Panhandle PCR for cDNA: A rapid method for isolation of *MLL* fusion transcripts involving unknown partner genes

Maureen D. Megonigal*†, Eric F. Rappaport†‡, Robert B. Wilson§, Douglas H. Jones¶, James A. Whitlock‖, Jorge A. Ortega, Diana J. Slater*†, Peter C. Nowell§, and Carolyn A. Felix*†,††‡‡**

*Division of Oncology, ‡Joseph Stokes, Jr., Research Institute, †The Children's Hospital of Philadelphia, Departments of §Pathology and Laboratory Medicine and ††Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; ¶University of Iowa, Iowa City, IA 52222; ‖Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232; and **Department of Pediatrics, Children's Hospital, Los Angeles, CA 90027

Contributed by Peter C. Nowell, May 25, 2000

Identifying translocations of the *MLL* gene at chromosome band 11q23 is important for the characterization and treatment of leukemia. However, cytogenetic analysis does not always find the translocations and the many partner genes of *MLL* make molecular detection difficult. We developed cDNA panhandle PCR to identify der(11) transcripts regardless of the partner gene. By reverse transcribing first-strand cDNAs with oligonucleotides containing coding sequence from the 5′ *MLL* breakpoint cluster region at the 5′ ends and random hexamers at the 3′ ends, known *MLL* sequence was attached to the unknown partner sequence. This enabled the formation of stem-loop templates with the fusion point of the chimeric transcript in the loop and the use of *MLL* primers in two-sided PCR. The assay was validated by detection of the known fusion transcript and the transcript from the normal *MLL* allele in the cell line MV4–11. cDNA panhandle PCR then was used to identify the fusion transcripts in two cases of treatment-related acute myeloid leukemia where the karyotypes were normal and the partner genes unknown. cDNA panhandle PCR revealed a fusion of *MLL* with *AF-10* in one case and a fusion of *MLL* with *ELL* in the other. Alternatively spliced transcripts and exon scrambling were detectable by the method. Leukemias with normal karyotypes may contain cryptic translocations of *MLL* with a variety of partner genes. cDNA panhandle PCR is useful for identifying *MLL* translocations and determining unknown partner sequences in the fusion transcripts.

Translocations of the *MLL* gene at chromosome band 11q23 occur in leukemias of infants (reviewed in ref. 1) and leukemias associated with DNA topoisomerase II inhibitors (reviewed in ref. 2). The ability to rapidly identify *MLL* translocations, whether by cytogenetic or molecular approaches, is relevant to diagnosis and prognosis and to treatment planning. *MLL* is an example of a gene involved in translocations with numerous different partner genes; many are still uncharacterized (1, 2). The specific partner gene with which *MLL* is fused may have an impact on the clinical response (3). Previously, we developed panhandle PCR approaches to identify *MLL* der(11) translocation breakpoints in genomic DNA (4–8). The salient features include attachment of known *MLL* sequence to the unknown partner gene, formation of a stem-loop template, and two-sided PCR. Because *MLL* sequences are at both ends of the template, all primers are derived from *MLL*. Panhandle PCR methods offer the advantage of amplifying *MLL* translocation breakpoints without primers from the partner genes.

Although panhandle PCR approaches are highly effective for genomic translocation breakpoint cloning (4–8), sometimes the genomic target sequence may be too large to amplify. In addition, if the amplicon contains intronic sequence only and not exonic sequence, panhandle PCR approaches may not reveal the partner gene (4, 8). *MLL* genomic translocation breakpoints occur within an 8.3-kb breakpoint cluster region (bcr) (9). Here, we targeted the corresponding 859-bp cDNA (9) for a panhandle PCR approach that would identify der(11) transcripts. We used cDNA panhandle PCR to investigate the nature of the *MLL* fusion transcripts in two cases of treatment-related acute myeloid leukemia (AML) with normal karyotypes but with *MLL* gene rearrangement detectable by Southern blot. Leukemias with normal karyotypes have been found to harbor *MLL* tandem duplications (6, 10–17). The results of this analysis show that leukemias with normal karyotypes may harbor *MLL* gene translocations. cDNA panhandle PCR can lead readily to discovery of partner genes.

Methods

The institutional review boards at Children's Hospital Los Angeles, Vanderbilt University Medical Center, and The Children's Hospital of Philadelphia approved this research. The AML cell line MV4–11 with known t(4;11) (18) and two cases of treatment-related AML (t-AML) (Table 1) were studied. Genomic DNA and total RNA were isolated from MV4–11 cells and cryopreserved leukemic marrow cells by using 5.7 M CsCl-4 M guanidine isothiocyanate gradients. *MLL* gene rearrangement was examined by using the B859 fragment of *ALL-1* cDNA (19).

cDNA Panhandle PCR Analysis of Normal *MLL* and der(11) Transcripts. Fig. 1 summarizes cDNA panhandle PCR. In step 1, a population of first-strand cDNAs is reverse-transcribed from total RNA with oligonucleotides containing known *MLL* coding sequence from the 5′ bcr at the 5′ ends and random hexamers at the 3′ ends. The random hexamers anneal to and prime from many complementary sequences in total RNA. This produces first-strand cDNAs of different sizes. Those derived from the normal *MLL* allele and from der(11) transcripts have a known *MLL* sequence and its inverse complement at the 5′ and 3′ ends, respectively (Fig. 1 *Upper Left*). The purpose of step 2 is to generate sense second strands by *MLL* primer 1 extension from the first-strand cDNAs (Fig. 1 *Upper Center*). In step 3, primer 2 is added and thermal cycling is begun. The first event during thermal cycling is formation of stem-loop "panhandle" templates from the second strands. Heat denaturation makes the double-stranded cDNAs single-stranded. Intrastrand annealing of complementary *MLL* sequences in the second strands and polymerase extension of the recessed 3′ ends complete forma-

Abbreviations: AML, acute myeloid leukemia; bcr, breakpoint cluster region; t-AML, treatment-related AML; TAP, tip-associated protein.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF272370–AF272385).

‡‡To whom reprint requests should be addressed at: Division of Oncology, Leonard and Madlyn Abramson Pediatric Research Center, Room 902B, The Children's Hospital of Philadelphia, 3516 Civic Center Boulevard, Philadelphia, PA 19104-4318. E-mail: felix@email.chop.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.150241797. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.150241797

Table 1. Cases of t-AML

	Patient t-36	Patient t-44
Race/gender	Hispanic/male	White/male
Primary cancer	Alveolar rhabdomyosarcoma	Ewing's sarcoma
Age at primary cancer diagnosis	1 yr, 4 months	10 yrs, 8 months
Prior chemotherapy	VCR, VP16, IFOS	VCR, VP16, IFOS, ADR, CPM, AMD, CBDCA
Prior radiation	No	No
Interval to t-AML	60 months	34 months
FAB	M4	M2
Karyotype	46,XY	46,XY

VCR indicates vincristine; VP16, etoposide; IFOS, ifosphamide; ADR, doxorubicin; CPM, cyclophosphamide; AMD, dactinomycin; CBDCA, carboplatin; FAB, French-American-British.

tion of the handles. Either normal *MLL* sequence or the point of fusion in the chimeric transcripts and coding sequence of the partner gene are within the loops (Fig. 1 *Upper Right*). Step 4 continues with primer 1 extension from the 3′ end of the stem-loop structure during the thermal cycling. This generates the double-stranded template with *MLL* sequence at both ends to be amplified exponentially with *MLL* primers 1 and 2 (Fig. 1 *Lower Left*). Step 5 entails nested PCR with *MLL* primers 3 and 4 (Fig. 1 *Lower Right*). Positions of the *MLL* primers relative to the *MLL* sequence in the oligonucleotides used to generate the first-strand cDNAs are shown in Fig. 1.

The specific protocol follows. First-strand cDNAs were synthesized from 1 μg of total RNA by using the Superscript Preamplification System (Life Technologies, Grand Island, NY), except that *MLL*-random hexamer oligonucleotides were used in place of random hexamers. The oligonucleotide sequences were 5′-CCTGAATCCAAACAGGCCACCACTCCAGCTTC-NNNNNN-3′ (8); the *MLL* sequence in the oligonucleotides corresponded to bcr cDNA positions 92–123 in exon 5 (8, 9). Two microliters of first-strand cDNAs was added to a 45.5 μl mixture containing 1.75 units *Taq*/Pwo DNA polymerase, 385 μM each dNTP, 1.1× buffer (Expand Long Template System, Boehringer Mannheim), and 12.5 pmols of *MLL* primer 1 (8). The mixture was heated to 80°C for 5 min before the cDNAs were added. Primer 1 corresponded to *MLL* bcr cDNA positions 34–55 in exon 5 (5′-TCCTCCACGAAAGCCCGTCGAG-3′) (8, 9), upstream of the *MLL* sequence in the oligonucleotides used to synthesize the first-strand cDNAs. To achieve primer 1 extension, the mixture was denatured at 94°C for 1 min followed by 1 cycle at 94°C for 10 sec and 68°C for 7 min. The sample was heated again to 80°C for 5 min and 2.5 μl (12.5 pmols) of *MLL* primer 2 were added. Primer 2 corresponded to *MLL* bcr cDNA positions 136–158 in exon 5 (5′-TCAAGCAGGTCTCCCAGC-CAGCAC-3′) (8, 9), downstream of the *MLL* sequence in the oligonucleotides used to synthesize the first-strand cDNAs. The final 50-μl PCRs contained 350 μM each dNTP and 1× buffer. PCR with primers 1 and 2, including the 7-min elongation and the increment in elongation times, was as described (4, 5, 8). One microliter of the products was used in nested PCR with primers 3 (5′-GGAAAAGAGTGAAGAAGGGAATGTCTCGG-3′) and 4 (5′-GTGGTCATCCCGCCTCAGCCAC-3′) corresponding to *MLL* bcr cDNA positions 55–83 and 159–179 in exon 5 (8, 9). Conditions were the same as above.

cDNA panhandle PCR products were subcloned by recombi-

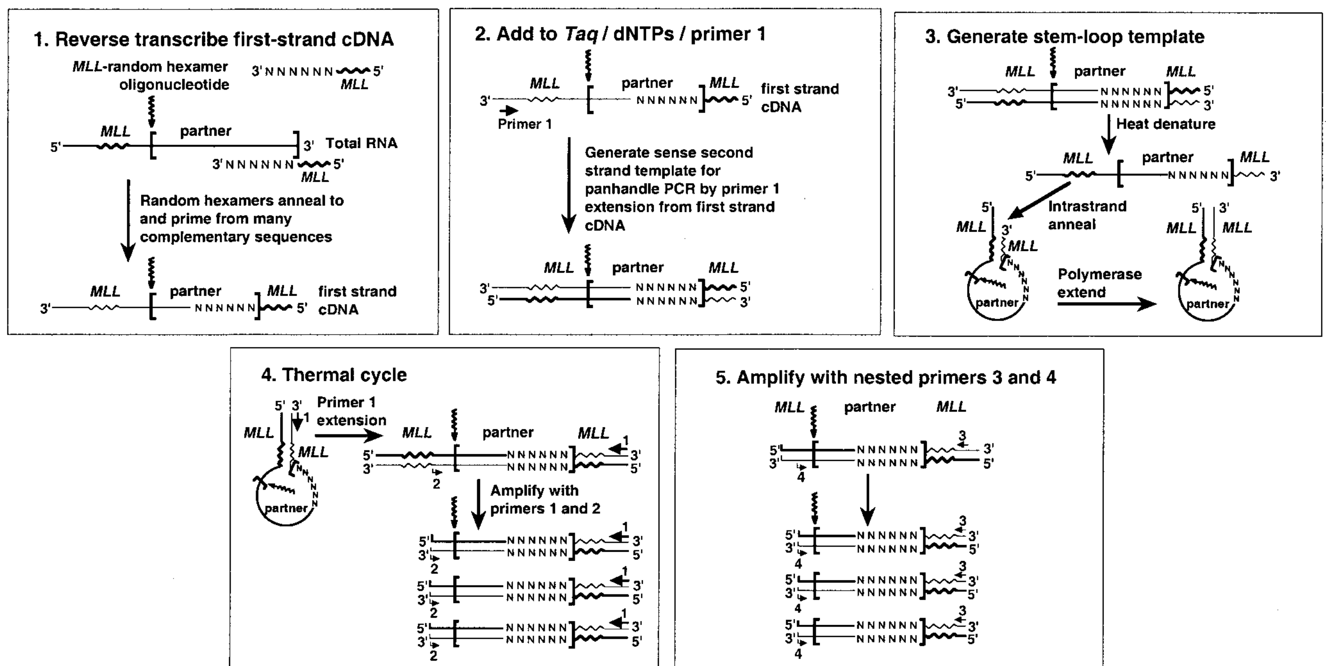

Fig. 1. Steps in cDNA panhandle PCR. Corkscrew arrow indicates fusion point in *MLL* chimeric transcript. A population of first-strand cDNAs of various sizes is expected because of *MLL*-random hexamer oligonucleotide design. Transcripts from normal *MLL* allele also would be amplified.

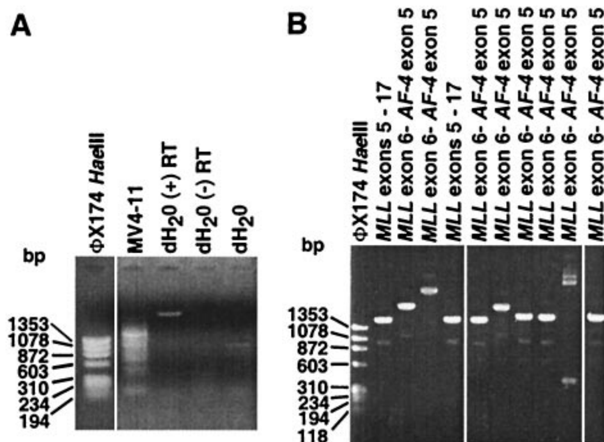

Fig. 2. (*A*) cDNA panhandle PCR analysis of total RNA from cell line MV4–11. Smear indicates products of various sizes (lane MV4-11). dH_2O control reactions with and without reverse transcriptase (RT) were included. (*B*) PCR screen of recombination PCR-generated subclones of cDNA panhandle PCR products from cell line MV4–11. *MLL*-containing subclones shown by sequencing are indicated above respective lanes. Two subclones contained normal *MLL* cDNA; eight showed in-frame fusion of *MLL* exon 6 to position 148 of 2,805-bp full-length *AF-4* cDNA (GenBank accession no. L13773).

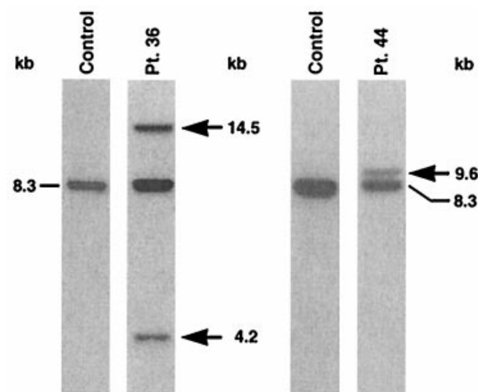

Fig. 3. Identification of *MLL* rearrangements in cases of t-AML by Southern blot analysis. *Bam*HI-digested marrow DNAs were hybridized with B859 fragment of *ALL-1* cDNA (9). Peripheral blood lymphocyte DNA from normal subject was used as the control. Dash shows germ-line band; arrows show rearrangements. Patient numbers correspond to text and Table 1.

nation PCR (7). pUC19 was linearized by *Hin*dIII digestion. *MLL* ends complementary to the ends of the cDNA panhandle PCR products that were to be inserted were added to the vector during PCR using primers 5′-ACATTCCCTTCTTCA-CTCTTTTCCTGGCGTAATCATGGTCATAGC-3′ and 5′-GTGGCTGAGGCGGGATGACCACCATGCCTGCAG-GTCGACTC-3′ (8) and described conditions (7). The PCR-amplified pUC19 and cDNA panhandle PCR products were purified by using Geneclean III reagents and resuspended in 10 μl of elution buffer (Bio 101). PCR-amplified, purified pUC19 (2.5 μl) and 2.5 μl of purified cDNA panhandle PCR products were mixed and added to 50 μl of MAX efficiency DH5α cells (Life Technologies) to recombine *in vivo*. The transformation was per manufacturer's instructions (Life Technologies). Subclones containing cDNA panhandle PCR products were identified by PCR with primers 3 and 4 (8).

Validation of der(11) Transcripts. The above-generated first-strand cDNAs were amplified with gene-specific primers. For the t-AML of patient 36, the sense primer 5′-CGCCCAAGTATC-CCTGTAAA-3′ from *MLL* exon 6 or 5′-TGAAGCA-GAAAATGTGTGGG-3′ from *MLL* exon 8 was used with the antisense primer 5′-GATTTCTTCCCTTTGCCCTC-3′ from *AF-10*. For the t-AML of patient 44, the sense primer 5′-AGTGAGCCCAAGAAAAAG-3′ from *MLL* exon 5 was used with the antisense primer 5′-GCCGATGTTGGAGAGG-TAGA-3′ from *ELL*.

Results

cDNA Panhandle PCR Detects the Known der(11) Transcript in MV4–11 Cell Line. To test cDNA panhandle PCR as a strategy for cloning der(11) transcripts, the cell line MV4–11 with a known transcript fusing *MLL* exon 6 to *AF-4* exon 5 (GenBank accession nos. L04284 and NM_005933) (20, 21) was studied. cDNA panhandle PCR products of various sizes were obtained (Fig. 2*A*). Recombination PCR gave 50 subclones, 12 of which were sequenced (Fig. 2*B*). Two subclones with 1,589-bp inserts contained *MLL* exons 5–17, suggesting that they were from transcripts from the normal *MLL* allele. The expected in-frame fusion of *MLL* exon 6 to *AF-4* exon 5 (20, 21) was present in eight of the 12 subclones. The insert sizes that contained this junction were from 381 to 2,904 bp; all but one were >1,565 bp and three were >2 kb. Because the *MLL* bcr cDNA sequence is only 859 bp (9), it was possible to identify the point of fusion in the 381-bp subclone. The sequences of two subclones were consistent with vector without insert.

cDNA Panhandle PCR Reveals a Cryptic t(10,11) Translocation in t-AML with Normal Karyotype. The karyotype was normal in the t-AML of patient 36 (Table 1) but 14.5- and 4.2-kb rearrangements on Southern blot analysis were consistent with a cryptic translocation involving *MLL* (Fig. 3). We used cDNA panhandle PCR to ascertain the partner gene. cDNA panhandle PCR products of various sizes were obtained (Fig. 4*A*). Recombination PCR gave 261 subclones (Fig. 4*B*); 25 were sequenced. Of the 25, 12 contained *MLL* sequence only, 12 revealed the unknown partner gene, and one was empty vector.

The subclones with *MLL* sequence only are summarized in Fig. 4*C*. The insert sizes were from 180 to 947 bp. These subclones were derived from fully processed transcripts, incompletely processed transcripts with intronic sequence, and alternatively spliced transcripts skipping exon 8. Transcripts fusing the end of an internal codon in exon 5 with the start of an internal codon in exon 3 suggested exon scrambling (22, 23).

Two in-frame chimeric transcripts were detected that fused either *MLL* exon 7 or *MLL* exon 8 to position 979 of the cDNA sequence of *AF-10* (GenBank accession no. U13948) (Fig. 4*C*). Five subclones with 605-bp inserts contained the *MLL* exon 7-*AF-10* junction. Seven subclones with 1,842- to 1,845-bp inserts contained the *MLL* exon 8-*AF-10* junction. *AF-10* is a known partner gene of *MLL* at chromosome band 10p12 (24, 25). In the subclones with the *MLL* exon 8-*AF-10* junction, the 48 bases from positions 1883 to 1930 of the corresponding *AF-10* cDNA, which are codons 567–582, were absent; the sequence then continued through *AF-10* cDNA position 2379 in the largest subclone. A recent sequence entry (GenBank accession no. AL161799) containing intron-exon junctions for a portion of *AF-10* suggests that the point of fusion in *AF-10* is the first base of an exon. The same entry suggests that the 48 bases absent from the transcripts fusing *MLL* exon 8 to *AF-10* may comprise an exon. These results indicate that both *MLL* and *AF-10* were alternatively spliced.

Amplification of the same first-strand cDNA with *MLL* exon 6- and *AF-10*-specific primers and sequencing confirmed both fusion transcripts. Predicted 335-bp and 447-bp products contained the *MLL* exon 7-*AF-10* and *MLL* exon 8-*AF-10* junctions. Sequencing of a 268-bp product obtained with *MLL* exon 8- and

MEDICAL SCIENCES

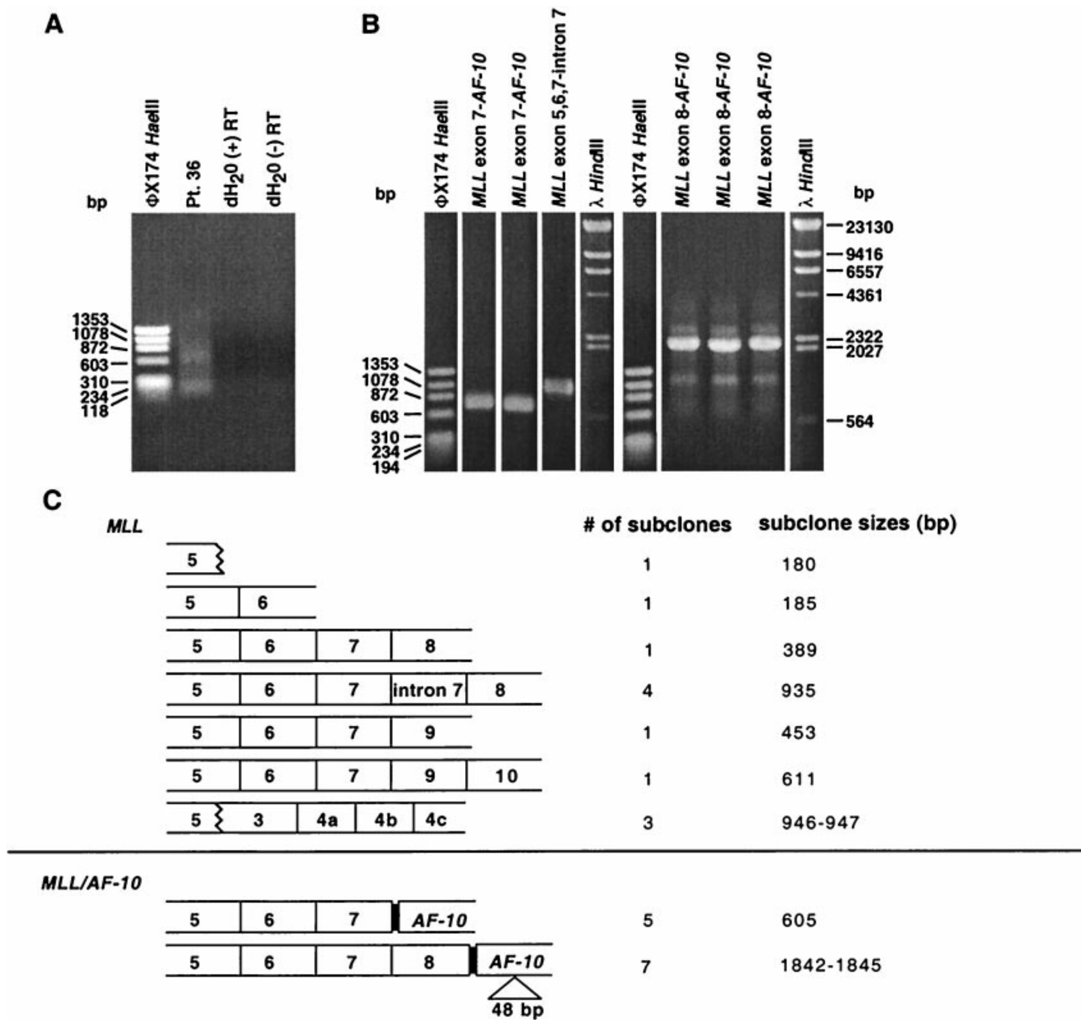

Fig. 4. (*A*) cDNA panhandle PCR analysis of total RNA from t-AML of patient 36. Smear indicates products of heterogeneous sizes (lane Pt. 36). (*B*) PCR screen of representative recombination PCR-generated subclones of cDNA panhandle PCR products shown in *A*. *MLL*-containing subclones confirmed by sequencing are shown above respective lanes. (*C*) Summary of sequences in recombination PCR-generated subclones. Twelve subclones contained *MLL* sequence only (*Upper*). Incompletely processed and alternatively spliced transcripts and transcripts suggesting exon scrambling were detected. In three subclones, exon 5 sequence extended to position 3945 of *MLL* cDNA; the 3′ sequence was from exons 3, 4a, 4b, and 4c starting at position 2782 of *MLL* cDNA (GenBank accession nos. LO4284 and NM_005933). Positions 3945 and 2782 are the end and start of internal codons in *MLL* exons 5 and 3. Chimeric transcripts contained in-frame fusions of either *MLL* exon 7 or *MLL* exon 8 to position 979 of *AF-10* cDNA (GenBank accession no. U13948) (*Lower*).

AF-10-specific primers further confirmed the fusion of *MLL* exon 8 with *AF-10*.

cDNA Panhandle PCR Reveals a Cryptic t(11;19) Translocation in t-AML with Normal Karyotype. The karyotype was also normal in the t-AML of patient 44 (Table 1), but Southern blot analysis of the *MLL* bcr revealed a single 9.6-kb rearrangement (Fig. 3)**.** We used cDNA panhandle PCR to investigate the fusion transcript. The cDNA panhandle PCR products of various sizes are shown in Fig. 5*A*. Recombination PCR gave 130 subclones (Fig. 5*B*), 23 of which were sequenced.

Fifteen of the 23 subclones contained *MLL* sequence only and were from 146 to 456 bp (Fig. 5 *B* and *C*)**.** One subclone ended within exon 5. Three subclones contained *MLL* exons 5–8. One subclone derived from an alternatively spliced transcript contained *MLL* exons 5, 6, 7, and 9, skipping exon 8 (Fig. 5*D*). Ten subclones contained *MLL* exons 5 and 6 and then exon 5 joined accurately at splice junctions, suggesting scrambled exons (22, 23).

Four subclones with 642-bp inserts revealed the unknown partner gene. Each contained a fusion of *MLL* exon 7 to position 148 of the cDNA sequence of *ELL* (GenBank accession no. U16282), a previously described partner gene of *MLL* at chromosome band 19p13 (26, 27). Because *MLL* exon 5 was joined to exon 7, skipping exon 6, this fusion transcript was alternatively spliced. Amplification of a 366-bp product with *MLL* exon 5- and *ELL*-specific primers and sequencing revealed a second chimeric transcript with the same *MLL* exon 7-*ELL* point of fusion, in which *MLL* exon 6 was present (Fig. 5 *C* and *D*)**.**

Three subclones contained *MLL* sequence as well as the reverse complement of *MLL* sequence and the reverse complement of *TAP* sequence (tip-associated protein) (GenBank accession nos. U80073 and AC015703), which is also from chromosome band 11q23 (Fig. 5*D*). One subclone contained the alternatively spliced *MLL* exon 7-*ELL* fusion and the reverse complement of *MLL* and *TAP* sequence. The significance of these unusual subclones is unclear because PCR with gene-specific primers did not verify the corresponding transcripts.

Discussion

The original panhandle PCR and panhandle variant PCR amplify genomic DNA with known 5′ and unknown 3′ sequences

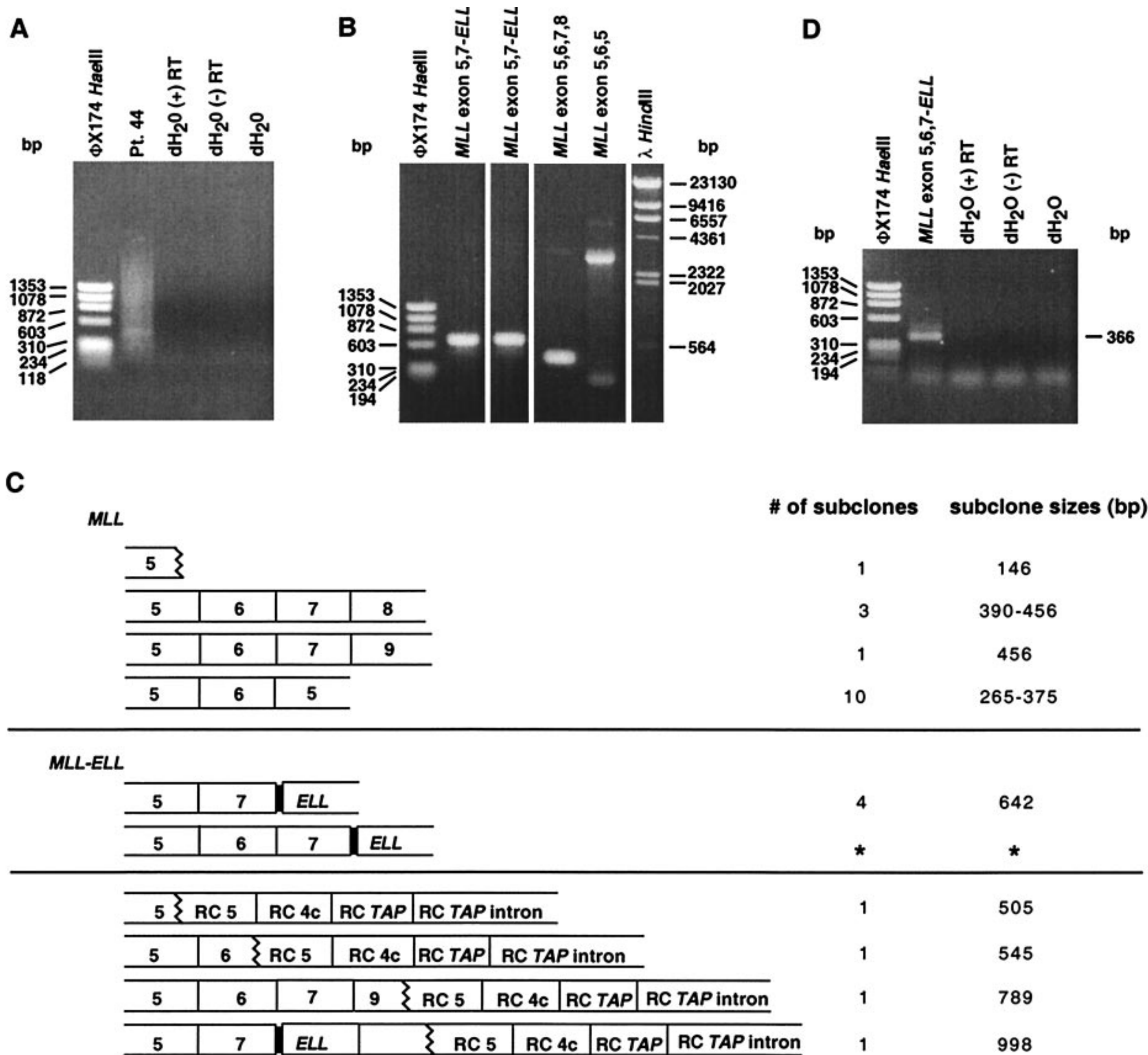

Fig. 5. (*A*) cDNA panhandle PCR analysis of total RNA from t-AML of patient 44. Smear indicates products of heterogeneous sizes (lane Pt. 44). (*B*) PCR screen of representative recombination PCR-generated subclones of cDNA panhandle PCR products shown in *A*. *MLL*-containing subclones shown by sequencing are indicated above respective lanes. (*C*) Summary of sequences in recombination PCR-generated subclones. Fifteen subclones contained *MLL* sequence alone (*Top*). Alternatively spliced and scrambled transcripts were detected. Alternatively spliced chimeric transcript identified by cDNA panhandle PCR in which *MLL* exon 6 was absent had in-frame fusion of *MLL* exon 7 to position 148 of *ELL* cDNA (GenBank accession no. U139480) (*Middle*). * shows second *MLL-ELL* chimeric transcript in which *MLL* exon 6 was present, which was identified by amplification of same first-strand cDNA with gene-specific primers (please see second lane, *D*). Transcripts with *MLL* or *MLL-ELL* and *TAP* sequences are unconfirmed (*Bottom*). RC indicates reverse complement. (*D*) Detection of second *MLL-ELL* chimeric transcript by PCR with gene-specific primers. Sequence of 366-bp product showed fusion of *MLL* exon 7 to *ELL* in transcript in which *MLL* exons 5–7 all were present (lane 2) (please see * in *C*).

(28, 29). Because *MLL* has many unknown partner genes, we previously adapted these methods to isolate *MLL* translocation breakpoints from genomic DNA (4–8). Both attach known *MLL* DNA to the unknown partner sequence. A stem-loop template forms and is amplified in two-sided PCR with primers all from *MLL*. In the present work, we developed cDNA panhandle PCR for *MLL* der(11) transcripts. The strategy has some features of the original and panhandle variant PCR approaches.

New strategies to detect translocations are essential because a growing number of genes in leukemia-associated translocations, including *MLL*, form fusions with multiple and/or uncharacterized translocation partners. Southern blot analysis identifies *MLL* translocations regardless of the partner gene, but Southern blot analysis is not as sensitive as PCR. Alternative approaches for detecting *MLL* translocations regardless of the partner gene are karyotype analysis and fluorescence *in situ* hybridization analysis with an *MLL*-specific probe; both have become routine tests when treatment-related leukemia is suspected so that, in many cases, such information is available. However, karyotype analysis detects only two-thirds of translocations involving *MLL* (30, 31) and, as was true in this study, has a high incidence of falsely negative results. Reverse transcriptase–PCR with gene-specific primers will not recognize all of the translocations because, for many of the partner genes, no primers are available.

Because the partner gene may be relevant to outcome (3), cDNA panhandle PCR is a simple approach to screening for *MLL* translocations that could be useful clinically. All three panhandle PCR-based strategies are well suited to *MLL* translocations. However, cDNA panhandle PCR has fewer steps, does not require restriction enzyme cleavage, and does not require a

ligation. Moreover, in cDNA panhandle PCR, smaller amplicons contain the informative exonic sequences and reveal the partner genes more readily. The steps entail reverse transcription of first-strand cDNAs from total RNA using *MLL*-random hexamer oligonucleotides, generation of second-strand cDNAs by *MLL* primer 1 extension, formation of stem-loop templates by intrastrand annealing of the second strands, and PCR with primers all from *MLL*. The random hexamers in the oligonucleotides used to generate the first-strand cDNAs prime from many complementary sequences in the total RNA; however, only second-strand cDNAs containing *MLL* sequence at both ends will form the stem-loop structure that is amplified in the PCR. This increases specificity.

cDNA panhandle PCR is distinct from 3′ rapid amplification of cDNA ends (RACE), which generates first-strand cDNA using an oligonucleotide with a sequence complementary to the cloning vector and an oligo(dT) sequence at its 5′ and 3′ ends (32–34). The oligo(dT) sequence anneals to and primes from poly(A) tails in heterogeneous full-length transcripts. cDNA panhandle PCR seems advantageous over 3′ RACE PCR where larger products are required to reach the point of fusion. Another method creates the first-strand cDNA by reverse-transcribing with oligonucleotides containing complementary sequence to the cloning vector at the 5′ ends and random hexamers at the 3′ ends (24); however, the obligate intrastrand loop formation in generation of cDNA panhandle PCR templates confers greater specificity.

We used cDNA panhandle PCR to identify the unknown partner genes in two cases of t-AML in which the karyotypes were normal. The results indicate that cDNA panhandle PCR will identify heterogeneous *MLL*-containing transcripts from the normal and translocated *MLL* alleles in the same reaction. Fully spliced transcripts, alternatively spliced transcripts, transcripts with exon scrambling, and incompletely processed transcripts were detectable. The transcripts with *MLL* exons accurately spliced but scrambled and in an order different from genomic DNA indicate complexity in the splicing process; the phenomenon has been described (22, 23). The scrambled transcripts in the leukemia of patient 36 joined codons in the middle of two exons. Similarly, Caldas *et al.* (23) reported exon scrambling that resulted from splicing at nonsplice consensus sites within exons of the *MLL* gene. Additional experiments using cDNA panhandle PCR may augment our understanding of the heterogeneity of *MLL*-containing transcripts.

In one case of t-AML with two *MLL* rearrangements at the level of the Southern blot, the partner gene was *AF-10* and two alternatively spliced transcripts were detected. The *AF-10* gene is a known partner gene of *MLL* at chromosome band 10p12 and encodes a putative transcription factor as its protein product (24, 25). In the transcripts with the *MLL* exon 8-*AF-10* junction, 48 bases of the contiguous *AF-10* cDNA were absent. Chaplin *et al.* (25) observed absence of the same 48 bases from another *MLL-AF-10* cDNA. The reading frame is not disrupted in these transcripts, and a recent *AF-10* sequence entry suggests that the 48 bases comprise an exon. Thus, both *MLL* and *AF-10* were alternatively spliced. In the second case, Southern blot analysis revealed a single *MLL* rearrangement. Although single rearrangements frequently are *MLL* tandem duplications (6, 10–17), cDNA panhandle PCR identified an *MLL-ELL* chimeric transcript. *ELL* is one of three known partner genes of *MLL* at chromosome band 19p13 and encodes an arginine-rich basic protein, the C terminus of which has homology to poly(ADP) ribose polymerase (26). Leukemias with normal karyotypes may harbor cryptic *MLL* translocations with a variety of partner genes.

C.A.F. is supported by National Institutes of Health Grants CA66140, CA80175, CA77683, and CA85469 and a Leukemia and Lymphoma Society Scholar Award.

1. Felix, C. A. & Lange, B. J. (1999) *Oncologist* **4,** 225–240.
2. Felix, C. A. (1998) *Biochim. Biophys. Acta* **1400,** 233–255.
3. Reaman, G. H., Sposto, R., Sensel, M. G., Lange, B. J., Feusner, J. H., Heerema, N. A., Leonard, M., Holmes, E. J., Sather, H. N., Pendergrass, T. W., *et al*. (1999) *J. Clin. Oncol.* **17,** 445–455.
4. Felix, C. A., Kim, C. S., Megonigal, M. D., Slater, D. J., Jones, D. H., Spinner, N. B., Stump, T., Hosler, M. R., Nowell, P. C., Lange, B. J. & Rappaport, E. F. (1997) *Blood* **90,** 4679–4686.
5. Felix, C. A. & Jones, D. H. (1998) *Leukemia* **12,** 976–981.
6. Megonigal, M. D., Rappaport, E. F., Jones, D. H., Kim, C. S., Nowell, P. C., Lange, B. J. & Felix, C. A. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 11583–11588.
7. Megonigal, M. D., Rappaport, E. F., Jones, D. H., Williams, T. M., Lovett, B. D., Kelly, K. M., Lerou, P. H., Moulton, T., Budarf, M. L. & Felix, C. A. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 6413–6418.
8. Megonigal, M. D., Cheung, N.-K. V., Rappaport, E. F., Nowell, P. C., Wilson, R. B., Jones, D. H., Addya, K., Leonard, D. G. B., Kushner, B. H., Williams, T. M., *et al*. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 2814–2819.
9. Gu, Y., Nakamura, T., Alder, H., Prasad, R., Canaani, O., Cimino, G., Croce, C. M. & Canaani, E. (1992) *Cell* **71,** 701–708.
10. Schichman, S. A., Caligiuri, M. A., Gu, Y., Strout, M. P., Canaani, E., Bloomfield, C. D. & Croce, C. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6236–6239.
11. Schichman, S. A., Caligiuri, M. A., Strout, M. P., Carter, S. L., Gu, Y., Canaani, E., Bloomfield, C. D. & Croce, C. M. (1994) *Cancer Res.* **54,** 4277–4280.
12. Caligiuri, M. A., Strout, M. P., Schichman, S. A., Mrozek, K., Arthur, D. C., Herzig, G. P., Baer, M. R., Schiffer, C. A., Heinonen, K., Knuutila, S., *et al*. (1996) *Cancer Res.* **56,** 1418–1425.
13. Caligiuri, M. A., Strout, M. P., Oberkircher, A. R., Yu, F., de la Chapelle, A. & Bloomfield, C. D. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 3899–3902.
14. So, C. W., Ma, Z. G., Price, C. M., Dong, S., Chen, S. J., Gu, L. J., So, C. K. C., Wiedemann, L. M. & Chan, L. C. (1997) *Cancer Res.* **57,** 117–122.
15. Yu, M., Honoki, K., Andersen, J., Paietta, E., Nam, D. K. & Yunis, J. J. (1996) *Leukemia* **10,** 774–780.
16. Wiedemann L. M., MacGregor, A. & Caldas, C. (1999) *Br. J. Haematol* **105,** 256–264.
17. Schnittger, S., Kinkelin, U., Schoch, C., Heinecke, A., Haase, D., Haferlach, T., Buchner, T., Wormann, B., Hiddemann, W. & Griesinger, F. (2000) *Leukemia* **14,** 796–804.
18. Lange, B., Valtieri, M., Santoli, D., Caracciolo, D., Mavillio, F., Gemperlein, I., Griffin, C., Emanuel, B., Finan, J., Nowell, P. & Rovera, G. (1987) *Blood* **70,** 192–199.
19. Felix, C. A., Hosler, M. R., Winick, N. J., Masterson, M., Wilson, A. E. & Lange, B. J. (1995) *Blood* **85,** 3250–3256.
20. Reichel, M., Gillert, E., Nilson, I., Siegler, G., Greil, J., Fey, G. H. & Marschalek, R. (1998) *Oncogene* **17,** 3035–3044.
21. Felix, C. A., Hosler, M. R., Slater, D. J., Megonigal, M. D., Lovett, B. D., Williams, T. M., Nowell, P. C., Spinner, N. B., Owens, N. L., Hoxie, J., *et al*. (1999) *Mol. Diagn.* **4,** 269–283.
22. Nigro, J. M., Cho, K. R., Fearon, E. R., Kern, S. E., Ruppert, J. M., Oliner, J. D., Kinzler, K. W. & Vogelstein, B. (1991) *Cell* **64,** 607–613.
23. Caldas, C., So, C. W., MacGregor, A., Ford, A. M., McDonald, B., Chan, L. C. & Wiedemann, L. M. (1998) *Gene* **208,** 167–176.
24. Chaplin, T., Bernard, O., Beverloo, H. B., Saha, V., Hagemeijer, A., Berger, R. & Young, B. D. (1995) *Blood* **86,** 2073–2076.
25. Chaplin, T., Ayton, P., Bernard, O. A., Saha, V., Valle, V. D., Hillion, J., Gregorini, A., Lillington, D., Berger, R. & Young, B. D. (1995) *Blood* **85,** 1435–1441.
26. Thirman, M. J., Levitan, D. A., Kobayashi, H., Simon, M. C. & Rowley, J. D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12110–12114.
27. Shilatifard, A., Lane, W. S., Jackson, K. W., Conaway, R. C. & Conaway, J. W. (1996) *Science* **271,** 1873–1876.
28. Jones, D. H. & Winistorfer, S. C. (1992) *Nucleic Acids Res.* **20,** 595–600.
29. Jones, D. H. & Winistorfer, S. C. (1997) *BioTechniques* **23,** 132–138.
30. Felix, C. A., Hosler, M. R., Slater, D. J., Parker, R., Masterson, M., Whitlock, J. A., Rebbeck, T. R., Nowell, P. C. & Lange, B. J. (1998) *J. Pediatr. Hematol. Oncol.* **20,** 299–308.
31. Martinez-Climent, J. A., Thirman, M. J., Espinosa, R., Le Beau, M. M. & Rowley, J. D. (1995) *Leukemia* **9,** 1299–1304.
32. Frohman, M. A., Dush, M. K. & Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8998–9002.
33. Frohman, M. A. (1990) in *PCR Protocols: A Guide to Methods and Applications*, eds. Innis, M. A., Gelfand, D. H. & Sninsky, J. J. (Academic, San Diego), pp. 28–38.
34. Frohman, M. A. (1993) *Methods Enzymol.* **218,** 340–356.