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30. We thank N. Aubonney, S. de Vevey, C. Losberger, L. Potier, and C. Siegfried for technical assistance; G. Mazzei for sequence analysis; H. Kikutani for the gift of human CD23 cDNA; M. Leeman-Husler for the graphics; and J. Knowles, K. Hardy, and J.-J. Mermod for discussions and support. The study in Brussels has been supported by the Fond de la Recherche Scientifique Médicale (Belgium).

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Fusion Between Transcription Factor CBFβ/PEBP2β and a Myosin Heavy Chain in Acute Myeloid Leukemia

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The pericentric inversion of chromosome 16 [inv(16)(p13q22)] is a characteristic karyotypic abnormality associated with acute myeloid leukemia, most commonly of the M4Eo subtype. The 16p and 16q breakpoints were pinpointed by yeast artificial chromosome and cosmid cloning, and the two genes involved in this inversion were identified. On 16q the inversion occurred near the end of the coding region for CBFβ, also known as PEBP2β, a subunit of a heterodimeric transcription factor regulating genes expressed in T cells; on 16p a smooth muscle myosin heavy chain (SMMHC) gene (*MYH11*) was interrupted. In six of six inv(16) patient samples tested, an in-frame fusion messenger RNA was demonstrated that connected the first 165 amino acids of CBFβ with the tail region of SMMHC. The repeated coiled coil of SMMHC may result in dimerization of the CBFβ fusion protein, which in turn would lead to alterations in transcriptional regulation and contribute to leukemic transformation.

Molecular analysis of specific chromosome abnormalities observed in tumor cells has led to the discovery of different genetic events leading to tumorigenesis. In leukemias, at least two mechanisms have been identified for the deregulation of cellular proto-oncogenes by chromosome rearrangements: (i) juxtaposition of a cellular proto-oncogene to the regulatory elements of a tissue-specific gene, particularly the immunoglobulin and T cell receptor genes, that leads to the inappropriate expression of the oncogene (*1*) and (ii) gene fusion at the junction of a translocation that generates a chimeric mRNA and a protein with transforming properties (*2*).

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A characteristic chromosome 16 pericentric inversion, inv(16)(p13q22), has been found in almost all patients with the M4Eo subtype, which constitutes about 8% of acute myeloid leukemia (AML) patients (*3*). Given the absence of other karyotypic abnormalities in many of these patients, a pathogenic relationship between inv(16) and AML M4Eo has been suggested (*3–5*).

Yeast artificial chromosomes (YACs) identified as containing the p arm inversion breakpoint (*6*) were used to screen the Los Alamos chromosome 16 cosmid library (*7*). To find which cosmids contained the breakpoint, we used clones so isolated as probes for fluorescence in situ hybridization (FISH). One of the cosmid clones, 16C3, generated single, discrete fluorescent signals on 16p from normal lymphoblastoid cells and one signal on each arm of the inverted 16 from the cell line ME-1, which was derived from a patient with AML M4Eo and inv(16) (*8*) (Fig. 1A). These data indicate that the 16p breakpoint lies within this cosmid (Fig. 2A). We confirmed this finding with peripheral leukemic cells from three additional patients who had inv(16) (*9*). Moreover, when a 1.2-kb Eco RI repeat-free fragment from cosmid 16C3 (named 16C3e) was used as a probe, rearranged fragments were detected by Southern (DNA) blot hybridization in multiple patients as well as when the DNA was

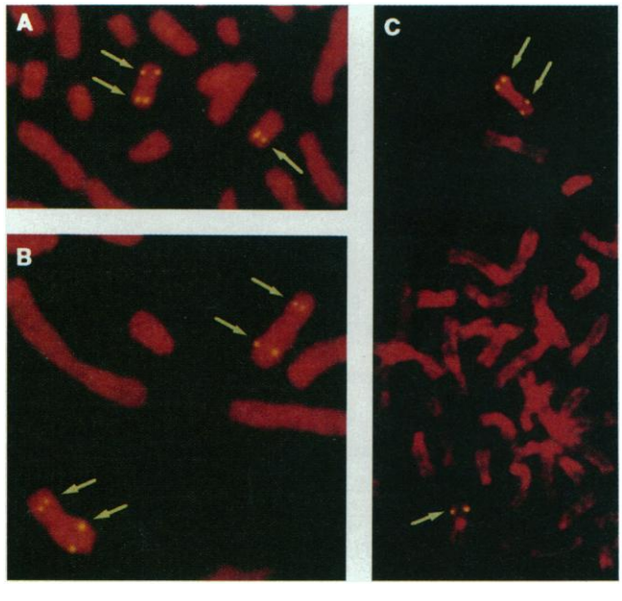

Fig. 1. Results from FISH with cosmids at the inv(16) breakpoint. (**A**) Cosmid 16C3 as a probe on a partial metaphase from the inv(16) cell line ME-1 (*8*). (**B**) A partial metaphase of a normal human lymphocyte probed with cosmid CC19. (**C**) Cosmids LA2-2 and LA4-1 on a partial metaphase from an inv(16) patient. We performed FISH as previously described (*6*).

digested with either Hind III or Kpn I (Fig. 3). By Southern blot hybridization against a regional assignment hybrid panel of human chromosome 16 (*10*), this cosmid was assigned to 16p13.12–p13.13, between the breakpoints of hybrids CY19 and CY185 (*11*). This is the same interval where the inv(16) p arm breakpoint has been mapped (*6*, *12*). A restriction map of the cosmid was generated, and the inv(16) breakpoints in five patients were deduced on the basis of the hybridization pattern of patient genomic DNA with 16C3e (Fig. 2B).

We screened a cosmid library from genomic DNA of peripheral leukemia cells of a patient with inv(16) (*13*) with 16C3e. The cosmid clones isolated were used as FISH probes to reveal that at least one of them, CC19, contained the fusion genomic DNA sequence. This clone generated split signals on both chromosome 16s from normal metaphases (Fig. 1B).

Restriction mapping of CC19 showed that it contained additional restriction fragments not found in 16C3 (Fig. 2B). One of these fragments, a single-copy 0.7-kb Hind III fragment, was used to hybridize to the chromosome 16 hybrid panel mentioned above. This probe was assigned to 16q13–q22.1, between hybrids CY7 and CY6 (*10*), which is consistent with the assignment of the inv(16) q arm breakpoint in previous studies (*12*).

To clone the q arm breakpoint region, we used this 0.7-kb Hind III fragment from cosmid CC19 to screen the Los Alamos chromosome 16 cosmid library. Two overlapping cosmid clones, LA2-2 and LA4-1, were identified. Both of them were shown to contain the q arm breakpoint of inv(16) when used as FISH probes. The clones produced a single signal on 16q of the normal chromosome and a signal on both arms of the inverted chromosome (Figs. 1C and 2A).

To identify genes affected by the inversion, we used single-copy fragments and whole cosmid DNA from cosmids 16C3, CC19, and LA2-2 to screen cDNA libraries. By screening a human fetal brain cDNA library (from Stratagene) and a human large intestine cDNA library (*14*) with cosmid 16C3, we identified several overlapping cDNA clones. The sequence of one of them, L11a, which had a 1.8-kb insert, showed a high degree of similarity to myosin heavy chain genes with the highest similarity to smooth muscle forms in other species [ranging from 77 to 90% at the DNA level (9)], suggesting that L11a contains the sequence of a human smooth muscle myosin heavy chain (SMMHC) gene. Hybridization with L11a against cosmid 16C3 DNA revealed that there were exons on both sides of the inv(16) breakpoints (Fig. 2B), indicating that this SMMHC gene is disrupted by the inversion. According to generally accepted nomenclature, this gene is named *MYH11* (*15*).

To identify the q arm component of the postulated fusion gene, we screened the same human fetal brain cDNA library and a HeLa expression cDNA library (*16*) with whole cosmid DNA or single-copy fragments of LA2-2 and LA4-1. We isolated several overlapping cDNA clones; their sequence showed a high degree of similarity to a newly described mouse DNA-binding factor CBFβ (*17*). The sequence similarity of RL9a, the longest cDNA clone, to mouse CBFβ at the DNA level is more than 90% in the coding region and more than 70% in the 3′ untranslated region. At the protein level, only three amino acid changes were identified out of the total of 182 (*18*). Therefore, RL9a contains the gene coding for the human counterpart of the mouse CBFβ. We designate this human gene as *CBFB*.

When a fragment of RL9a was used to probe patient genomic DNA, rearranged bands were detected (9). Mapping on cosmids showed that the breakpoint in the inv(16) patient whose DNA was used to make the cosmid library falls in an intron of *CBFB* (Fig. 2B).

Because both *CBFB* on the q arm and *MYH11* on the p arm are disrupted by the inversion, a fusion transcript could potentially be made by splicing the exons of the two genes together on the inversion chromosome. Two fusions are possible: one containing the 5′ portion of the *CBFB* gene and the 3′ portion of *MYH11*, and the other composed of the *MYH11* 5′ region and the *CBFB* 3′ region. The former fusion is probably more important for leukemogenesis because in two AML M4Eo patients with inv(16) there is an associated deletion centromeric to the p arm breakpoint, which would truncate the 5′ portion of *MYH11* (*19*).

We designed primers from the middle of the *CBFB* coding sequence and the 3′ region of *MYH11* (*20*). Amplification by reverse transcriptase–polymerase chain reaction (RT-PCR) was conducted with total cellular RNA from the cell line ME-1 and peripheral leukemia cells of five patients with inv(16). PCR products were generated in all six samples using different combinations of *CBFB* and *MYH11* primers (Fig. 4A). Sequencing of the PCR products con-

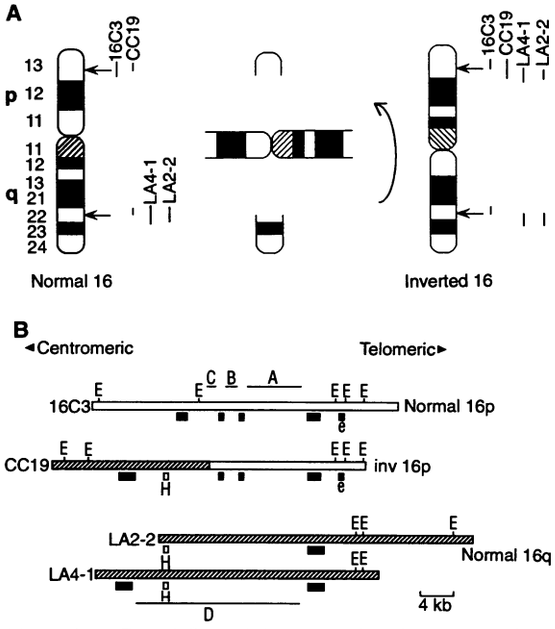

Fig. 2. Cosmids spanning the inv(16) breakpoints. (**A**) Schematic representations of the locations of the cosmids on normal and inverted chromosome 16s (not to scale). (**B**) Restriction map of cosmids 16C3, CC19, LA2-2, and LA4-1. Unfilled horizontal bars represent sequences from the p arm and hatched horizontal bars are sequences from the q arm. Solid boxes are probable exon locations for *MYH11*, and dotted boxes are probable exon locations for *CBFB* (the solid boxes with an "e" designate the location of 16C3e). The open boxes labeled with "H" refer to the repeat-free 0.7-kb Hind III fragment used to identify LA2-2 and LA4-1. Horizontal lines labeled with letters A through D indicate intervals containing p and q arm breakpoints. The cell line ME-1 and three patients have p arm breakpoints located in the A region, one patient each has a p arm breakpoint located in regions B and C, and the q arm breakpoints in all six samples are located in region D. E, Eco RI.

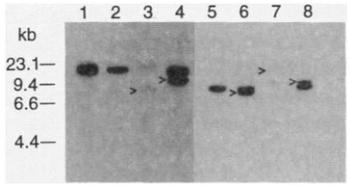

Fig. 3. Autoradiograph of Southern blot hybridization with ^{32}P-labeled 16C3e DNA. Lanes 1 and 5: DNA from a normal human fibroblast cell line; lanes 2 and 6: DNA from inv(16) patient A; lanes 3 and 7: DNA from inv(16) patient B; lanes 4 and 8: DNA from the inv(16) cell line ME-1. DNA in lanes 1 through 4 was digested with Hind III and DNA in lanes 5 through 8 was digested with Kpn I. Abnormal bands in patient sample lanes are indicated by arrowheads. The DNA in lanes 3 and 7 is underloaded, resulting in the apparent slower migration of the hybridized bands. Southern blot hybridizations were performed as described (*6*).

firmed that they are all in-frame fusion gene transcripts derived from *CBFB* and *MYH11* (Fig. 4B). The *CBFB* breakpoints in all six samples are the same, located close to the 3′ end of the coding region with only the last 17 of the 182 amino acids of the potential CBFβ protein deleted (*18*). Interestingly, this breakpoint is located at a sequence that serves as an alternative splice donor in both mouse and human (*9, 17*).

We identified three different breakpoints in the *MYH11* coding region in the six patients. The cell line ME-1 and three patients shared the same breakpoint (see Fig. 4A, lanes 3 through 6), whereas two other patients had their breakpoints at 927 and 720 bp upstream from the first one, respectively (Fig. 4A, lanes 1 and 2). All of these rearrangements maintain the reading frame of the fusion transcript (*21*).

By analogy to the molecular events revealed in other leukemias, it is likely that the fusion of *CBFB* and *MYH11* produces a protein that contributes to leukemogenesis. Moreover, this fusion protein must have a dominant effect as only one of the two chromosome 16s is inverted in leukemic cells. The mouse CBFβ gene has been cloned and analyzed recently (*17, 22–24*). Core-binding factor (CBF) binds to the core site of murine leukemia virus and also to the enhancers of the T cell receptor genes (*22*). The core site appears to be a major genetic determinant of the tissue specificity of leukemias induced by the murine leukemia virus (*23*). Affinity-purified CBF contains at least two subunits, CBFα and CBFβ (*17, 24*). It has been shown that CBFα is identical to AML1, the gene found to be disrupted in the characteristic t(8;21) translocation in the M2 subtype of AML (*17*). Another DNA-binding factor named PEBP2, which binds to similar core sequences in the enhancer of the polyomavirus, has been identified in NIH 3T3 cells (*25–28*). PEBP2 contains two subunits, PEBP2α and PEBP2β. Two α subunits have been identified: one is identical to CBFα (*27*) and the other shares a 130–amino acid homology with CBFα (AML1) and with *runt*, a *Drosophila* segmentation gene (*28, 29*). However, PEBP2β and CBFβ are identical (*17, 26*).

The CBFβ protein does not contain any known DNA-binding motifs or transcriptional activation domains, and no significant similarity was found to any gene or protein in GenBank. In vitro analysis indicates that the mouse CBFβ/PEBP2β does not bind to DNA sequences directly; rather, it forms a heterodimeric complex with CBFα or PEBP2α and stabilizes the interaction of the α subunits with DNA (*17, 26*).

Thus, separate subunits of a transcription factor can be involved in different leukemias. CBF must be crucial for the control of cell division or differentiation, or both, of the myeloid lineage because the expression of either subunit as a fusion protein is associated with the blockage of differentiation and uncontrolled expansion of leukemia cells. Cytologically the two types of AMLs in which inv(16) and t(8;21) take place are different: inv(16) is a marker for the M4Eo subtype of AML, which shows both granulocytic and monocytic differentiation, and is characterized by abnormal eosinophilia in bone marrow and peripheral blood; in contrast, t(8;21) is highly predictive for the M2 subtype of AML, which is characterized by granulocyte maturation (*4, 30*). Both types of leukemia have a relatively favorable prognosis.

The consistent involvement of *MYH11* in inv(16) suggests that both partners in the fusion event play a significant role. Muscle genes have been found to be fused to oncogenes in at least two reports: one between actin and the v-*fgr* oncogene, the other between tropomyosin and the *trk* oncogene (*31*). In both events, which represented isolated occurrences, truncated muscle genes were fused to a tyrosine kinase. However, neither of these two muscle gene components was found to be indispensible to the transforming capability of the oncogenes, and the actin part of the v-*fgr* gene actually inhibits the kinase and its transforming activity (*32*).

It is not immediately obvious, therefore, what the contribution of *MYH11* is to the pathogenesis of inv(16) leukemia. All three breakpoints in *MYH11* are located in the conserved tail or rod region. This tail region of the protein contains a repeated α-helical structure, the major function of which is to form a coiled coil with another molecule in the assembly of a myosin-thick filament (*33*). This could result in dimerization of two CBFβ-SMMHC molecules, whereas the normal CBFβ is thought to function as a heterodimer with CBFα or PEBP2α (*17, 22–28*). There are several possible mechanisms whereby this could result in a dominant transforming phenotype. The dimerized CBFβ-SMMHC protein may be able to form a more stable complex with the α subunits than that formed by wild-type CBFβ, augmenting the effect of this complex on the target genes. Conversely, the CBFβ-SMMHC dimer may have a dominant negative effect by

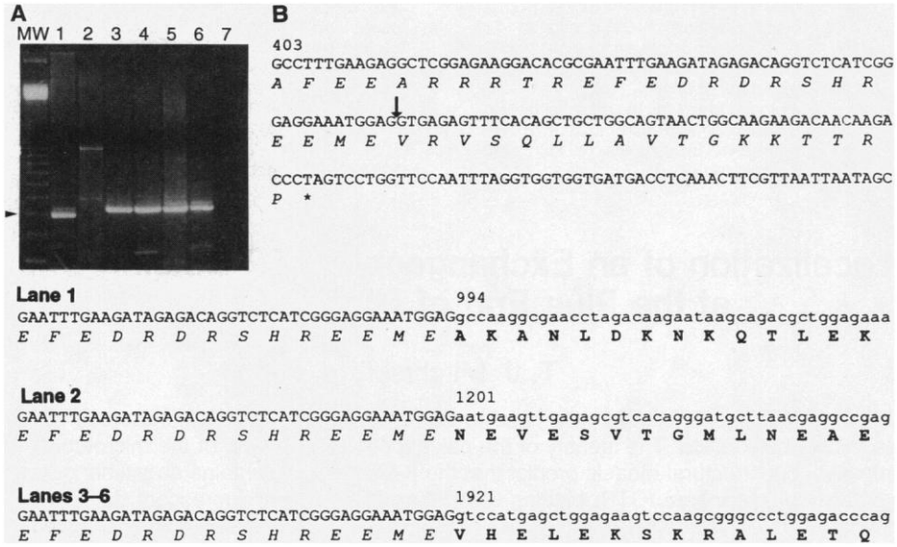

Fig. 4. Detection of fusion transcripts by RT-PCR. (**A**) Ethidium bromide–stained agarose gel separating the RT-PCR products. Lanes 1 through 5: RT-PCR using RNA from peripheral leukemic cells of five inv(16) patients; lane 6: RT-PCR using RNA from the cell line ME-1; lane 7: RT-PCR with no template. Primers C1 and M1 were used for reactions in lanes 2 through 7 and primers C1 and M2 were used for lane 1 [see (*20*) for the sequences of the primers]. MW, molecular weight standards; the arrow indicates 369 bp. (**B**) The upper sequence is the 3′ end coding region of *CBFB*. The arrow indicates the inv(16) breakpoint, the nucleotide number on top of the sequence is from (*18*), and the asterisk denotes the stop codon. The lower part shows sequences of the RT-PCR products surrounding the fusion junctions. Sequences originated from *CBFB* are in capital letters and those from *MYH11* in small letters. The deduced amino acid sequences are in italics for CBFβ and in bold type for SMMHC (*35*). Lane numbers from (A) are in front of each corresponding sequence. The locations of breakpoints in *MYH11* are indicated by nucleotide numbers [as in (*15*)] on top of the sequences. We isolated RNA from cells using RNAzol (Cinna/Biotecx, Friendswood, Texas) and performed RT-PCR as described (*34*). Products from PCR were separated on a low melting point agarose gel (NuSieve GTG; FMC, Rockland, Maine), excised from the gel under long wavelength ultraviolet light, and used directly for sequencing with the Sequenase kit (U.S. Biochemical, Cleveland, Ohio).

binding α subunits with high affinity, preventing binding of the DNA target sequence. It is also possible that the SMMHC protein contributes a domain that results in inappropriate transcriptional regulation by the α/β complex. Finally, the CBFβ-SMMHC dimers may acquire some new activity in transcriptional regulation.

The elucidation of these two genes as the fusion partners in an inversion leading to a common form of adult leukemia should also allow the development of a mouse model and a sensitive RT-PCR test for specific diagnosis and assessment of residual disease after treatment. Complete elucidation of the mechanisms by which CBFβ-SMMHC transforms a particular hematopoietic lineage may eventually lead to new and more effective therapies for this relatively common form of adult leukemia.

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20. The sequences of the primers are the following: the sense primer, C1, GCAGGCAAGGTATATTTGAAGG [nt 253 through 274 of *CBFB*, (*18*)]; antisense primer 1, or M1, CTCTTCTCCTCATTCTGCTC [reverse sequence of nt 2095 through 2114 of *MYH11*, (*15*)]; antisense primer 2, or M2, ACTGCAGCTCCTGCACCTGC [reverse sequence of nt 1119 through 1138 of *MYH11*, (*15*)].
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Localization of an Exchangeable GTP Binding Site at the Plus End of Microtubules

T. J. Mitchison

Microtubule polarity arises from the head-to-tail orientation of α-β tubulin heterodimers in the microtubule lattice. The identity of the polypeptide at each end of the microtubule is unknown, but structural models predict that the β-tubulin end contains an exchangeable guanosine triphosphate (GTP) binding site. When GTP-coated fluorescent beads were incubated with microtubules, they bound specifically to plus ends, suggesting that tubulin is oriented in microtubules with β-tubulin toward the plus end.

Microtubules are polar polymers of the protein tubulin. In most cells, the minus ends of microtubules are attached to an organizing center near the cell center, whereas the more peripheral plus ends grow and shrink by dynamic instability (*1*). Tubulin is a heterodimer of α- and β-tubulin subunits, which are 36 to 42% identical in sequence and have similar structures (*2*). The tubulin polypeptides are arranged head-to-tail in the heterodimer and the heterodimers are arranged head-to-tail in the microtubule lattice (*3*), creating a polar lattice. So far it has not been determined which subunit type is exposed at the plus end and which is exposed at the minus end of microtubules.

The polypeptides α- and β-tubulin each bind one molecule of guanine nucleotide with high affinity. The nucleotide binding site on α-tubulin binds GTP nonexchange-

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