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The t(10;11)(p13;q14) in the U937 cell line results in the fusion of the *AF10* gene and *CALM*, encoding a new member of the AP-3 clathrin assembly protein family

M. H. DREYLING*†, J. A. MARTINEZ-CLIMENT*‡, M. ZHENG§, J. MAO§, J. D. ROWLEY*, AND S. K. BOHLANDER*¶

*Section of Hematology/Oncology, The University of Chicago, Chicago, IL 60637; and §Genome Therapeutics, Waltham, MA 02154

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ABSTRACT **The translocation t(10;11)(p13;q14) is a recurring chromosomal abnormality that has been observed in patients with acute lymphoblastic leukemia as well as acute myeloid leukemia. We have recently reported that the monocytic cell line U937 has a t(10;11)(p13;q14) translocation. Using a combination of positional cloning and candidate gene approach, we cloned the breakpoint and were able to show that *AF10* is fused to a novel gene that we named *CALM* (*C*lathrin *A*ssembly *L*ymphoid *M*yeloid leukemia gene) located at 11q14. AF10, a putative transcription factor, had recently been cloned as one of the fusion partners of *MLL*. *CALM* has a very high homology in its N-terminal third to the murine *ap-3* gene which is one of the clathrin assembly proteins. The N-terminal region of ap-3 has been shown to bind to clathrin and to have a high-affinity binding site for phosphoinositols. The identification of the *CALM/AF10* fusion gene in the widely used U937 cell line will contribute to our understanding of the malignant phenotype of this line.**

The cloning of recurring chromosomal translocations in hematologic and solid malignancies has led to the discovery of many genes that play a pivotal role in the regulation of cell growth and differentiation. Translocations disturb normal gene function mainly by two distinct mechanisms: (*i*) They deregulate the expression of genes near the translocation breakpoints, but the protein is not altered; (*ii*) They lead to the expression of fusion proteins. The latter mechanism is commonly found in myeloid malignancies, whereas the first mechanism predominates in lymphoid tumors (1).

Recently, we and others have shown by molecular cytogenetic techniques that the U937 cell line, which was derived from a diffuse histiocytic lymphoma (2), has a t(10;11)(p13;q14) translocation (3, 4). This translocation is distinct from the t(10;11)(p11–13;q23) found in acute myeloid leukemia (5) which results in the fusion of the *MLL* gene at 11q23 (6–9) to the recently cloned *AF10* gene at 10p12 (10, 11). The t(10;11)(p13;q14–21) has been observed as a rare but recurring translocation both in acute lymphoblastic leukemia (12, 13), in acute myeloid leukemia (13–16), and in acute eosinophilic leukemia (17).

Although U937 was isolated from what was described as a diffuse histiocytic lymphoma, the cell line expresses myeloid markers and is widely used in the study of myeloid differentiation (18, 19). Little is known about the genetic abnormalities of U937. Its karyotype is complex and the true identity of many rearrangements has not yet been resolved. We and others have shown by fluorescence *in situ* hybridization (FISH) that one of these rearrangements is a reciprocal (10;11)(p13;q14) translocation (3, 4). We now report that this translocation leads to the fusion of *AF10* at 10p13 to a novel gene, *CALM*, at 11q14. The molecular characterization of one of the rearrangements of U937 and the observation that one of the fusion partners of *MLL*, namely *AF10*, is fused to another gene in a different translocation has not, to our knowledge, been previously reported.

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MATERIALS AND METHODS

Cell Lines. Metaphase cells from the U937 cell line were prepared for analysis according to standard cytogenetic procedures (20). The identity of the cell line had been verified by cytogenetic analysis (4). The following cell lines were used as controls in the Northern blot analysis: K562 (erythroleukemia), HeLa (cervical carcinoma), Jurkat (T-cell acute lymphoblastic leukemia), HepG2 (hepatoblastoma), and Clone 13.

FISH Probes and FISH Procedure. The Centre d'Etude Polymorphisme Humain Mega yeast artificial chromosome (YAC) library was used as a source for YAC FISH probes. Table 1 summarizes the YACs and their location relative to the breakpoint. The YACs were prepared as FISH probes and FISH was performed as described (21, 22). The Spectrum-Orange directly-labeled chromosome 10 centromeric probe was obtained from Vysis (Downers Grove, IL). The biotinylated YAC probes were detected with fluorescein isothiocyanate-conjugated avidin (Vector Laboratories). Chromosomes were counterstained with 4′,6′-diamidino-2-phenylindole dihydrochloride after hybridization. Separate 4′,6′-diamidino-2-phenylindole dihydrochloride and fluorescein isothiocyanate images of the same metaphase cell were captured with a charge-coupled device camera (Photometrics, Tucson, AZ). The images were pseudocolored and merged with Adobe Photoshop (Adobe, Mountain View, CA) and the National Institutes of Health image program (Bethesda, MD).

Mapping Technique. Data from the Genethon YAC mapping effort and the CLONESPATH program were used extensively to identify candidate YACs that were likely to span the translocation breakpoint (23). The program and the YAC data were obtained by means of anonymous File Transfer Protocol (ftp) from Genethon.

Northern Blot Analysis. Poly(A) mRNA for Northern blot analysis was prepared from cell lines using the FastTrack mRNA isolation kit (Invitrogen). Multiple tissue Northern blots were used according to the manufacturer's instructions (Clontech). The *AF10* probe used was prepared from PCR amplified fragments of the *AF10* gene using primers designed according to the published cDNA sequence (11). The probe included nucleotides 492-2849 of *AF10*.

The 3′ *CALM* probe spanned nucleotides 1730–2047 of *CALM* that was amplified with primers NG.T45 and NG.B362

Abbreviations: FISH, fluorescence *in situ* hybridization; YAC, yeast artificial chromosome; STS, sequence-tagged site; EST, expressed sequence tag.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. 445976).

†Present address: Universitätsklinikum Göttingen, 37075 Göttingen, Germany.
‡Present address: Late Children's Hospital, 46009 Valencia, Spain.
¶To whom reprint requests should be addressed at: Institute for Human Genetics, University of Göttingen, Gosslerstrasse 12D, 37073 Göttingen, Germany.

Table 1. YACs and their location relative to breakpoints

CEPH* address	Size, kb	Genes contained	STSs/ genes contained	FISH result	Chimerism
817e11	1380		D10S548	10p, distal	ND
950f2	1250		D10S203	10p, distal	ND
807b3	1050	*AF10*	NR	10p, split	ND
815c7	1180	*BMI1*	NR	10p, prox.	ND
887b6	610	*BMI1*	NR	10p, prox.	ND
936g7	1650	*BMI1*	D10S563	10p, prox.	ND
965d10	1270		D10S211	10p, prox.	ND
934e11	NTR		D10S563 D10S582 D10S586	10p, prox.	ND
950d2	1520		D10S582 D10S586	10p, prox.	Chimeric

ND, not detected; NTR, not reported; NR, none reported; prox., proximal.
*Centre d'Etude Polymorphisme Humain.

from the U937 cDNA. The *CALM* probe that included the more conserved 5′ portion extended from nucleotide 307 to nucleotide 2092.

The probes were labeled with [^{32}P]dCTP according to the oligonucleotide primed-labeling protocol (24). Hybridization was carried out at 42°C in 50% formamide, 1 M NaCl, 1% (wt/vol) sodium dodecyl sulfate (SDS), 10% (wt/vol) dextran sulfate, 50 mM sodium phosphate (pH 6.5), and 250 μg/ml sheared salmon sperm DNA and filters were washed at a final stringency of 1X SSC (150 mM sodium chloride/15 mM sodium citrate, pH 7.5) and 1% SDS at 65°C. Filters were exposed to x-ray films for from 2 to 8 days at −70°C.

cDNA Cloning and Sequence Analysis. A cDNA library from U937 poly(A)-selected mRNA was constructed at Stratagene. The cDNA was cloned nondirectionally into the Lambda Zap II vector. The cDNA library was screened with the *AF10* probe or the 3′ *CALM* probe by standard protocols. Positive phage clones were purified and *in vivo* excised according to the manufacturer's protocol (Stratagene).

Double-stranded sequencing was performed with the dideoxy chain terminating method (25) with the Sequenase kit (United States Biochemicals) using deoxyadenosine 5′[α-[^{35}S]thio] triphosphate incorporation. Newly determined sequences were compared to GenBank using the BLASTN program on the Blast server at the National Institutes of Health (26). Contigs were assembled with the help of the ASSEMBLYLIGN and MACVECTOR (Kodak) programs on a Macintosh computer.

Chromosomal Assignment of *CALM*. The chromosomal assignment of the 3′ *CALM* probe was determined with the use of a somatic cell hybrid panel blot (Oncor). A genomic sequence-tagged site (STS) was designed for *CALM* using the information about one of the alternatively spliced exons (nucleotides 1407–1543). These primers as well as the others used are summarized in Table 2.

RESULTS

We had previously shown that the U937 cell line did not contain a del(11)(q14qter) but rather a translocation t(10;11)(p13;q14–21). Using mega YACs from the Centre d'Etude Polymorphisme Humain library we mapped the translocation breakpoint on the short arm of chromosome 10. We determined first that the breakpoint was located between YACs 817e11 and 950d2 which contained the STSs D10S548 and D10S550, respectively. The estimated genetic distance between these loci was 3 centimorgans according to the Centre d'Etude Polymorphisme Humain reference map (23). We then used the CLONESPATH program to identify those YACs that would lie between these markers and would help us to define the breakpoint more precisely. Reiterating this process we were able to identify one YAC (807b3; 1050 kb) that spanned the breakpoint. This YAC hybridizes to the der(10) as well as to the der(11) chromosome in U937 as shown in Fig. 1.

We also employed a candidate gene approach to examine genes that had been mapped to 10p and that might be involved in the translocation. One of these genes was *BMI1*, which had been cloned as the site of a retroviral integration that augmented the tumorigenic effect of a *MYC* transgene (27–29). However, two YACs that contained *BMI1* (815c7 and 936g7) clearly mapped proximal to the translocation breakpoint. This observation is consistent with the results of Shipley *et al.* (3) who used a phage insert containing the *BMI1* gene for FISH analysis in U937. Fig. 2 shows the genomic map of 10p which we constructed from our FISH results and from the data from the previously mapped STSs.

Another candidate gene in the region was *AF10*. *AF10* had recently been cloned as the gene that fuses to *MLL* in the t(10;11)(p12;q23) translocation (11). Although the breakpoint in this translocation had been reported as being located at band 10p12 or 10p11, we had reason to suspect that the genomic location of *AF10* was very close to the U937 breakpoint. Southern analysis showed (data not shown) that *AF10* was indeed contained within YAC 807b3 which made this gene a very strong candidate for being involved in the translocation.

Northern analysis of U937 confirmed the involvement of *AF10* in the t(10;11)(p13;q14). The normal *AF10* transcript of ≈5.5 kb was absent and two new transcripts of slightly larger and slightly smaller size were found. The total amount of *AF10* message was reduced (Fig. 3*A*). These findings suggested that this alteration of the *AF10* mRNA might be due to the formation of a fusion mRNA between *AF10* and another gene from 11q14. The complete absence of the normal *AF10* mRNA is an interesting observation and cannot be adequately explained at present. FISH analysis using YAC 807b3 did not detect a deletion of the *AF10* region on the normal chromosome 10. However, partial deletions of the YAC target region, which is 1050 kb in size, would not have been detected.

We screened a U937 cDNA library with the *AF10* probe (nucleotides 492-2849) and identified three clones. Mapping and partial sequencing of these clones showed that two contained only part of *AF10*, whereas the third clone with an insert size of 4.2 kbp contained 43 bp of novel sequence at the 5′ end joined to *AF10* sequences at nucleotide 423 (numbering according to ref. 11).

Comparison of the novel 43-bp fragment to sequences in GenBank revealed that it was identical to two expressed sequence tags (EST) (GenBank accession numbers R58653 and R67661) that had been generated by random cDNA sequencing projects (University of Washington, St. Louis). These ESTs extended the unknown sequence 5′ and 3′. Further

Table 2. Summary of primers

Name	Position	Sequence
NG.T45	*CALM* 1730–1753	5′-CCAAACTCCCACCTAGCAAGTTAG-3′
NG.B362	*CALM* 2047–2023	5′-CAGGCTGGCTGTATATTAAGGTTGG-3′
AN.B497	*CALM* 2165–2144	5′-AAGGATTTTGCTGCTTGAGCAC-3′
AN.T288	*AF10* 288–268	5′-CGAGAACCCGCTGGTTTATTG-3′
NA.T501	*CALM* 1998–2023	5′-GGAAGTGTTCCTGTAATGACGCAAC-3′
NA.B628	*AF10* 458–437	5′-AAAGCTCCATCCTTATGGGGAC-3′

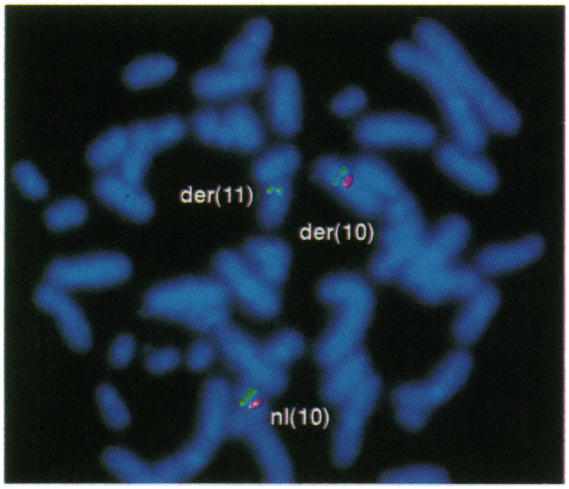

FIG. 1. FISH analysis of a metaphase cell from U937 hybridized with the YAC 807b3 probe and a chromosome 10 centromere probe. Three green signals can be seen for the YAC probe: on the normal chromosome 10 [nl(10)], on the der(10), and on the der(11) chromosome. The signals on the der(10) and the der(11) are slightly weaker than the signal on the normal chromosome 10 indicating the splitting of the YAC by the translocation. The chromosome 10 centromere is also labeled (red).

searching of GenBank with these ESTs revealed another EST (T34093) that extended further 5′. The sequence could be extended through two more ESTs (T11671 and T11670) found in a third round of GenBank searching for a total of 550 bp 5′ and 250 bp 3′ of the original 43 bp sequence. Analysis of these 800 bp showed that they contained an open reading frame of 609 bp from the 5′ end of the clone that was terminated by a stop codon four codons downstream of the breakpoint. This indicated that the open reading frame was the 3′ end of an unknown gene. PCR primers designed from this sequence (NG.T45 and NG.B362) gave an amplification product of the expected size (317 bp) when we used cDNA as template, thus confirming the validity of the contig assembly.

Hybridization of this PCR product (3′ *CALM* probe) to a somatic cell hybrid panel, confirmed that the sequence was—as expected—derived from chromosome 11 and was conserved in hamster and mouse.

The 3′ *CALM* probe was used to screen the U937 cDNA library to obtain a full-length cDNA. Several positive phage clones were isolated that had inserts ranging from 1.3 to 4.2 kbp. Sequencing of several overlapping clones revealed an open reading frame of 652 amino acids (Fig. 4). There is no

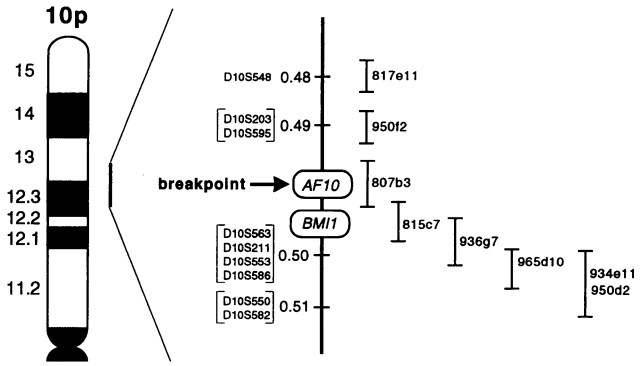

FIG. 2. This figure shows the relative position of some of the YACs used for mapping of the t(10;11)(p13;q14) breakpoint on the short arm of chromosome 10. The position of the STS markers is derived from the Genethon map. *BMI1* was assigned to YACs 815c7 and 936g7 slightly centromeric to the breakpoint. These YAC are very close to YAC 807b3 that spans the breakpoint and contains *AF10*. The figure is not drawn to scale.

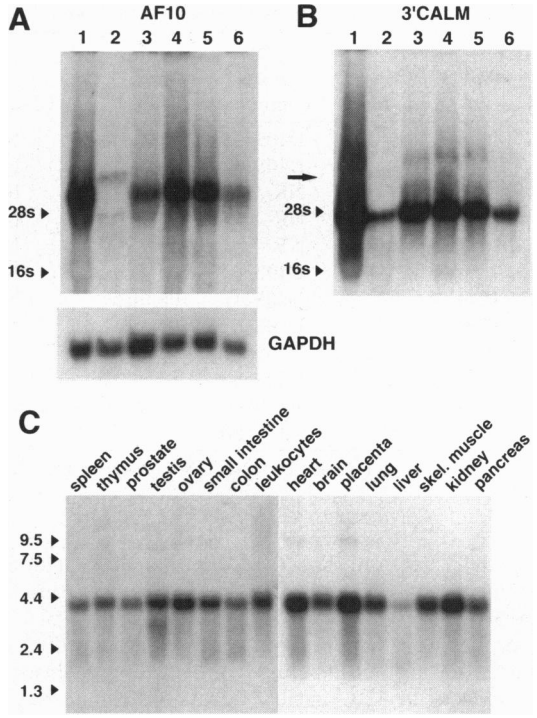

FIG. 3. Northern blotting with RNA from the following cell lines. Lanes: 1, K562; 2, U937; 3, HeLa; 4, HepG2; 5, Clone 13; 6, Jurkat. (*A*) Hybridization to the *AF10* probe: A strong hybridization signal is seen in all cell lines except for U937. Here two weaker bands of larger and smaller size than the normal *AF10* message are detected. Lower panel shows the same blot hybridized to the GAPDH probe to control for RNA loading. There is slightly less RNA in the U937 lane than in the other lanes. (*B*) Hybridization to the 3′ *CALM* probe: The normal size *CALM* message is reduced in intensity in U937 and a very weak band (barely visible, arrow) is present that corresponds in size to the larger transcript detected by the *AF10* probe in *A*. (*C*) Multiple tissue Northern blot hybridized with the 3′ *CALM* probe. *CALM* is expressed in all tissues, a major transcript of about 4 kbp and a minor transcript of about 9 kbp is seen. There is also a smaller sized transcript of 3 kbp in testis. This probe does not cross hybridize to the murine *ap-3* gene.

in-frame stop codon upstream of the first ATG codon at position 148. However, we think that this ATG is the start codon because it corresponds to the start codon of the murine *ap-3* gene (see below) and, as in the *ap-3* gene, there is a very G-C rich region 5′ of the start codon (30).

The 652 amino acids show a remarkable homology to the murine, bovine, and rat clathrin assembly protein ap-3 (also called AP180, NP185, F1–20, and synapse specific phosphoprotein) (30, 31). The homology ranges from 18 to 81% in different portions of the protein. The most striking homology of more than 80% is found between the first 289 amino acids of CALM and ap-3. A detailed comparison between these two proteins is shown in Fig. 5. In light of this homology, we chose the name *CALM* for *C*lathrin *A*ssembly protein-like *L*ymphoid *M*yeloid leukemia gene.

In contrast to ap-3, which is a neuron-specific phosphoprotein (33), CALM is expressed in all tissues examined (Fig. 3*C*). The size of the predominant mRNA is ≈4 kb. There is also a larger mRNA species of ≈9 kb and a smaller mRNA species of ≈3 kb in testes.

Using the 3′ *CALM* probe on a Zooblot (Bios, New Haven, CT), *CALM* was conserved in all mammals examined (dog, cat, rabbit, cow, sheep, mouse, rat, hamster, pig, and marmoset). Because this probe does not cross-hybridize with the murine *ap-3* gene (there is no significant homology at the nucleotide level), the bands detected in the lane with the mouse DNA must be the murine homolog of *CALM*. When a probe was used that included the more conserved 5′ part of the gene, a band could also be detected in chicken DNA (data not shown). A GenBank search

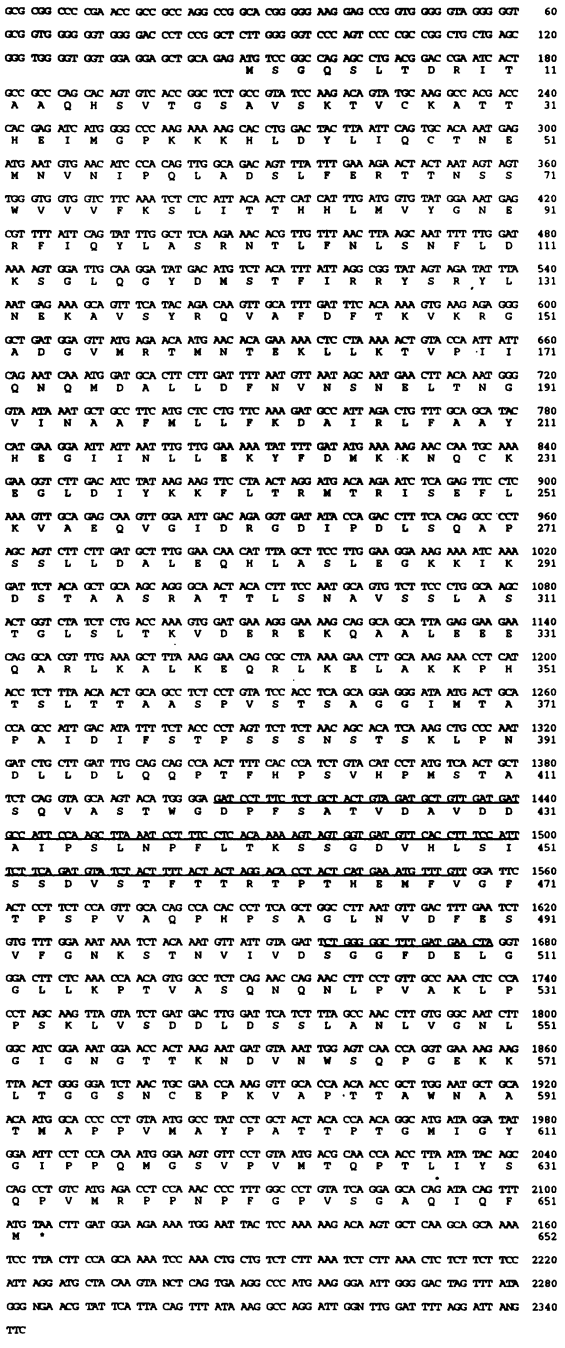

FIG. 4. cDNA and the deduced amino acid sequence of *CALM*. The translation starts with the first ATG found in the sequence. This start codon corresponds to the first methionine in the murine *ap-3* gene. The fusion point with *AF10* is denoted with a dot. Two alternatively spliced exons are underlined (positions 1405 to 1554 and positions 1658 to 1678). The sequence from positions 1405 to 1554 was present in the clones derived from the U937 library but absent in a sequence from GenBank (accession number T11670). PCR done with primers designed from this sequence (CALM1407T and CALM1543B) amplified a 137-bp fragment from genomic DNA suggesting that there are no additional exon–intron boundaries between positions 1405 and 1554. Nucleotides 1658 to 1678 were absent in one of our clones but present in others. Alternative splicing of these two exons does not change the reading frame.

also revealed homology to a putative yeast protein (YHR161c gene product of unknown function; SwissProt accesion number P38856). This homology was highest in the N-terminal 300 amino acids (25% identity) and extended throughout the two proteins.

Both a *CALM/AF10* fusion mRNA and an *AF10/CALM* fusion mRNA are expressed. The two reciprocal fusion mRNA

FIG. 5. Amino acid sequence comparison between the human *CALM* and the murine *ap-3* gene (Protein Identification Resource accession number S27866). The rat *ap-3* gene (Protein Identification Resource accession numbers S36326 and S36327) and the bovine *ap-3* gene (Protein Identification Resource accession number S39150) are nearly identical to the murine gene and were omitted from this comparison. There is only very low homology between *CALM* and ap-3 from amino acids 329 to 472 (*CALM* numbering). Identical amino acids are marked with an asterisk, conservative substitutions with a dot. The sequences were compared using the CLUSTAL V program (32).

can be amplified by PCR from the U937 cDNA library using a top primer for *AF10* (AN.T288) and a bottom primer for *CALM* (AN.B497) for the *AF10/CALM* fusion and a top primer for *CALM* (NA.T501) and a bottom primer for *AF10* (NA.B628) for the *CALM/AF10* fusion.

The nucleotide sequence at the breakpoint and the structure of these fusion genes is depicted in Fig. 6. The break occurs in the zinc finger motif of *AF10* between nucleotides 422 and 423 and between nucleotides 2090 and 2091 of *CALM*. The *CALM/AF10* mRNA would code for a protein in which the last four amino acids of CALM are replaced by nearly the full-length AF10 protein starting from amino acid 81 (nucleotide 423). The *AF10/CALM* fusion mRNA in contrast codes for a small AF10 protein which is truncated after 84 amino acids by the stop codon from *CALM*.

DISCUSSION

The t(10;11)(p13;q14) translocation and the genes involved are remarkable in several ways. It is the first reported translocation in which a fusion partner of *MLL* is found to fuse with a different gene with the fusion transcript leading to leukemia. It had also not been noted previously that a gene with a putative clathrin binding domain is involved in a translocation. Furthermore, the same

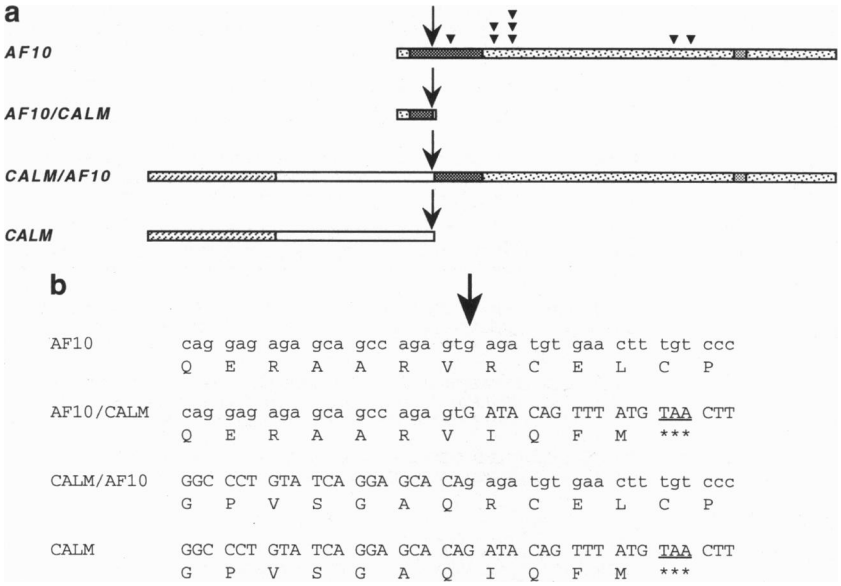

FIG. 6. (*a*) Diagrammatic representation of *AF10, CALM*, and the two fusion genes resulting from the translocation. The various protein domains are indicated. Previously described breakpoints in *AF10* are indicated with small triangles (10). ▨, Putative clathrin binding domain; ▩, zinc fingers; □, leucine zipper. (*b*) Nucleotide and amino acid sequence of *AF10, CALM*, and the two reciprocal fusion genes *AF10/CALM* and *CALM/AF10*. The *CALM* sequence is in uppercase. The Stop codon is underlined.

translocation (at least at the FISH level) occurs in myeloid as well as in lymphoid lineage leukemia.

The first 289 amino acids of CALM show a high homology (81%) to the clathrin assembly protein ap-3 which has been cloned in mice and rats (30, 31). Partial sequence of the bovine *ap-3* gene is deposited in GenBank (31). The mouse, rat, and the fragments of the bovine *ap-3* gene are highly homologous to each other with more than 97% identical amino acids. The human homolog of *ap-3* has not yet been cloned. We considered the possibility that *CALM* might be the human homolog of *ap-3*. However, several lines of evidence lead us to conclude that *CALM* cannot be the human homolog of the murine or rat *ap-3*. The interspecies conservation of *ap-3*—for example between mouse, rat, and bovine—is extremely high (greater than 99%) and is present throughout the protein, whereas the 3′ *CALM* cDNA probe, which has no significant nucleotide homology to the murine or rat *ap-3*, readily detects a band in genomic mouse DNA (data not shown), showing that there must be a murine homolog of *CALM* which cannot be *ap-3*. Expression of *CALM* is found in all tissues examined, which is in contrast to the murine *ap-3* gene which has been reported to be restricted to neurons in its expression (33). Therefore CALM and ap-3 appear to form a new family of proteins that we will call the AP-3-like clathrin assembly protein family. We also found a presumptive yeast protein with significant homology to CALM in the first 290 amino acids. This yeast protein is slightly more homologous to CALM than it is to murine or rat ap-3.

One of the putative functions of ap-3 is to assemble clathrin triskelia, which consist of three clathrin light chains and three clathrin heavy chains, into clathrin coats and presumably to promote the formation of clathrin-coated vesicles at presynaptic membranes (34–36). Clathrin-coated vesicles play a mayor role in receptor mediated endocytosis as well as in trans-Golgi network vesicle traffic [for review of clathrin function see ref. 37). Functional studies of ap-3 have shown that a clathrin-binding domain resides in the 33-kDa N-terminal portion of the protein (31, 34, 38), which binds to clathrin triskelia, probably through interaction with the clathrin heavy chains (39, 40). This 33-kDa region is thought to have a globular structure and to consist mainly of alpha helices (30); it also constitutes the region of the highest homology to *CALM*. Although this N-terminal domain is able to bind clathrin triskelia (38) it is unable to assemble them into clathrin cages and to bind to preassembled cages; this latter function resides in the 58-kDa C-terminal region of *ap-3*, which has a lower homology to *CALM* (34).

Another important function of the 33-kDa amino terminal region of *ap-3* is its high affinity binding of both inositol hexakisphosphate and diphosphoinositol pentakisphosphate (35, 41). Binding of either of these ligands inhibits the ability of *ap-3* to assemble clathrin (35). Thus, inositol hexakisphosphate and diphosphoinositol pentakisphosphate may regulate clathrin-coated vesicle assembly (or disassembly) by means of the clathrin assembly protein ap-3.

Other clathrin assembly protein complexes (AP-1 and AP-2), which consist of several subunits, mediate between integral membrane proteins and the clathrin triskelia to direct clathrin assembly at special sites of the plasma membrane called coated pits (37). One function of the C-terminal half of *CALM* could be to interact with proteins in the plasma membrane, possibly contributing to the regulation of the endocytotic activity and receptor turnover of the cell. This activity could in turn be regulated by means of the phosphoinositol pathways.

AF10 has recently been cloned as the fusion partner of the *MLL* gene in the t(10;11)(p12;q23) (10, 11). *MLL* at band 11q23 is involved in many translocations with many different partner genes in both acute myeloid leukemia as well as in acute lymphoblastic leukemia (5). At least 11 partner genes that form fusions with *MLL* have been cloned to date (7, 42–47). There are no common motifs between most of these partner genes, which has made it difficult to postulate a mechanism for the action of these fusion genes. The normal function of *MLL* is also poorly understood. The AT-hook domain of *MLL* has been shown to bind to cruciform DNA (48, 49) and—by analogy to its *Drosophila* homolog *trithorax*—*MLL* could be responsible for the positive regulation of homeotic genes (48).

The t(10;11)(p12;q23) translocations that lead to the *MLL/AF10* fusion are often complex and involve chromosomal insertions and inversions (50) because the direction of transcription of *AF10* with respect to the telomere centromere–orientation is opposite to that of *MLL* (51). *AF10,* which has a 1027-amino acid open reading frame, has two major protein motifs: a potential zinc finger domain (amino acids 25–194) and a potential leucine zipper motif (amino acids 766–794) (11). The zinc finger domain of *AF10* is nearly identical to the zinc finger domain of *AF17* (93% amino acid identity) (11), which is fused to *MLL* in a t(11;17) (45). *AF10* is also homologous to a protein named BR140 and to an uncharacterized gene in *Caenorhabditis elegans* named *cezf* (11).

It is thought that *AF10* functions as a transcription factor and that the zinc finger region might constitute a DNA binding domain. Chaplin *et al.* (11) mention that the zinc finger and leucine zipper motifs are unlike other zinc finger or leucine zipper motifs found in known transcription factors. However, at present, the function of *AF17*, BR140, or of *cezf* is unkown.

The breakpoint of *AF10* in U937 is further 5′ in *AF10* than the breakpoints described in the *MLL/AF10* fusions. In the *MLL/AF10* fusion several different breakpoints have been observed in *AF10* and all occur (with one exception) between the N-terminal zinc finger motif and the C-terminal leucine zipper motif (10). The *MLL/AF10* fusion protein contains the N-terminal *MLL* AT-hook and repressor domain joined to the C-terminal leucine zipper of *AF10*. In contrast, the U937 *AF10* breakpoint at nucleotide 423 disrupts the 5′ zinc finger motif and is so far the most 5′ breakpoint observed in *AF10*.

Two fusion genes are expressed in U937: a *CALM/AF10* fusion mRNA, which would code for a protein of 1595 amino acids and an *AF10/CALM* fusion mRNA, which would code for a short protein of 84 amino acids. The larger CALM/AF10 fusion protein would contain essentially all the domains of both proteins with the exception of the amino terminal zinc finger motif of *AF10* which is truncated at the 5′ end. It is reasonable to assume that this CALM/AF10 fusion protein is the critical fusion partner responsible for the malignant phenotype. However, we cannot discount the possibility that the truncated *AF10* protein plays the pivotal role. A critical unanswered question is how and to what extent the *CALM/AF10* fusion contributes to the malignant phenotype of U937. The marker 10 chromosome described by Shipley and coworkers (52), which represents the der(10), is present in all sublines of U937 examined. The fact that this translocation occurs in a cell line that is used frequently in various biological analyses enhances its importance.

Beverloo and coworkers (51) have convincingly shown that the direction of transcription of the *AF10* gene is 5′–3′ telomere to centromere, opposite to the direction of *MLL*. This suggests that the *CALM/AF10* fusion is transcribed from the der(10) and the *AF10/CALM* fusion from the der(11) chromosome barring any complicated rearrangements that we should have detected during our initial positional cloning approach. *CALM* appears to be important in the integration of signals from various signaling pathways and proteins (clathrin, phosphoinositols, and receptor-mediated endocytosis). A CALM/AF10 fusion protein might interfere with the normal function of *CALM* or—even more likely—establish cross talk between otherwise independent signaling pathways.

The *CALM/AF10* fusion could also offer an opportunity to learn more about the role *MLL* plays in the *MLL/AF10* fusion. The reciprocal *AF10/MLL* fusion mRNA has not yet been found (11); this is presumably due to the complicated rearrangements that are necessary to fuse two genes such as *MLL* and *AF10* whose transcription has an opposite orientation relative to the telomere (51). Its absence focuses attention on the *MLL/AF10* fusion as being the event critical in leukemogenesis. This is in contrast to the *CALM/AF10* fusion in which both fusion products are expressed.

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