity or 43% similarity in the conserved helicase domain.

To map the human p54 gene locus on chromosome 11, we examined the relationship of 3′ (probe pHE0.8; figure 3A) and 5′ (probe p11HS0.6; figure 1) DNA segments of p54 to the 11q23.3 breakpoint found in three different hematologic malignancies. The schematic representation in figure 6C illustrates that the 5′ (untranslated region) of the p54 gene has translocated from the telomeric end of chromosome 11 to the 14q+ chromosome and, therefore, the 5′ end of the gene has a telomeric orientation. Furthermore, we found that the breakpoint of a t(4;11)(q21;q23.3) acute lymphocytic leukemia, and of an inv ins (X;11)(q24;q23.3q21) acute myelomonocytic leukemia is different than the one found in t(11;14), since both the 5′ and 3′ p54 cDNA probes hybridized to the telomeric segment of the chromosome 11 involved in t(4;11) and inv ins (X;11) [Figure 6]. The findings suggest that either there is a difference in the site of p54 gene disruption or a different type of genetic heterogeneity in the 11q23.3 breakpoint found in hematologic malignancies (24).

cDNA sequence analysis of the 1.8 kb abnormal mRNA found in the t(11;14) RC-K8 cells suggests that it is a fusion product of the 5′ region of the p54 gene with the exons M1 and M2 of the Igγ2 gene. Such nucleotide sequence predicts an unusual truncated surface immunoglobulin C2 protein with only 17 extracellular, 25 transmembrane and 28 amino acids belonging to the intracytoplasmic domains of an Igγ gene (Figure 3C). Since p54 was found to express an abundant transcript in various tissues, including B-lymphocytes (Fig 2), it is plausible that a strong promoter/enhancer region of p54 may serve as a deregulatory element for the truncated B-cell receptor, which in turn may provide proliferative advantage to the RC-K8 lymphoma cells.

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