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Cloning of *ELL*, a gene that fuses to *MLL* in a t(11;19)(q23;p13.1) in acute myeloid leukemia

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ABSTRACT **To characterize the functions of *MLL* fusion transcripts, we cloned the gene that fuses to *MLL* in the translocation t(11;19)(q23;p13.1). This translocation is distinct from another type of 11;19 translocation with a 19p13.3 breakpoint that results in the fusion of *MLL* to the *ENL* gene. By PCR screening of a cDNA library prepared from a patient's leukemia cells with this translocation, we obtained a fusion transcript containing exon 7 of *MLL* and sequence of an unknown gene. The sequence of this gene was amplified and used as a probe to screen a fetal brain cDNA library. On Northern blot analysis, this cDNA detected a 4.4-kb transcript that was abundant in peripheral blood leukocytes, skeletal muscle, placenta, and testis and expressed at lower levels in spleen, thymus, heart, brain, lung, kidney, liver, and ovary. In addition, a 2.8-kb transcript was present in peripheral blood, testis, and placenta. On "zoo blots," this gene was shown to be evolutionarily conserved in 10 mammalian species as well as in chicken, frog, and fish. We have named this gene *ELL* (for eleven–nineteen lysine-rich leukemia gene). A highly basic, lysine-rich motif of the predicted ELL protein is homologous to similar regions of several proteins, including the DNA-binding domain of poly(ADP-ribose) polymerase. The characterization of the normal functions of *ELL* as well as its altered function when fused to *MLL* will be critical to further our understanding of the mechanisms of leukemogenesis.**

Aberrations in chromosome 11 band q23 are frequent cytogenetic abnormalities in hematologic malignancies, especially in acute leukemias. Previously, we identified a yeast artificial chromosome that contained the breakpoint region in leukemias with several common 11q23 translocations (1). Subsequently, we cloned a gene named *MLL* (for myeloid–lymphoid leukemia or mixed-lineage leukemia) that spans this breakpoint (2). Other groups have also cloned this gene and have called it *Htrx*, *ALL-1*, and *HRX* (3–5). A 0.74-kb *Bam*HI fragment isolated from *MLL* detects gene rearrangements on Southern blot analysis in all patients with the five most common types of 11q23 translocations as well as a large number of other, less common 11q23 abnormalities (6). All breakpoints in *MLL* occur within an 8.3-kb breakpoint cluster region. The *MLL* gene encodes an A-T hook domain at the N terminus and several regions of homology to the *Drosophila* trithorax gene, including a series of zinc fingers in the region immediately 3′ to the breakpoint region and 210 C-terminal amino acids that do not contain a known motif. Mapping of functional domains of *MLL* with yeast *GAL4* hybrids in transient transfections has revealed that *MLL* contains a strong activation domain 3′ of the breakpoint and a repression domain 5′ of the breakpoint region (7).

Six genes at 11q23 partner chromosomal breakpoints have been cloned. These include *AF4* in t(4;11)(q21;q23) (4, 8), *ENL* in t(11;19)(q23;p13.3) (5), *AF9* in t(9;11)(p22;q23) (9), *AF6* in t(6;11)(q27;q23) (10), *AF1p* in t(1;11)(p32;q23) (11), and *AFX* in t(X;11)(q13;q23) (12). The functions of these fusion partner genes have not been determined. Translocations at 11q23 result in the formation of two derivative chromosomes that encode chimeric transcripts. The der(11) transcript contains 5′ *MLL* sequences fused to 3′ sequences of the gene located on the partner chromosome, whereas the other derivative chromosome contains the 5′ sequence of the partner gene potentially fused to 3′ sequence of *MLL*. However, in 25% of patients, translocations are associated with deletions of *MLL* sequence that is 3′ to the breakpoint (13). Thus, in these cases, a fusion transcript from the other derivative chromosome cannot be formed. In addition, analysis of complex 11q23 translocations reveals that the der(11) junction is always conserved (14). These data indicate that the fusion transcript encoded by the der(11) must be critical to leukemogenesis.

Although earlier cytogenetic studies did not discriminate between different breakpoints within band 19p13 in cases with a t(11;19)(q23;p13), it has become clear with more precise cytogenetic techniques that two breakpoints exist within band 19p13 (Fig. 1). These two breakpoints are easily distinguishable by fluorescence *in situ* hybridization (FISH) (15). The t(11;19)(q23;p13.3) breakpoint is common in acute lymphoblastic leukemia, primarily in infants and children. The translocation t(11;19)(q23;p13.1) is a recurring abnormality in both *de novo* and therapy-related acute myeloid leukemia and it has been observed in infants, children, and adults. The gene in t(11;19)(q23;p13.3) has already been cloned and named *ENL* (for eleven–nineteen leukemia).

To characterize the functions of *MLL* fusion transcripts further, we have cloned the gene that fuses to *MLL* in t(11;19)(q23;p13.1). The open reading frame of this gene codes for a hydrophilic, highly basic protein. We have named this gene *ELL* (for eleven–nineteen lysine-rich leukemia gene).§ The *ELL* gene has a broad tissue distribution and is conserved in all mammalian species as well as in chicken, fish, and frog.

MATERIALS AND METHODS

Cytogenetic Analysis and FISH. Cytogenetic analysis was performed with a trypsin–Giemsa banding technique. Chromosomal abnormalities are described according to the International System for Human Cytogenetic Nomenclature. FISH was performed with biotin-labeled probes (16).

Construction and Screening of cDNA Libraries. Poly(A)$^+$ RNA was isolated with the FastTrack kit (Invitrogen) from cryopreserved bone marrow from a 41-year-old woman with

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Abbreviations: FISH, fluorescence *in situ* hybridization; 5′ RACE, rapid amplification of cDNA 5′ ends.

§The sequence reported in this paper has been reported in the GenBank data base (accession no. U16282).

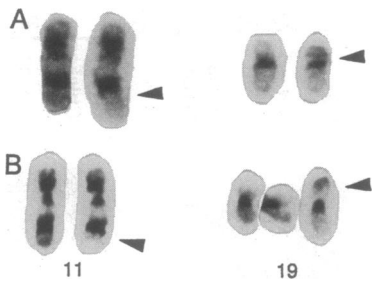

FIG. 1. Partial karyotype of chromosome pairs 11 and 19 from two cells from patients with a t(11;19) with a 19p13.1 breakpoint (*A*) and with 19p13.3 breakpoint (*B*). The normal chromosome is on the left in each pair. Arrowheads indicate the breakpoints in the translocation chromosome on the right. Note that there is a much larger amount of pale material at the end of the long arm of the der(11) chromosome in *A* than in *B*, and conversely, a much smaller segment of pale material at the end of the short arm of the der(19) chromosome. There is an additional normal chromosome 19 in *B*.

acute myeloid leukemia (FAB-M4) with a karyotype of 46,XX,t(11;19)(q23;p13.1)(100%). First-strand cDNA synthesis was catalyzed with Moloney murine leukemia virus reverse transcriptase using random hexamers (Pharmacia). The second strand was synthesized with RNase H and DNA polymerase I (Pharmacia). *Eco*RI/*Not* I adaptors were blunt-end ligated to the cDNA with T4 DNA ligase (TimeSaver cDNA library kit; Pharmacia) and then ligated into the *Eco*RI site of the λZAP II vector (Stratagene). The library was initially screened with the 0.74-kb *Bam*HI cDNA fragment of *MLL*. Subsequently, the library was screened by PCR with nested primers from *MLL* exon 7 and the vector. PCR products were ligated into the pT7-Blue T vector (Novagen). A commercially prepared human fetal brain cDNA library (Stratagene) was screened with probes amplified from the *MLL/ELL* fusion transcript.

Nucleotide Sequencing. cDNA clones were sequenced entirely on both strands with Sequenase (United States Biochemical) using doubled-stranded templates. The DNA sequence and the predicted open reading frame were compared with GenBank data bases by using the BLAST and TFASTA programs.

Southern and Northern Blot Analysis. DNA was extracted and digested by standard techniques. RNA was isolated from cell lines with TriReagent (Molecular Research Center, Cincinnati, OH). The multiple-tissue Northern blots (Clontech), the somatic cell hybrid panel (Oncor), and the "zoo blots" (Bios, New Haven, CT) were used according to the manufacturers' suggestions.

Rapid Amplification of cDNA 5′ Ends (5′ RACE). First-strand cDNA was synthesized from 2 μg of placental poly(A)$^+$ RNA with avian myeloblastosis virus reverse transcriptase (Clontech) and an antisense primer 150 bp downstream of the beginning of the 2.8-kb clone isolated from the fetal brain cDNA library. An anchor oligonucleotide was ligated to the 3′ end of the cDNA by T4 RNA ligase (Clontech). After 30 cycles of amplification using the anchor primer (Clontech) and a primer 130 bp from the beginning of the 2.8-kb cDNA clone, a second round of 30 cycles of PCR was performed with a 17-mer oligonucleotide from the internal portion of the anchor primer and two different internal primers from the 2.8-kb clone (110 and 90 bp from the beginning of the clone).

RESULTS

Cloning of t(11;19)(q23;p13.1) Fusion Transcript. The 0.74-kb *Bam*HI cDNA fragment from the *MLL* gene detected two rearranged bands in the *Bam*HI digest of the DNA from the patient with the translocation t(11;19)(q23;p13.1) (Fig. 2*A*). The cDNA library prepared from the patient's leukemia cells was initially screened with the 0.74-kb *Bam*HI cDNA fragment of *MLL* used to detect gene rearrangements. Three clones were isolated from the library but none contained a fusion transcript. To facilitate the cloning of this fusion transcript, we devised a PCR screening method using nested *MLL*-specific and vector primers. Detailed mapping of the genomic breakpoint within *MLL* in this patient's leukemia revealed a break between exons 7 and 9. Thus the *MLL*-specific primers were chosen approximately 50 and 30 bp upstream of the end of exon 7. After two rounds of amplification, several PCR products were obtained. Sequence analysis revealed normal *MLL* transcripts as well as a clone with a 270-bp insert that contained 30 bp of exon 7 and 240 bp that diverged from *MLL* sequences. The 240 bp that diverged from *MLL* were amplified by PCR and subsequently used as a probe to screen a fetal brain cDNA library. Three clones were isolated and sequenced. The largest of these clones (2.8 kb) contained the entire 240 bp found in the fusion transcript. The 2.2-kb clone contained a similar sequence (16 of 17 bp) to part of the 240-bp probe but was otherwise completely different from the rest of the probe or from any sequences in the 2.8-kb fragment. The 1.9-kb clone contained 48 bp that were present in the 2.8-kb clone and in the 240-bp fragment but diverged both 5′ and 3′ to this sequence. Preliminary sequence analysis revealed *Alu* sequences within this clone as well as consensus splice acceptor and splice donor sites flanking this 48-bp sequence, suggesting that this 1.9-kb clone represents a partially processed transcript or a genomic clone. The 1.9-kb and 2.2-kb clones were not characterized further.

5′ RACE. An additional 23 nt that are upstream of the beginning of the 2.8-kb cDNA fragment were obtained with the 5′ RACE strategy. These sequences contained a potential start codon flanked by an adenine in the −3 position and a guanine in the +4 position, thus following Kozak's consensus sequence for an initiation codon (17). Twelve base pairs of 5′ untranslated sequence was obtained from the 5′ RACE, but we cannot rule out the possibility that the 5′ untranslated region may be longer.

Chromosomal Localization. The 240-bp fragment that diverged from *MLL* in the fusion transcript was found to hybridize to chromosome 19 in a somatic cell hybrid panel (data not shown). The entire 2.8-kb clone was labeled for FISH and it hybridized exclusively to chromosome 19, band p13.1 (Fig. 3).

Northern Blot Analysis. Multiple-tissue Northern blots were hybridized with a probe containing the first 500 bp of the

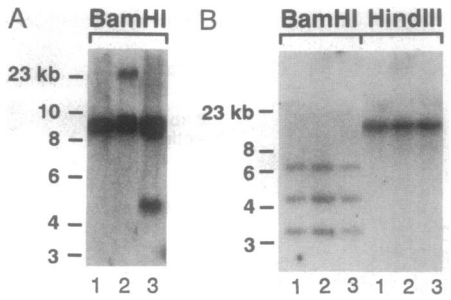

FIG. 2. (*A*) Southern blot of DNA digested with *Bam*HI and probed with the 0.7-kb fragment of the *MLL* gene. Lanes: 1, control; 2, leukemia cell DNA with the t(11;19)(q23;p13.1) used to prepare the cDNA library; 3, DNA from a patient with a t(11;19)(q23.p13.3). Both patients exhibit two rearranged bands with this probe. (*B*) Southern blot of DNA digested with *Bam*HI or *Hin*dIII and probed with the first 500 bp of the 2.8-kb *ELL* clone. This fragment contains cDNA sequences from both sides of the breakpoint. Lanes 1–3 are as in *A*. No rearrangements were detected with these digests.

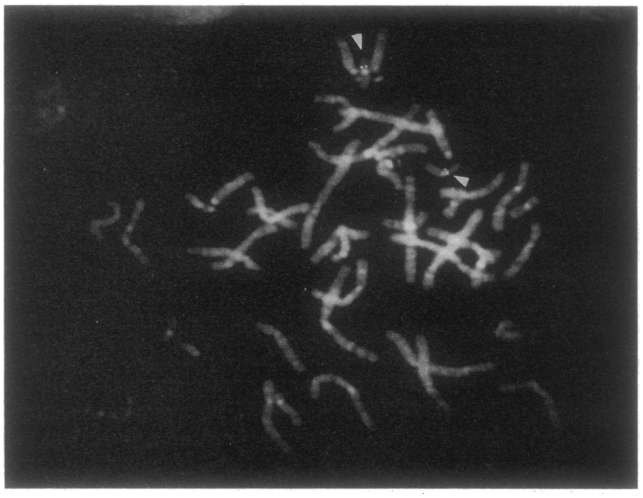

FIG. 3. FISH in normal metaphase cells with the 2.8-kb fragment of *ELL* labeled with biotin and detected with fluorescein-conjugated avidin. Images were captured with a CCD (charge-coupled device) camera. Chromosome 19 band p13.1 is identified with an arrowhead.

2.8-kb cDNA (Fig. 4 *A* and *B*). A 4.4-kb transcript appeared to be expressed ubiquitously, with the highest level of expression in skeletal muscle, placenta, testis, and peripheral blood leukocytes. A 2.8-kb transcript was also present in placenta, peripheral blood, and testis. On prolonged exposures of the autoradiographs, this 2.8-kb transcript was also visualized in other tissues. Hybridization of this same probe to a Northern blot of hematopoietic cell lines revealed expression of both transcripts in T-cell, B-cell, and myeloid lineages (Fig. 4*C*).

Evolutionary Conservation. To determine whether this gene was evolutionarily conserved, blots containing DNA from multiple mammalian and other species were hybridized with a probe containing the first 500 bp of the 2.8-kb cDNA (Fig. 5). The equivalent intensity of the bands in all mammalian species suggests a high degree of conservation. In addition to mammalian species, this gene appears to be present in chicken, fish, and frog.

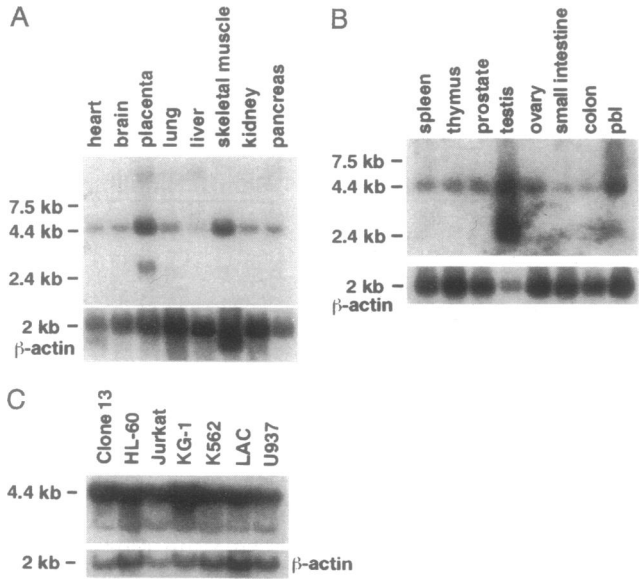

FIG. 4. Northern blots of RNA from multiple human tissues (*A* and *B*) and hematopoietic cell lines (*C*) hybridized with the first 500 bp of the 2.8-kb *ELL* clone. All blots were reprobed with β-actin cDNA. pbl, Peripheral blood leukocytes.

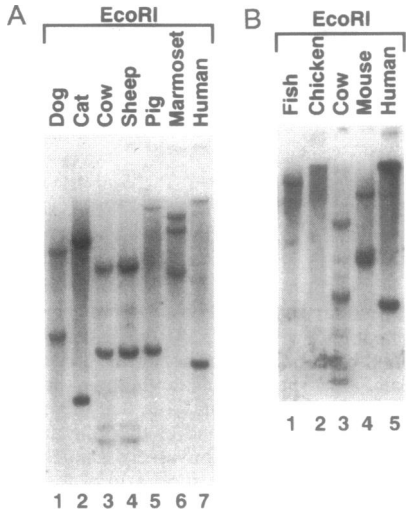

FIG. 5. Zoo blots showing *Eco*RI-digested DNA probed with the first 500 bp of the 2.8-kb *ELL* clone.

Southern Blot Analysis. To determine whether DNA rearrangements could also be observed in patient samples by probes from the partner gene on 19p13.1, the blot that was used to detect *MLL* rearrangements was rehybridized with a probe containing the first 500 bp of the 2.8-kb cDNA (Fig. 2*B*). This probe contains the sequences that are immediately 5′ and 3′ to the breakpoint within the *ELL* cDNA and could thus potentially detect both the der(11) and the der(19). However, no gene rearrangements could be identified, suggesting that the exons of *ELL* contained within the probe may be far from the genomic translocation breakpoint junction on chromosome 19.

Sequence Analysis. The entire 2.8-kb cDNA fragment was sequenced on both strands. The sequence that diverged from *MLL* in the fusion transcript begins at nt 148 of the 2.8-kb cDNA (Fig. 6*A*). The chimeric transcript generated by this translocation consists of 5′ *MLL* through exon 7 fused in frame to 2747 nt of *ELL* (Fig. 6*B*). The *ELL* sequence in the fusion transcript contains a predicted open reading frame of 576 aa followed by 930 nt of 3′ untranslated sequence. In addition to the sequence present in the fusion transcript, the 2.8-kb cDNA contains 124 nt 5′ of the breakpoint on chromosome 19. When combined with the 23 nt obtained from 5′ RACE, the sequence 5′ of the breakpoint codes for 45 aa. The open reading frame of the unrearranged *ELL* gene codes for a predicted protein of 621 aa.

Homology Searches. There are no significant nucleotide or amino acid homologies to the other *MLL* partner genes that have been cloned. However, *ELL* contains several regions that are rich in basic amino acids as has been noted in *AF4*, *ENL*, and *AF9*. The highest area of homology exists in a highly basic, lysine-rich region beginning at amino acid 442 of *ELL*. This region is homologous to the basic region of the DNA-binding domain of poly(ADP-ribose) polymerase (Fig. 7) (18–20). In addition, this region is also homologous to a lysine-rich region of arginine-rich nuclear protein (21). Data base searches also revealed a long stretch of limited homology to the Engrailed gene family, primarily upstream of the homeobox domain (22). A stretch of amino acids in this lysine-rich region is identical to a repeated element in the knob-associated histidine-rich protein of *Plasmodium falciparum* (23, 24). However, the adjacent amino acids are not highly conserved. In addition, the bovine leukemia virus receptor has a similar basic region near its N terminus (25).

DISCUSSION

Although *MLL* encodes DNA-binding motifs suggestive of a transcription factor, the normal functions of *MLL* have not

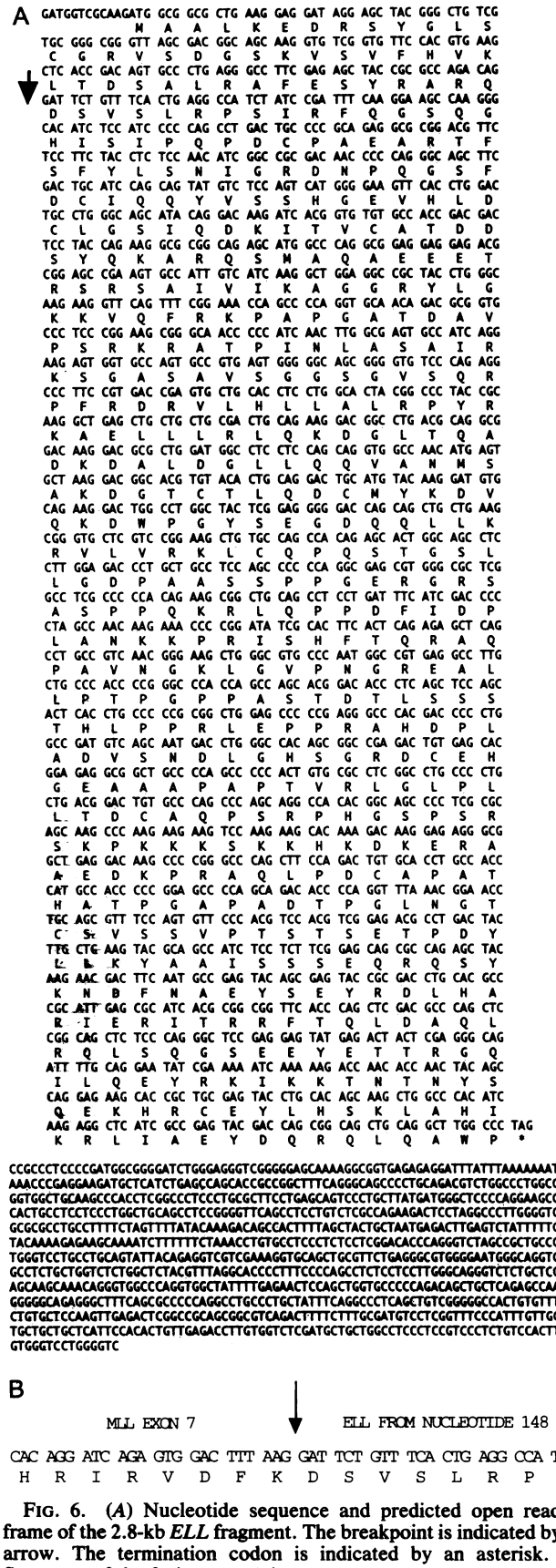

FIG. 6. (*A*) Nucleotide sequence and predicted open reading frame of the 2.8-kb *ELL* fragment. The breakpoint is indicated by an arrow. The termination codon is indicated by an asterisk. (*B*) Sequence of the fusion transcript.

yet been determined. Moreover, little is known about the altered function of *MLL* that is a consequence of 11q23 translocations. The creation of in-frame chimeric transcripts as a result of these translocations suggests that the sequences contributed by the partner genes are also critical to this process. Because of the large number of 11q23 aberrations that involve *MLL*, we reasoned that comparison of *MLL* partner genes might define common domains that are essential for the leukemogenic function of the fusion transcript. To facilitate the characterization of the nature of the chimeric transcripts that form in these translocations, we have cloned the gene (*ELL*) that fuses to *MLL* in t(11;19)(q23;p13.1), a recurring cytogenetic abnormality in acute myeloid leukemia. The *ELL* gene is highly conserved and expressed ubiquitously as a 4.4-kb message and, in certain tissues, also as a 2.8-kb transcript. Previous studies of deletions that occur in the formation of 11q23 translocations have shown that the der(11) encodes the fusion transcript that is critical to leukemogenesis. Thus, in t(11;19)(q23;p13.1), this transcript contains 4.2 kb of 5′ *MLL* sequence juxtaposed to at least 2.7 kb of 3′ *ELL* sequence and codes for a predicted fusion protein of 2015 aa. Additional 3′ untranslated *ELL* sequence may also be present.

Data base searches of the nucleotide sequence and the predicted protein of *ELL* reveal that it is a novel gene. The region with the most significant homologies to previously identified genes lies in a highly basic, lysine-rich region near the C terminus of the ELL protein. Utilizing the Chou–Fasman (26) algorithm, computer modeling of the three-dimensional structure of *ELL* places this domain at a helical portion of the predicted protein. The best-characterized gene with a strong homology to *ELL* is poly(ADP-ribose) polymerase, a protein that ADP-ribosylates various nuclear proteins, including histones, DNA topoisomerases, and DNA polymerases. Poly(ADP-ribose) polymerase is involved in cellular proliferation and is critical to the repair of double-strand DNA breaks and single-strand nicks (27). The C-terminal portion of the DNA-binding domain of poly(ADP-ribose) polymerase contains a highly basic, lysine-rich motif that is homologous to ELL, but the N-terminal portion contains two zinc fingers that are not present in ELL. The zinc fingers are required for recognition of double-strand breaks and single-strand nicks. Whereas deletion or mutation of both zinc fingers eliminates recognition of strand breaks or nicks, binding to intact DNA does not require the zinc fingers (28). Moreover, studies of the C-terminal portion of the DNA-binding domain of poly(ADP-ribose) polymerase by Southwestern blot analysis have revealed that DNA binding is preserved in the absence of the N-terminal portion of the DNA-binding domain (29). In addition, a bipartite nuclear localization signal has been identified in the C-terminal portion of the DNA-binding domain of poly(ADP-ribose) polymerase (30). The second portion of this sequence (KKKSKK) is identical to a segment of the lysine-rich motif of ELL, but the first portion of the bipartite signal is not present in ELL. Functional studies will be necessary to determine whether this region of ELL possesses the capacity for either DNA binding or nuclear localization.

Homologies to other genes with highly basic, lysine-rich motifs have been identified. Arginine-rich nuclear protein was cloned from an expression library screened with an antibody to nuclear proteins. The precise function of arginine-rich nuclear protein has not been determined, but it contains a region of homology to the U1 protein of small nuclear ribonucleoprotein particles and is thus thought to be involved with RNA processing. The homology to the engrailed family of genes spans a region in the EH-4 domain and the beginning of the homeodomain. Taken together, these homologies suggest that ELL may also be a nuclear protein that potentially has DNA binding properties. A similar lysine-rich motif is also present in the bovine leukemia virus receptor and the knob-associated histidine-rich protein of *Plasmodium falciparum*. The significance of the homologies to these two genes is not clear.

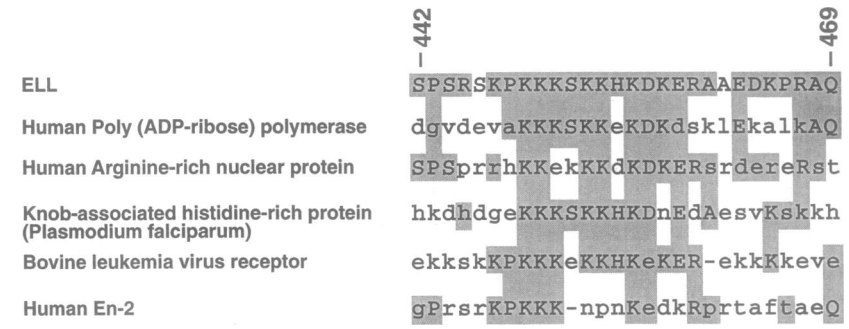

FIG. 7. Amino acid homologies. Identical amino acids shown as uppercase letters and divergent amino acids as lowercase letters. Identical and conserved amino acids are on a shaded background.

None of the six *MLL* fusion partner genes cloned to date had previously been identified. The potential normal functions of these genes have not been characterized and thus comparisons of these genes can be based only on their nucleotide sequence and predicted proteins. The proteins encoded by *AF4, AF9,* and *ENL* contain nuclear targeting sequences and regions rich in serine and proline residues, suggesting that these proteins may act as transcription factors. These three proteins also have segments that are rich in basic amino acids, but none of these proteins contains a known DNA-binding domain. *AF6* and *AF1p* do not appear to be similar to *AF4, AF9,* and *ENL. AF6* encodes short stretches rich in prolines, charged amino acids, serines, and glutamines and does not have a nuclear localization signal. *AF6* also encodes a GLGF motif found in proteins thought to be involved in signal transduction. *AF1p* encodes three acidic domains, a glutamine-rich region, and 15 copies of a DPF tripeptide repeat of unknown significance. *AF1p* is highly homologous to the murine eps 15 gene that encodes a cytoplasmic phosphoprotein. Only partial sequence of the *AFX* gene has been published. *ELL* is not homologous to any of the partner genes that have been cloned. Like *AF4, AF9,* and *ENL, ELL* encodes segments that are rich in basic amino acids, although this is a more prominent feature of *ELL* than of the other translocation partners. In addition, *ELL* also encodes a potential nuclear localization sequence.

Further work will be necessary to elucidate the normal functions of *ELL* as well as the contribution of its 3′ sequences to leukemogenesis. The homology of its highly basic, lysine-rich region to a DNA-binding domain suggests that ELL may also be a nuclear protein with DNA-binding properties. One potential consequence of the formation of the *MLL/ELL* fusion transcript may be an altered DNA-binding specificity that results from the substitution of the DNA-binding domain of ELL for the loss of the zinc fingers encoded by 5′ *MLL*. The characterization of *ELL* should provide insights into its function and into the pathogenesis of acute leukemia.

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