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Genetics

Acute mixed-lineage leukemia t(4;11)(q21;q23) generates an *MLL–AF4* fusion product

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ABSTRACT **A chromosomal translocation, t(4;11)-(q21;q23), is associated with an aggressive mixed-lineage leukemia. A yeast artificial chromosome was used to clone the chromosomal breakpoint of this translocation in the RS4;11 cell line. The breakpoint sequences revealed an inverted repeat bordered by a consensus site for topoisomerase II binding and cleavage as well as χ-like elements. The der(11) chromosome encodes a fusion RNA and predicted chimeric protein between the 11q23 gene *MLL* and a 4q21 gene designated *AF4*. The sequence of the complete open reading frame for this fusion transcript reveals the MLL protein to have homology with DNA methyltransferase, the *Drosophila* trithorax gene product, and the "AT-hook" motif of high-mobility-group proteins. An alternative splice that deletes the AT-hook region of MLL was identified. AF4 is a serine- and proline-rich putative transcription factor with a glutamine-rich carboxyl terminus. The composition of the complete *MLL–AF4* fusion product argues that it may act through either a gain-of-function or a dominant negative mechanism in leukemogenesis.**

Specific interchromosomal translocations are associated with distinct hematologic neoplasms and have led to the identification of new oncogenes (1, 2). One such translocation is the t(4;11)(q21;q23) seen in an aggressive mixed-lineage leukemia (3). This translocation is one of a number of 11q23 translocations associated with leukemia (4) which appear to involve the *MLL* (mixed-lineage leukemia) (5–8) gene on 11q23.

The majority of t(4;11) leukemias occur in infancy and have a poor prognosis (3, 9). The leukemic cells have a lymphoblastic morphology and typically express the stem-cell marker CD34, HLA-DR, and the B-cell marker CD19 but fail to express CALLA (CD10). In addition, the myelomonocytic marker CD15 is typically present (9). The biphenotypic nature of these blasts suggests that the t(4;11) transforms a multipotential progenitor cell. In addition to the t(4;11), other 11q23 abnormalities can produce the same clinical and immunophenotypic disease (4). *MLL* on 11q23 is also involved in primary and secondary acute nonlymphocytic leukemia (ANLL) (10, 11). The secondary ANLL is associated with prior therapy with inhibitors of DNA topoisomerase II (11).

Isolation of a yeast artificial chromosome (YAC), yB22B2, that spans the 11q23 breakpoint region (5) has demonstrated that 11q23 breakpoints of both acute lymphocytic leukemia (ALL) and ANLL are clustered within a 7-kb region. A large transcript spans this breakpoint cluster region and was first named *MLL* (7, 8, 12). This gene has extensive homology to a *Drosophila* homeotic regulator of development, trithorax (12–14). Molecular analysis of the t(4;11) and t(11;19) translocations indicates that *MLL* fuses with genes from 4q21 and 19p13 to generate chimeric transcripts from both the der(11) and non-der(11) chromosomes (12, 13). Complex translocations involving 11q23 selectively retain the der(11) (17). Molecular analysis indicates that a portion of the non-der(11) *MLL* sequences is deleted in up to 30% of cases (18). This argues that the der(11) encodes the critical product for leukemogenesis.‡

MATERIALS AND METHODS

Cells. RS4;11 (19), B1 (20), and MV411 (21) cell lines have been described. Patient material was provided by the Children's Cancer Study Group and the Pediatric Oncology Group.

YAC Characterization and Breakpoint Cloning. The *CD3D*- and *CD3G*-positive YAC, yB22B2, was isolated as described (5). Southern analysis was performed after pulsed-field gel electrophoresis (22). Partial *Mbo* I or complete *Xba* I digests of yB22B2-containing yeast DNA were cloned into λDash II (Stratagene). The P/S4 probe was used to isolate a der(4) breakpoint-containing phage from an RS4;11 genomic library cloned into λZap II (Stratagene).

Transcript Analysis. Two micrograms of poly(A)$^+$ RNA was isolated with the Micro FastTrack kit (Invitrogen) and analyzed by Northern blotting. RS4;11 poly(A)$^+$ RNA was converted to first-strand cDNA with Moloney murine leukemia virus reverse transcriptase primed with oligo(dT) and random hexamer. The double-stranded cDNA was cloned into λZap II. The clone pTA1 is a reverse-transcription–PCR product. All inserts were completely sequenced on both strands. Homologies to known proteins were identified with the BLAST, FASTA, and MOTIFS programs (Genetics Computer Group).

RESULTS

YAC Cloning of the t(4;11) Breakpoint. When these studies began there were no known genes close enough to the t(4;11) breakpoint to allow its cloning by conventional techniques. Therefore, YAC clones for 11q23 genes known to be either centromeric (*CD3D*, *-G*, and *-E*) or telomeric (*THY1*, *CBL2*, and *ETS1*) to the t(4;11) breakpoint were obtained (23). YAC yB22B2, which contains *CD3D* and *CD3G*, was shown to span the breakpoint of several 11q23 translocations (5). We constructed a rare-cutting restriction map and cloned the ends of the insert (Fig. 1*A*). The *CD3* genes were next to the left vector arm. A subclone of the right end of the insert (RE1.1) mapped telomeric to the t(4;11) breakpoint. The genomic insert was subcloned into λ phage and probes were isolated that localized the t(4;11) breakpoint to a central 70-kb *Not* I fragment (Fig. 1*B*). Probes 98.40 and P/S4 mapped

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Abbreviations: YAC, yeast artificial chromosome; HMG, high mobility group.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. L22179).

breakpoints of the RS4;11, MV-411, and B1 cell lines and nine patient specimens (Fig. 1*C*). These 11q23 breakpoints clustered within a 7-kb region, consistent with results from our earlier studies (24, 25).

The probe P/S4 hybridized to a normal 4.6-kb *Eco*RI fragment and a rearranged 3.7-kb *Eco*RI fragment of der(4) origin in the cell line RS4;11. This rearranged *Eco*RI fragment was cloned and shown by PCR-based somatic cell hybrid analysis to span the RS4;11 breakpoint. A PCR product derived from the 4q21 portion was used to clone normal chromosome 4 sequences. DNA sequence comparison of der(4) with germ-line 4q21 and 11q23 revealed the presence of a 43-bp inverted repeat of chromosome 11 origin at the der(4) breakpoint (Fig. 2). At the border of this repeated sequence is a 14-of-18 match with the *in vitro* vertebrate topoisomerase II binding and cleavage consensus (26). This sequence also has features of *in vivo* topoisomerase II binding and cleavage sites (27), having a G+C-rich core surrounded by A+T-rich tracks. Sequences conforming to the χ-like element (28) [GC(A/T)GG(A/T)GG] are present 48 bp from the breakpoint in the 11q23 germ-line sequence and at the breakpoint in the 4q21 germ-line sequence.

***MLL* Sequence Homologies and Alternative Processing.** Evolutionarily conserved probes were used to isolate clones from a RS4;11 cDNA library and a contig of >9 kb was assembled (Fig. 3*A*). Northern analysis using chromosome 11-encoded cDNAs revealed predominant MLL transcripts of 14.5 and 12.0 kb in cell lines without the t(4;11). In contrast, the t(4;11)-bearing cell lines RS4;11 and B1 also demonstrated an abundant altered transcript of 11 kb (Fig. 3*B*).

The cDNA contig revealed a 2304-aa open reading frame running in a 5′ centromeric to 3′ telomeric orientation (Fig. 4). The sequence encoded 5′ of the breakpoint is of 11q23/*MLL* origin. The BLAST program identified regions of homology with DNA-(cytosine-5)-methyltransferase (29) (Poisson probability, P = 0.0013) and the *Drosophila* zinc-finger protein trithorax (14) (P = 0.0072) (Figs. 4 and 5). The homology with methyltransferase is in a zinc-finger-like motif. Sequence homology to trithorax was noted in two regions of *MLL* proximal to the breakpoint. Homology with trithorax telomeric of the breakpoint has been reported (12, 13, 30). Also present are homologies between MLL and the 70-kDa protein of U1 small nuclear ribonucleoprotein particles (31) and the AT-hook DNA-binding motif of the high-mobility-group (HMG)-I and HMG-Y transcriptional activating proteins (15, 16) (Figs. 4 and 5). The homology with the snRNP protein is in an evolutionarily conserved arginine-rich region of unknown function.

Examples of alternative splicing within MLL were identified. An alternative exon of 33 aa starts at residue 145 in Fig. 4. Clone pc902, isolated from a regenerating marrow cDNA library, demonstrated the molecular basis for the alternative 14.5- and 12.0-kb normal *MLL* transcripts (Figs. 3*A* and 4). Alternative use of donor and acceptor splice sites removes 2.5 kb of coding sequence. A probe derived from the spliced region (3B) hybridizes only to the larger, 14.5-kb normal *MLL* transcript (Fig. 3*B*). This splice removes the MLL AT-hook motifs (Fig. 4).

t(4;11) Produces a Fusion Transcript with a 4q21 Gene. Extension of the cDNA contig in the telomeric direction demonstrated the presence of a fusion transcript encoded on the der(11) chromosome. Probes from cDNA sequences located telomeric to the breakpoint mapped to chromosome 4. Gu *et al.* (13) have named this gene *AF-4* (here referred to as *AF4*).

Northern analysis using a cDNA probe of chromosome 4 origin identified a predominant 9.5-kb transcript and a faint 10.8-kb transcript in cell lines and a wide range of tissues (Fig. 3*B*). An additional 11-kb RNA was present only in the cell lines with the t(4;11) and represents the fusion transcript with *MLL* encoded on the der(11). Sequence analysis indicates an

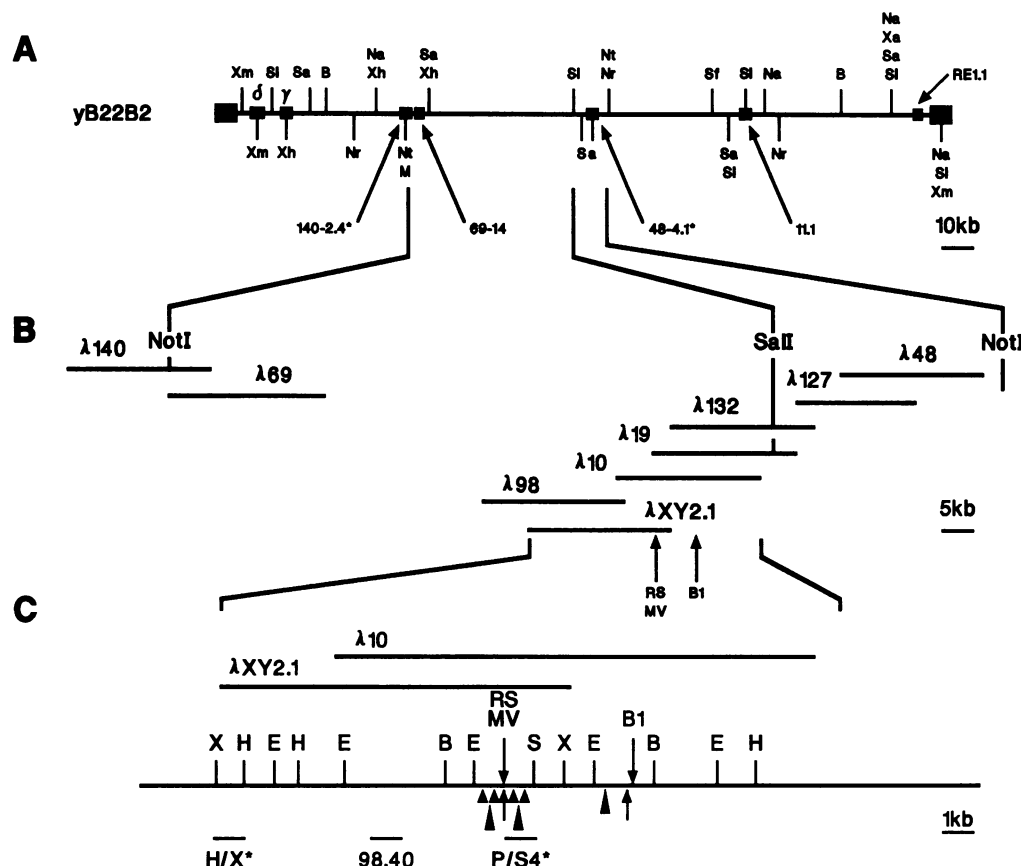

FIG. 1. (*A*) Rare-cutting restriction endonuclease map of yB22B2 insert in a centromeric (left) to telomeric (right) orientation. Thick boxes, vector arms; intermediate-thickness boxes, unique-sequence probes; thin line, human insert; δ, *CD3D*; γ, *CD3G*; B, *Bss*hII; M, *Mlu* I; Na, *Nae* I; Nr, *Nru* I; Nt, *Not* I; Sa, *Sac* II; Sf, *Sfi* I; Sl, *Sal* I; Xh, *Xho* I; Xm, *Xma* I. (*B*) λ phage contig across a 70-kb *Not* I fragment containing the breakpoint. RS, MV, and B1 are the breakpoint sites of the RS4;11, MV-411, and B1 cell lines, respectively. (*C*) Restriction map of the breakpoint region on 11q23. Probes H/X, 98.40, and P/S4 are shown below the map. RS, MV, and B1 are the sites of the RS4;11, MV-411, and B1 breakpoints. Small arrowheads are the sites of t(4;11), large arrowheads the sites of t(9;11), and the upward arrows the sites of t(11;19) patient breakpoints. B, *Bam* HI; E, *Eco*RI; H, *Hin*dIII; S, *Sac* I; X, *Xba* I.

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Ch 4    CTCTGTAAAAGAAGGGATTAGGGGATTAGGATGTAATGGTTTAACTGCTCCCCTTTTGGAAAACCTCCTTCCAGTGGCAT
der (4) CTCTGTAAAAGAAGGGATTAGGGGATTAGGATGTAAatttcctctttcaacacctttggtagtagcattttctttatttA
Ch 11   CTAAAGGTGTTCAGTGATCATAAAGTATATTGAGTGTGAAAGACTTTAAATAAAGAAAATGCTACTACCAAAGGTGTTGA

Ch 4    GCTTGGAGGTGCCAAGTTGCACTTTATGAGACGAGGCAGCCTGTCCTTGTAGATCTTTC
der (4) AAGAGGAAATCAGCACCAACTGGGGGAATGAATAAGAACTCCCATTAGCAGGAGGGTTT
Ch 11   AAGAGGAAATCAGCACCAACTGGGGGAATGAATAAGAACTCCCATTAGCAGGAGGGTTT
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FIG. 2. Breakpoint sequence, centromeric to telomeric, of normal chromosome 4 (Ch 4), RS4;11 der(4), and normal chromosome 11 (Ch 11). Arrows indicate the position and orientation of the inverted repeat present in both the der(4) and normal 11 sequences. Lowercase, der(4) inverted repeat; bracket, topoisomerase II binding site; boxes, χ-like elements.

in-frame fusion of *MLL* and *AF4*. The predominant 11-kb *MLL–AF4* fusion RNA contains the AT-hook motifs (Fig. 3*B*), although we cannot exclude the presence of a minor 9.5-kb species that would lack that motif. An extra glutamine can occur at the point of fusion as a result of the alternative use of two adjacent CAG trinucleotides as a splice acceptor site.

The 865 aa contributed to the der(11) fusion product by *AF4* are rich in serine (17%) and proline (11%). Interestingly, the carboxyl terminus of AF4 contains a glutamine-rich segment with homology to a larger glutamine-rich region in the trithorax protein (Figs. 4 and 5). There are two areas of concentrated basic charge. A putative nuclear localization signal is present in the second basic region (32). Although proline-rich, the AF4 sequence does not have a region where the prolines are as concentrated as is typical of a transcriptional activation domain (33).

DISCUSSION

In this analysis of the t(4;11) we provide additional data to support 11q23 breakpoint clustering, identify genomic se-

FIG. 3. (*A*) cDNA contig. Ch, chromosome; Cen, centromere; Tel, telomere. Filled bars, 11q23-encoded; open bars, 4q21 encoded. (*B*) Northern blots of poly(A)$^+$ RNA from the t(4;11) cell lines RS4;11 (RS) and B1 (B) and the control lines THP-1 (T), Nall-1 (N), and REH (R). Blots were probed successively with a probe of pure *MLL*/11q23 cDNA origin (probe 11), a probe of pure *AF4*/4q21 cDNA origin (probe 621), and then an *MLL* cDNA probe (probe 3B) containing sequence deleted by alternative processing.

1 MAHSCRWRFPARPGTTGGGGGGGRRGLGGGPRQRVPALLLPPGPPVGGGG

51 PGAPPSPPAVAAAAAAAGSSGAGVPGGAAAASAASSSSASSSSSSSSSAS

101 SGPALLRVGPGFDAALQVSAAIGTNLRRFRAVFGESGGGGGSGELTTQIP

151 CSWRTKGHIHDKKTEPFRLLAWSWCLNDEQFLGFGSDEEVRVRSPTRSPS

201 (VKTSPRKPRGRPRSGSDRNSAILSDPSVFSPLNKSETKSGDKIKKKDSKS

251 IEKKRGRPPTFPGVKIKITHGKDISELPKGNKEDSLKKIKRTPSATFQQA

301 TKIKKLRAGKLSPLKSKFKTGKLQIGRKGVQIVRRRGRPPSTERIKTPSG

351 LLINSELEKPQKVRKDKEGTPPLTKEDKTVVRQSPRRIKPVRIIPSSKRT

401 DATIAKQLLQRAKKGAQKKIEKEAAQLQGRKVKTQVKNIRQFIMPVVSAI

451 SSRIIKTPRRFIEDEDYDPPIKIARLESTPNSRFSAPSCGSSEKSSAASQ

501 HSSQMSSDSSRSSSPSVDTSTDSQASEEIQVLPEERSDTPEVHPPLPISQ

551 SPENESNDRRSRRYSVSERSFGSRTTKKLSTLQSAPQQQTSSSPPPPLLT

601 PPPPLQPASSISDHTPWLMPPTIPLASPFLPASTAPMQGKRKSILREPTF

651 RWTSLKHSRSEPQYFSSAKYAKEGLIRKPIFDNFRPPPLTPEDVGFASGF

701 SASGTAASARLFSPLHSGTRFDMHKRSPLLRAPRFTPSEAHSRIFESVTL

751 PSNRTSAGTSSSGVSNRKRKRKVFSPIRSEPRSPSHSMRTRSGRLSSSEL

801 SPLTPPSSVSSSLSISVSPLATSALNPTFTFPSHSLTQSGESAEKNQRPR

851 KQTSAPAEPFSSSSPTPLFPWFTPGSQTERGRNKDKAPEELSKDRDADKS

901 VEKDKSRERDREREKENKRESRKEKRKKGSEIQSSSALYPVGRVSKEKVV

951 GEDVATSSSAKKATGRKKSSSHDSGTDITSVTLGDTTAVKTKILIKKGRG

1001 NLEKTNLDLGPTAPSLEKEKTLCLSTPSSSTVKHSTSS)GSMLAQADKLP

1051 MTDKRVASLLKKAKAQLCKIEKSKSLKQTDQPKAQGQESDSSETSVRGPR

1101 IKHVCRRAAVALGRKRAVFPDDMPTLSALPWEEREKILFSMGNDDKSSIA

1151 GSEDAEPLAPPIKPIKPVTRNKAPQEPPVKKGRRSRRCGQCPGCQVPEDC

1201 GVCTNCLDKPKFGGRNIKKQCCKMRKCQNLQWMPSKAYLQKQAKAVKKKE

1251 KKSKTSEKKDSKESSVVKNVVDSSQKPTPSAREDPAPKKSSSEPPPRKPV

1301 EEKSEEGNVSAPGPESKQATTPASRKSSKQVSQPALVIPPQPPTTGPPRK

1351 EVPKTTPSEPKKKQPPPPESGPEQSKQKKVAPRPSIPVKQKPKEKEKPPP

Ch 11 ↓ Ch 4

1401 VNKQENAGTLNIFSTLSNGNSSKQKIPADGVHRIRVDFKQTYSNEVHCVE

1451 EILKEMTHSWPPPLTAIHTPSTAEPSKFPFPTKDSQHVSSVTQNQKQYDT

1501 SSKTHSNSQQGTSSMLEDDLQLSDSEDSDSEQTPEKPPSSSAPPSAPQSL

1551 PEPVASAHSSSAESESTSDSDSSSDSESESSSSDSEENEPLETPAPEPEP

1601 PTTNKWQLDNWLTKVSQPAAPPEGPRSTEPPRRHPESKGSSDSATSQEHS

1651 ESKDPPPKSSSKAPRAPPEAPHPGKRSCQKSPAQQEPPQRQTVGTKQPKK

1701 PVKASARAGSRTSLQGEREPGLLPYGSRDQTSKDKPKVKTKGRPRAAASN

1751 EPKPAVPPSSEKKKHKSSLPAPSKALSGPEPAKDNVEDRTPEHFALVPLT

1801 ESQGPPHSGSGSRTSGCRQAVVVQEDSRKDRLPLPLRDTKLLSPLRDTPP

1851 PQSLMVKITLDLLSRIPQPPGKGSRQRKAEDKQPPAGKKHSSEKRSSDSS

1901 SKLAKKRKGEAERDCDNKKIRLEKEIKSQSSSSSSSHKESSKTKPSRPSS

1951 QSSKKEMLPPPPVSSSSQKPAKPALKRSRREADTCGQDPPKSASSTKSNH

2001 KDSSIPKQRRVEGKGSRSSSEHKGSSGDTANPFPVPSLPNGNSKPGKPQV

2051 KFDKQQADLHMREEKKMKQKAELMTDRVGKAFKYLEAVLSFIECGIATES

2101 ESQSSKSAYSVYSETVDLIKFIMSLKSFSDATAPTQEKIFAVLCMRCQSI

2151 LNMAMFRCKKDIAIKYSRTLNKHFESSSKVAQAPSPCIARSTGTPSPLSP

2201 MPSPASSVGSQSSAGSVGSSGVAATISTPVTIQNMTSSYVTITSHVLTAF

2250 DLWEQAEALTRKNKEFFARLSTNVCTLALNSSLVDLVHYTRQGFQQLQEL

2301 TKTP*

FIG. 4. Derived amino acid sequence (single-letter code) of the der(11) fusion protein. Arrow indicates the chromosome (Ch) 11–Ch 4 junction. Parentheses indicate amino acids removed in the alternatively processed MLL transcript. Sequence homologies: dashed underline, AT-hook motif; thin underline, U1 small nuclear ribonucleoprotein; thick underline, DNA methyltransferase; boxes, trithorax. AF4 basic regions are bracketed. Nuclear localization consensus sequence is overlined.

quence motifs that may be involved in the mechanism of translocation, and describe the complete open reading frame of a der(11) fusion transcript. This fusion transcript juxtaposes 5′ sequence from the *MLL* gene on 11q23 and 3′ sequence from the gene on 4q21 encoding the putative transcription factor AF4.

The 11q23 breakpoints from a range of leukemias are clustered within a 7-kb region of *MLL*. Comparison of the cDNA and genomic sequences from both 11q23 and 4q21 reveals that the RS4;11 breakpoint has occurred within introns of *MLL* and *AF4*. Our breakpoint mapping of cell lines and patient material with *MLL* translocations indicates that the *MLL* exon just centromeric and the *AF4* exon just telomeric of the RS4;11 breakpoint are alternatively incorporated into the fusion transcript without changing the reading frame.

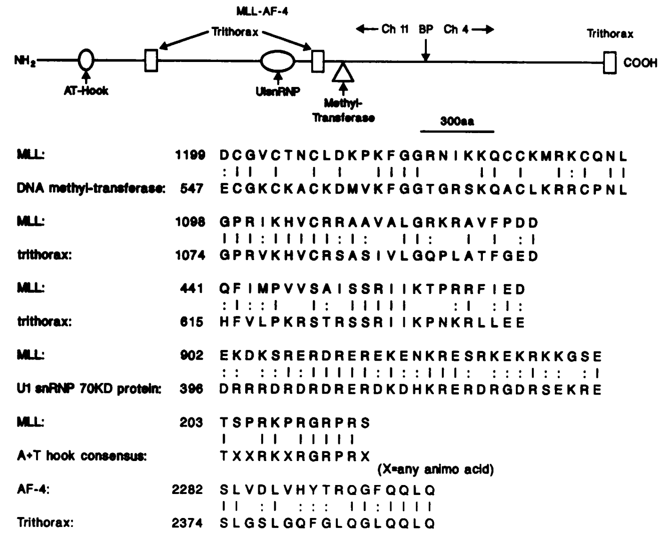

FIG. 5. Sequence homologies. At the top, a schematic representation of the MLL-AF-4 fusion protein shows the location of the homologies. Vertical line, identity; colon, conservative amino acid change; snRNP 70KD, small nuclear ribonucleoprotein 70-kDa.

At the der(4) breakpoint in the RS4;11 cell line there is an inverted repeat of chromosome 11 origin. At the telomeric border of this repeat is a sequence with the characteristics of *in vitro* vertebrate (26) and *in vivo* (27) consensus topoisomerase II binding and cleavage sites. This is quite interesting in that *MLL* breakpoints also occur in the same region in leukemias that follow treatment with topoisomerase II inhibitors (P.H.D., unpublished data). χ-like elements, which have been found near translocation breakpoints in other types of hematologic malignancies, are present in the region of the RS4;11 breakpoint (28). Our analysis of the flanking chromosome 4 and 11 sequences and analysis of the normal 11 by Djabali *et al.* (30) indicate they are rich in *Alu* and LINE-1 repetitive elements. However, the sequences immediately adjacent to the breakpoint do not have significant homology to these repetitive elements. In addition, heptamers resembling those in antigen-receptor genes have also been noted in the breakpoint regions on 4q21 and 11q23 (34), but they are not in the orientation typical of recombination sites (35).

The MLL sequence reported here is homologous with the *Drosophila* trithorax gene product and the HMG protein AT-hook (12, 13). In addition, we have noted a cysteine-rich, zinc-finger-like region of MLL encoded 5′ of the breakpoint cluster which has homology to a DNA methyltransferase. Antibodies to this motif of the methyltransferase can inhibit enzymatic function, although it is not the catalytic site. It has been postulated the motif takes part in protein–protein interactions which activate enzymatic function (29). Of interest, this region is retained in both alternatively processed forms of MLL. The homology to trithorax and the AT hook of the HMG proteins suggests that MLL also functions to control transcription. Both the zinc fingers and AT hooks in MLL could bind DNA. MLL appears to be widely expressed, but if its function is analogous to that of trithorax its effect could vary markedly from tissue to tissue (36).

AF4 has a number of features which suggest it also functions as a transcription factor. It has a putative nuclear localization signal and two basic regions. The carboxyl terminus contains a glutamine-rich possible transcriptional activation domain with homology to trithorax.

Cytogenetic and molecular studies argue that the der(11) (17, 18) is required for leukemogenesis. Our data suggest several possible roles for the MLL–AF4 product in transformation. The MLL zinc fingers are downstream of the break-

point cluster region and are not retained in the der(11) product. DNA binding could then be directed by the AT hooks. Either the absence of the zinc fingers or the presence of AF4 sequence in the fusion protein may redirect the sites or alter the effects of DNA binding. Either scenario would be consistent with a gain-of-function mechanism for leukemogenesis. The region of DNA methyltransferase homology might function in protein–protein interactions (37) that could produce a dominant negative effect by trapping normal MLL in functionally inactive complexes.

The cloning and description of the t(4;11) breakpoint and the complete der(11) fusion product in this report offer insight into the pathogenesis of an important class of leukemias. This will enable studies with models of altered MLL which will determine whether gain-of-function, loss-of-function, or dominant negative mechanisms are operative in leukemogenesis.

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1. Rowley, J. D. (1982) *Science* **216**, 749–751.
2. Korsmeyer, S. J. (1992) *Annu. Rev. Immunol.* **10**, 785–807.
3. Parkin, J. L., Arthu, D. C., Abramson, C. S., McKenna, R. W., Kersey, J. H., Heideman, R. L. & Brunning, R. D. (1982) *Blood* **60**, 1321–1331.
4. Ramimondi, S. C., Peiper, S. C., Kitchingman, G. R., Behm, F. G., Williams, D. L., Hancock, M. L. & Mirro, J. (1989) *Blood* **73**, 1627–1634.
5. Rowley, J. D., Diaz, M. O., Espinosa, R., Patel, Y. D., van Melle, E., Zieman, S., Tallon-Miller, P., Lichter, P., Evans, G., Kersey, J. H., Ward, D. C., Domer, P. H. & LeBeau, M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9358–9362.
6. Cimino, G., Moir, D. R., Canaani, O., Williams, K., Crist, W. M., Katzav, S., Cannizzaro, L., Lange, B., Nowell, P. C., Croce, C. M. & Canaani, E. (1991) *Cancer Res.* **51**, 6712–6714.
7. Cimino, G., Nakamura, T., Gu, Y., Canaani, O., Parsad, R., Crist, W. M., Carroll, A. J., Baer, M., Bloomfield, C. D., Nowell, P. C., Croce, C. M. & Canaani, E. (1991) *Cancer Res.* **52**, 3811–3813.
8. Zieman-van der Poel, S., McCabe, N. R., Gill, H. J., Espinosa, R., Patel, Y., Harden, A., Rubinelli, P., Smith, S. D., LeBeau, M., Rowley, J. D. & Diaz, M. O. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10735–10739.
9. Pui, C.-H., Frankel, L. S., Carroll, A. J., Raimondi, S. C., Shuuster, J. J., Head, D. R., Crist, W. M., Land, V. J., Pullen, D. J., Steuber, C. P., Behm, F. G. & Borowitz, M. J. (1991) *Blood* **77**, 440–447.
10. Kalwinsky, D. K., Raimondi, S. C., Schell, M. J., Mirro, J., Santana, V. M., Behm, F., Dahl, G. V. & Williams, D. (1990) *J. Clin. Oncol.* **8**, 75–83.
11. Pui, C. H., Riberio, R. C., Hancock, M. L., Rivera, G. K., Evans, W. E., Raimondi, S. C., Head, D. R., Behm, F. G., Mahmound, M. H., Sandlund, J. T. & Crist, W. M. (1991) *N. Engl. J. Med.* **325**, 1682–1687.
12. Tkachuck, D. C., Kohler, S. & Cleary, M. L. (1992) *Cell* **71**, 691–700.
13. Gu, Y., Nakamura, T., Alder, H., Prasad, R., Canaani, O., Cimino, G., Croce, C. M. & Canaani, E. (1992) *Cell* **71**, 701–708.
14. Mazo, A., Der-Hwa, H., Mozer, B. A. & Dawid, I. B. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2112–2116.
15. Reeves, R. & Nissen, M. S. (1990) *J. Biol. Chem.* **265**, 8573–8582.
16. Fashena, S. J., Reeves, R. & Ruddle, N. H. (1992) *Mol. Cell. Biol.* **12**, 894–903.
17. Rowley, J. D. (1992) *Genes Chrom. Cancer* **5**, 264–266.
18. Thirman, M. J., Gill, H. J., Burrent, R. C., Mbangkollo, D., McCabe, N., Kobayashi, H., Diaz, M. O. & Rowley, J. D. (1992) *Blood* **80**, 254a.
19. Stong, R. C., Korsmeyer, S. J., Parkin, J. L., Arthur, D. C. & Kersey, J. H. (1985) *Blood* **65**, 21–31.
20. Cohen, A., Grunberger, T., Vanek, W., Duke, I. D., Doherty, P. J., Letarte, M., Roifman, C. & Freedman, M. H. (1991) *Blood* **78**, 94–102.
21. Lange, B., Valtieri, M., Santoli, D., Caraccicolo, D., Mavillo, F., Gemperlein, I., Griffin, C., Emanuel, B., Finan, J., Nowell, P. & Rovera, G. (1987) *Blood* **70**, 192–198.
22. Brownstein, B. H., Silverman, G. A., Little, R. A., Burke, D. T., Korsmeyer, S. J., Schlessinger, D. & Olson, M. V. (1989) *Science* **244**, 1348–1351.
23. Savage, P. D., Shapiro, M., Langdon, W. Y., Geurts van Kessel, A. H. M., Seuanez, H. N., Akao, Y., Croce, C. M., Morse, H. C. & Kersey, J. H. (1991) *Cytogenet. Cell Genet.* **56**, 112–116.
24. Morgan, G. J., Cotter, F., Katz, F. E., Ridge, S. A., Domer, P., Korsmeyer, S. & Wiedmann, L. M. (1992) *Blood* **80**, 2172–2175.
25. Chen, C.-S., Sorensen, P., Domer, P., Heerema, N., Reaman, G. H., Korsmeyer, S. J., Hammond, D. & Kersey, J. H. (1993) *Blood* **81**, 2386–2393.
26. Spitzner, J. R. & Muller, M. T. (1988) *Nucleic Acids Res.* **16**, 5533–5556.
27. Kas, E. & Laemmli, U. K. (1992) *EMBO J.* **11**, 705–716.
28. Wyatt, R. T., Rudders, R. A., Zelenetz, A., Delellis, R. A. & Kontiris, T. G. (1992) *J. Exp. Med.* **175**, 1575–1588.
29. Bestor, T., Laudano, A., Mattaliano, R. & Ingram, V. (1988) *J. Mol. Biol.* **203**, 971–983.
30. Djabali, M., Selleri, L., Parry, P., Bower, M., Young, B. D. & Evans, G. A. (1992) *Nat. Genet.* **2**, 113–118.
31. Theissen, J., Etzerodt, M., Reuter, R., Schneider, C., Lottspeich, F., Argos, P., Luhrmann, R. & Philipson, L. (1986) *EMBO J.* **5**, 3209–3217.
32. Chelsky, D., Ralph, R. & Jonak, G. (1989) *Mol. Cell. Biol.* **9**, 2487–2492.
33. Latchman, D. S. (1990) *Biochem. J.* **270**, 281–289.
34. Gu, Y., Cimino, G., Adler, H., Nakamura, T., Prasad, R., Canaani, O., Moir, D. T., Jones, C., Nowell, P. C., Croce, C. M. & Canaani, E. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10464–10468.
35. Gellert, M. (1992) *Trends Genet.* **8**, 408–412.
36. Breen, T. R. & Harte, P. J. (1993) *Development* **117**, 119–134.
37. Berg, J. M. (1990) *J. Biol. Chem.* **265**, 6513–6516.