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Mapping chromosome band 11q23 in human acute leukemia with biotinylated probes: Identification of 11q23 translocation breakpoints with a yeast artificial chromosome

(chromosomal translocations/lymphoid leukemias/myeloid leukemias/fluorescence *in situ* hybridization/gene mapping)

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ABSTRACT **Translocations involving chromosome 11, band q23, are frequent recurring abnormalities in human acute lymphoblastic and acute myeloid leukemia. We used 19 biotin-labeled probes derived from genes and anonymous cosmids for hybridization to metaphase chromosomes from leukemia cells that contained four translocations involving band 11q23: t(4;11)(q21;q23), t(6;11)(q27;q23), t(9;11)(p22;q23), and t(11;19)(q23;p13). The location of the cosmid probes relative to the breakpoint in 11q23 was the same in all translocations. Of the cosmid clones containing known genes, *CD3D* was proximal and *PBGD*, *THY1*, *SRPR*, and *ETS1* were distal to the breakpoint on 11q23. Hybridization of genomic DNA from a yeast clone containing yeast artificial chromosomes (YACs), that carry 320 kilobases (kb) of human DNA including *CD3D* and *CD3G* genes, showed that the YACs were split in all four translocations. These results indicate that the breakpoint at 11q23 in each of these translocations occurs within the 320 kb encompassed by these YACs; whether the breakpoint within the YACs is precisely the same in the different translocations is presently unknown.**

Cloning of the genes involved in recurring chromosome translocations in leukemia and lymphoma has provided not only significant insights into the various mechanisms for rearrangements, but also DNA probes for diagnosis and monitoring of disease (1–3). The isolation of translocation breakpoints in human acute myeloid leukemia (AML) has proceeded extremely slowly. One reason is the lack of cell lines that contain the relevant translocations; thus, investigators are restricted to using the limited amount of material available from patient samples. Somatic cell hybrids retaining the translocation or rearrangement of interest have had some limited usefulness. Another problem is the absence of a known gene, such as the immunoglobulin or T-cell receptor genes, that is located at the breakpoint on one of the involved chromosomes. A related problem is that DNA probes for a particular chromosome region are not available in sufficient numbers to offer a high likelihood of identifying a DNA rearrangement on Southern blot analysis either by conventional electrophoresis or by pulsed-field gel electrophoresis. We and others have used radiolabeled probes for *in situ* hybridization to identify genes that bracket the translocation breakpoints, although, in some cases, they showed no rearrangements on Southern blot analysis.

The use of cosmids or yeast artificial chromosomes (YACs) as probes to screen much larger segments of DNA for rearrangements provides an additional strategy for the analysis of chromosomal abnormalities; we have used this strategy for the analysis of one group of chromosome translocations—namely, those involving band 11q23. This chromosome band is of great interest in human acute leukemia for at least three reasons. First, there are a large number of different recurring rearrangements that involve 11q23, and thus, along with band 14q32, 11q23 is one of the bands most frequently involved in rearrangements in human tumor cells (4). The breakpoints in the 11q23 translocation partners include 1p32, 4q21, and 19p13 in acute lymphoblastic leukemia (ALL) and 1q21, 2p21, 6q27, 9p22, 10p11, 17q25, and 19p13 in AML, especially the monoblastic and myelomonocytic subtypes. Second, these translocations occur primarily in two morphologic types of leukemia that are different but have similar features. One common translocation in infants, t(4;11)(q21;q23), usually has a lymphoblastic phenotype, although the leukemia cells may express some myeloid surface markers; in some cases, variable numbers of monocytoid blast cells have been identified (5, 6). A cell line with the 4;11 translocation (RS4;11) has rearranged immunoglobulin heavy-chain and κ light-chain genes and yet can be induced to express monoblastic features on exposure to phorbol esters (7). These data suggest that a gene at 11q23 may be involved in determining the differentiation of primitive hematopoietic stem cells into lymphoblasts or monoblasts, or that it may be a gene that is active in both cell lineages. Third, translocations involving 11q23 have a very unusual age distribution; they comprise about two-thirds of the chromosome abnormalities in leukemia cells of children under 1 year of age (8–10).

We report here on our use of a series of cosmid probes and fluorescence *in situ* hybridization techniques to map the breakpoint at 11q23 in four different recurring translocations. These studies were undertaken to determine the position of the cosmid probes relative to the breakpoints at 11q23, to determine the order of the cosmids if the breakpoints were not identical, and to bracket the translocation breakpoints with cosmid probes to facilitate additional molecular studies.

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Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; YAC, yeast artificial chromosome; FITC, fluorescein isothiocyanate; DAPI, 4',6-diamidino-2-phenylindole dihydrochloride.

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We expanded these studies to include YAC clones identified by DNA sequences for known genes at 11q23; we found that YACs containing the *CD3D* and *CD3G* genes and flanking DNA sequences were split by all four translocation breakpoints. Thus, the use of YACs provides a very powerful tool for identifying previously uncharacterized translocation breakpoints.

MATERIALS AND METHODS

Patient Material and Cell Lines. The clinical and cytogenetic features of four patients and of a cell line with abnormalities involving 11q23 are listed in Table 1. The RS4;11 cell line was provided by one of us (J.H.K.) (6, 7). We also examined leukemia samples from two patients with a t(9;11), from one patient with a t(11;19), and from one patient with a t(6;11).

Metaphase Cell Preparation. Metaphase cells were prepared from bone marrow aspirates of leukemia patients as described (11). The RS4;11 cell line was cultured for 24 hr at 5×10^6 cells per ml; Colcemid (0.05 μg/ml) was added for 10–25 min and the cells were processed as described (11).

Probes. Most of the cosmid probes for the long arm of chromosome 11 were isolated from a genomic library constructed in the cosmid vector Cos-1 (12) by using DNA from a somatic cell hybrid, TG5D1-1, containing 11q13–qter as the only human material in a mouse erythroleukemia cell background (13). Additional cosmid clones containing known genetic loci were isolated by using cDNA or oligonucleotide probes from this library or from a human genomic cosmid library constructed in the cosmid vector pWE15. The cosmid probes we used had been localized to 11q22–25 by fluorescence *in situ* hybridization (14).

The two yeast clones were isolated from the YAC library that was described previously (15). The YAC library was screened by one of us (P.T.-M.). One yeast clone (yB22B2) was detected by using polymerase chain reaction primers (16) (sense, GACTATCAAGAAGATGGTTC; antisense, AAGGGGATTACATACTTCTG) specific for the *CD3G* gene (17), which had previously been mapped to 11q23 (18); this clone was initially requested by P.H.D. This yeast clone (yB22B2) appears to contain two YACs. One YAC contains a 320-kilobase (kb) insert of human DNA sequences, including the *CD3D* and *CD3G* genes, and the second YAC contains the same sequences with a 45-kb deletion in the end of the insert opposite to *CD3D* and *CD3G*. The *CD3E* gene is not present in the YACs. The second yeast clone (A73B12) was detected with consensus polymerase chain reaction primers for the α-interferon (*IFNA*) gene family; it contains 350 kb of human DNA, including the distal part of the *IFNA* gene cluster and the β_1-interferon (*IFNB1*) gene.

Repetitive DNA probes derived from pericentromeric satellite sequences of chromosomes 9 (pHur98; kindly provided by R. K. Moyzis, Los Alamos National Laboratory) and 11 (Oncor) were cohybridized with cosmid or YAC clones in some experiments for unambiguous identification of the normal and derivative chromosomes.

Fluorescence *in Situ* Hybridization. The procedure used for fluorescence *in situ* hybridization is a modification of the method described by Lichter *et al.* (14). Biotin-labeled probes were prepared by nick-translation using Bio-11-dUTP (Enzo Diagnostics). Slide preparations were treated with preboiled RNase [100 μg/ml in 2× SSC (pH 7.0); Sigma; 1× SSC = 0.15 M NaCl/0.015 M trisodium citrate] at 37°C for 1 hr, rinsed extensively in 2× SCC, and dehydrated in a graded ethanol series (70%, 85%, 95%). Chromosomal DNA was denatured by immersion of the slides in 70% formamide/4× SSC, pH 7.0, at 70°C for 2 min, followed by dehydration in an ethanol series. Prior to hybridization, unlabeled, sonicated total human genomic DNA (final concentration, 150 μg/ml) was added to the hybridization mixture (50% formamide/1× SSC/10% dextran sulfate, pH 7.0) containing the labeled probe (5–10 ng of cosmid probes per μl, 0.1 μg of total yeast DNA per μl). The hybridization mixture was heated at 75°C for 5 min and then incubated at 37°C for 5–10 min to promote partial reannealing. Ten microliters was applied to each slide, the slides were covered with coverslips (22 mm^2), and the edges were sealed with rubber cement. The slides were transferred to sealed containers and incubated for 16 hr at 37°C. After hybridization, the slides were washed in 50% formamide/4× SSC (three washes, 5 min each) at 40°C, and then in 4× SSC at 40°C (three washes, 3 min each). Thereafter, the slides were incubated with 3% bovine serum albumin (BSA) in 4× SSC for 30 min at 37°C.

The biotinylated probe was detected with fluorescein isothiocyanate (FITC)-conjugated avidin DCS (Vector Laboratories). Detection reagents were prepared in 4× SSC/0.1% Triton X-100/1% BSA, and the washes were performed in 4× SSC/0.1% Triton X-100 at 40°C (three washes, 3 min each). For detection of the fluorochrome, slides were incubated with 5 μg of FITC-avidin DCS per ml at 37°C for 30 min, followed by washes. In some cases, the fluorochrome signal was amplified by incubation with 5 μg of biotin-conjugated goat anti-avidin D antibodies per ml (Vector Laboratories) at 37°C (30 min), followed by washes, a second incubation with 5 μg of FITC-conjugated avidin DCS per ml at 37°C (30 min), and a final wash. Metaphase cells were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma; 200 ng/ml in 2× SSC, 5 min at room temperature) and mounted in 20 mM Tris·HCl, pH 8.0/90% (vol/vol) glycerol containing 2.3% DABCO antifade (Sigma). The slides were examined with a Zeiss standard 16 microscope equipped with epifluorescence optics (BP365, FT395, LP397 for DAPI;

Table 1. Clinical and cytogenetic features of patients or cell lines with abnormalities involving 11q23

Patient or cell line	Age, yr/sex	Hematologic disease	Stage	Karyotype
RS4;11*	32/F	ALL†	Relapse	46,XX,i(7q),t(4;11)(q21;q23) (100%)
1	44/M	AML-M1	Diagnosis	46,XY(16%)/46,XY,t(9;11)(p22;q23) (84%)
2	64/M	t-MDS	Diagnosis	46,XY,t(9;11)(p22;q23) (100%)
3	12/F	ALL/AMoL‡	Diagnosis	47,XX,+8,t(11;19)(q23;p13) (100%)
4	48/M	AMoL-M5	Relapse	46,XY(3%)/46,XY,t(6;11)(q27;q23) (55%)/46,XY,del(7)(q31;q36),t(6;11)(q27;q23) (35%)/two nonclonal cells with related abnormalities (7%)

AML-M1, acute myeloblastic leukemia; t-MDS, therapy-related myelodysplastic syndrome; AMoL, acute monoblastic leukemia.
*Described in refs. 6 and 7.
†ALL with monocytic features.
‡Biphenotypic acute leukemia with lymphoblastic and monoblastic features.

BP450-490, FT510, LP520 for FITC). Photographs were taken with Kodak Ektachrome P800/1600 film.

RESULTS

The results of the hybridizations of the cosmid probes to the RS4;11 cell line and to leukemia cells from the two patients with a t(9;11), one patient with a t(11;19), and one patient with a t(6;11), are shown in Table 2 and in Fig. 1 *A* and *B*. The location of the probes on either the derivative 11 [der(11)] or the translocation partner was the same in the different translocations. In the single exception (patient 1), their location did not differ; however, seven probes distal to the 11q23 breakpoint were deleted. This abnormality appeared to be a deletion because the probes were not identified on another chromosome in these cells. The order of these probes has not been established, although, based on the findings in the aforementioned patient, probes 9.4, ZA7, XH5, XB1, ZC9, *PBGD*, and *THY1* may be more proximal than the remainder of the probes that were translocated to the other chromosome involved in the rearrangement, assuming that the deletion is adjacent to the breakpoint. Similar results have been obtained using many of these same probes with another t(4;11) cell line (19) (J. Lu and D.C.W., unpublished observations). At present, we do not know the distance between the most distal of the probes that are proximal to the breakpoint at 11q23 and the most proximal of the distal probes.

YAC clones were requested for all known genes in 11q23. The yeast clone containing the *CD3G* YACs was the first one used for *in situ* hybridization and it contains the *CD3δ* and γ genes and lacks *CD3ε*. A single labeled locus on the chromosome 11 homologs was observed when DNA from the yeast clone was hybridized to normal human metaphase chromosomes. However, hybridization of these YACs to the RS4;11 cell line showed intense labeling on the normal chromosome 11 and a less intense signal on both of the rearranged der(11) and der(4) chromosomes, indicating that the breakpoint was within the 320 kb encompassed by the YACs (Fig. 1*E*). These YAC probes were then hybridized to leukemia cells with a t(9;11), t(6;11), or t(11;19) (Fig. 1 *C*, *D*, and *F*). The YACs were split in each instance; that is, there was a clear fluorescence signal both on the rearranged chromosome 11 and on the translocation partner (chromosome 6, 9, or 19). No signals were observed on any other chromosome. These data enabled us to localize the breakpoint in each translocation to within the 320 kb homologous to these YACs and distal to *CD3G*. We tested several of the cosmid probes (4.13, ZC9, *PBGD*, ZB6) to determine whether they were contained within the YACs; no positive hybridization was found.

The YAC identified with the *IFNA* gene was hybridized to cells from one of the patients who had a t(9;11); the YAC probe remained on the der(9). This confirmed our results of *in situ* hybridizations in which we used a radiolabeled *IFNA* probe (20). This YAC also contained the *IFNB1* gene located on 9p, which suggests that the breakpoint in 9p is distal to the entire *IFN* gene cluster.

Table 2. Chromosomal localization of probes on 11q

Probe	Abnormality				
	t(4;11)	t(9;11)*	t(9;11)†	t(11;19)	t(6;11)
3.16	der(11)				
23.20		der(11)		der(11)	
1.16	der(11)	der(11)	der(11)	der(11)	der(11)
4.13	der(11)	der(11)	der(11)	der(11)	der(11)
ZB6	der(11)	der(11)	der(11)	der(11)	der(11)
CD3D	der(11)	der(11)	der(11)	der(11)	der(11)
9.4	der(4)	Deleted	der(9)	der(19)	der(6)
ZA7	der(4)	Deleted	der(9)	der(19)	der(6)
XH5	der(4)	Deleted	der(9)	der(19)	der(6)
XB1	der(4)	Deleted	der(9)	der(19)	der(6)
ZC9	der(4)	Deleted	der(9)	der(19)	der(6)
PBGD	der(4)	Deleted	der(9)	der(19)	der(6)
THY1	der(4)	Deleted	der(9)	der(19)	der(6)
8.5	der(4)	der(9)	der(9)		
SRPR		der(9)			
XB2	der(4)	der(9)			
ETS1			der(9)	der(19)	
23.2	der(4)				
5.8	der(4)				
CD3G YAC	der(4)/ der(11)	der(9)/ der(11)	der(9)/ der(11)	der(11)/ der(19)	der(6)/ der(11)
IFN YAC		der(9)			

*Patient 1.
†Patient 2.

The use of the *CD3G* YACs has allowed us to identify the translocation breakpoint in band 11q23 in four different chromosomal translocations. The 4;11 translocation is seen primarily in ALL, whereas the 6;11 and 9;11 translocations are generally restricted to AML, most often of the monoblastic or myelomonocytic subtypes. The t(11;19) has been observed with about equal frequency in both ALL and AML. We recognize that the identification of the breakpoint within this yeast clone containing 320 kb of human DNA does not necessarily imply that the breakpoint is precisely the same in all four translocations. The situation could be analogous to the 9;22 translocation in chronic myeloid leukemia and in Ph^1-positive ALL, in which the same gene, *BCR*, is involved but the breakpoints are separated by at least 50–100 kb (21). Alternatively, several different, but closely linked genes could be involved in the various translocations. We have evidence that at least one other translocation breakpoint in 11q23 is not contained within the *CD3G* YAC (R. Burnett, M.M.L.B., J.D.R., and M.O.D., unpublished data).

A number of different laboratory groups have mapped genes and anonymous DNA probes relative to various 11q23 translocation breakpoints. Most investigators have used the RS4;11 cell line for these studies. It is not possible to compare the location of the anonymous cosmid DNA probes that we have used with the anonymous DNA probes analyzed by others. However, we can compare our results for defined genes. With regard to the *CD3* gene cluster, Evans *et al.* (22) and Tunnacliffe *et al.* (23) have shown that the three genes—γ, δ, and ε—lie within a 50-kb segment. The γ and δ genes are separated by 1 kb, whereas δ and ε are 22–26 kb apart. In an earlier study, Gold *et al.* (24) showed that *CD3D* and *CD3E* remained on the der(11) chromosome in somatic cell hybrids derived from the RS4;11 cell line. These results are consistent with our findings with the *CD3D* cosmid, which hybridized to the der(11) in each of the four translocations that we examined. Our results establish that the order of these genes is cen–*CD3E*–*CD3D*–*CD3G*–tel.

Savage *et al.* (25) and Wei *et al.* (26) examined somatic cell hybrids derived from the RS4;11 cell line (27) and showed that *NCAM*, *CD3D*, and *CD3E* remained on the der(11) chromosome, whereas *THY1* and *ETS1* were translocated to the der(4) chromosome. Yunis *et al.* (28) obtained the same results using a t(4;11) hybrid cell line derived from another patient. These data are concordant with the results we obtained with cosmid probes. In addition, our data as well as those of Yunis *et al.* (28) indicate that the *PBGD* and *SRPR* genes are distal to the breakpoint.

By using fluorescence *in situ* hybridization of probes to normal metaphase cells, Lichter *et al.* (14) prepared a high-resolution map of chromosome 11. Our analysis of cells with translocations involving 11q23 using the same probes has allowed us to refine the map of this band. The 4.13, 9.4, and

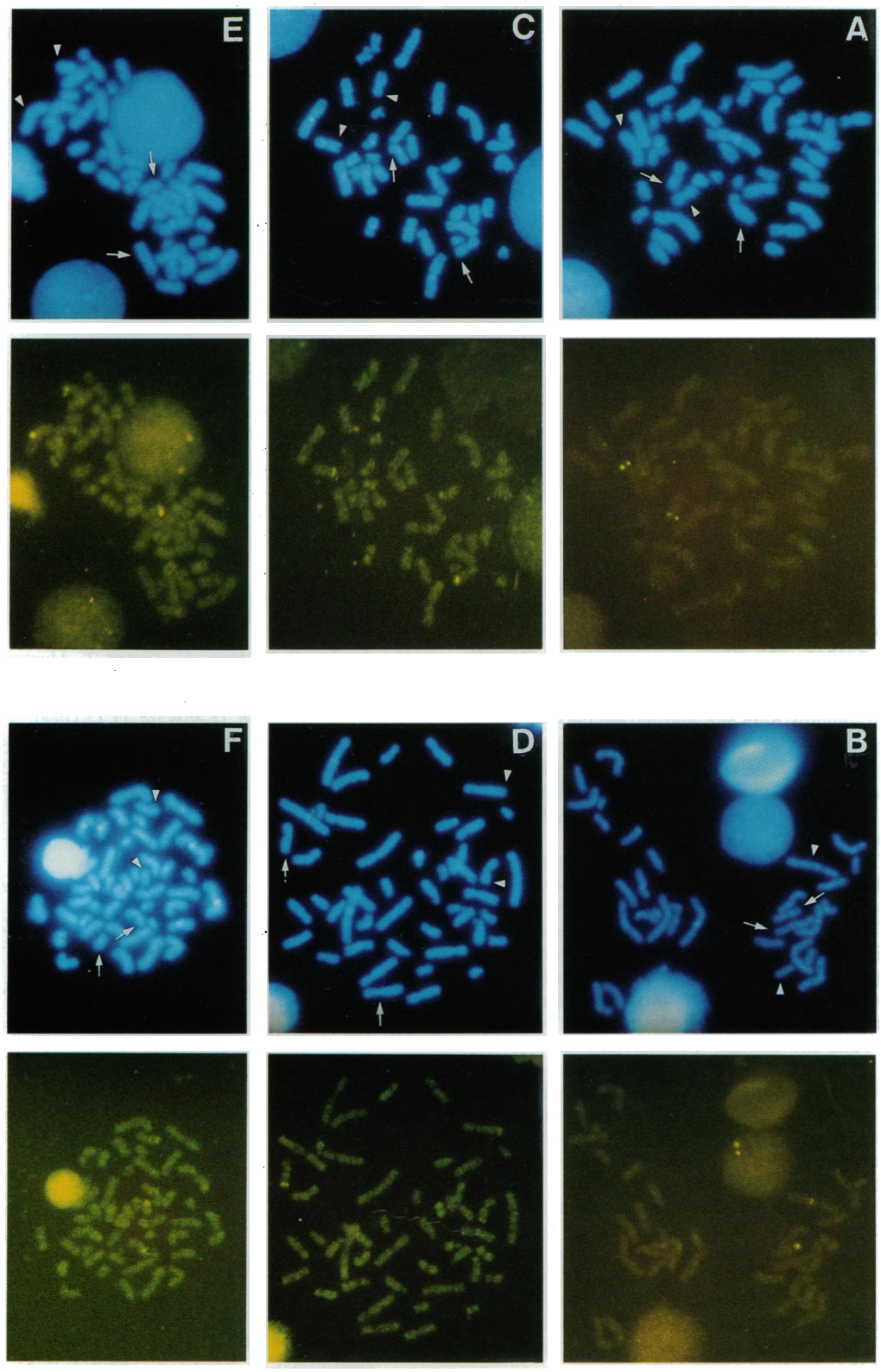

FIG. 1. Illustration of *in situ* hybridization of cosmid or YAC probes to metaphase cells containing translocations involving chromosome band 11q23. In each pair, the top photograph shows the cell stained with DAPI and the bottom photograph shows the hybridization of the biotinylated probe detected with FITC-conjugated avidin. The normal chromosome homologs are identified with arrows and the rearranged chromosomes are identified with arrowheads. (*A*) Mapping of cosmid ZC9 to a cell with a t(6;11); the cosmid has been translocated to the der(6) chromosome. (*B*) Cosmid ZB6 remains on the der(11) in a cell with the t(4;11). The location of the *CD3G* YACs on the normal 11 at band q23 and on both derivative translocation chromosomes is illustrated in *C–F*. The YACs are split as a result of the translocations; therefore, the signal is less intense on the rearranged chromosomes. (*C*) In this cell with a t(9;11), the normal 9 is in the center. The der(11) is on the left and the der(9) is at the top of the cell. (*D*) In this cell with a t(6;11), the normal 6 is on the perimeter at the bottom. The der(11) is on the right and the der(6) is near the top right of the cell. (*E*) In this t(4;11) cell, the normal 4 is on the perimeter near the bottom. The der(11) is on the left and the der(4) is on the perimeter near the top of the cell. (*F*) In this t(11;19) cell, the normal 19 is on the perimeter at the bottom. The der(19) is in the center and the der(11) chromosome is at the top in this cell.

ZA7 cosmids were previously mapped together within the region containing 1.16. The results of our hybridizations indicate that 4.13 and 1.16 are proximal, whereas 9.4 and ZA7 are distal to the 11q23 breakpoints. Similarly, the *CD3D*, *PBGD*, XB1, and XH5 probes were grouped together (14); however, our results show that *CD3D* is proximal to *PBGD*, XB1, and XH5, which are distal to the translocation breakpoints. Moreover, the deletion of seven cosmid probes from the der(9) chromosome in one patient with the t(9;11) suggests that they are nearer to the breakpoint than are *SRPR* and other cosmids not included in the deletion.

We have previously studied the 9;11 translocation by using probes for the interferon gene cluster on 9p and for the *ETS1* gene (20). Our data appeared to show that the α-interferon (*IFNA*) gene cluster remained on 9p, whereas the *IFNB1* gene moved to 11q. *ETS1* was translocated to 9p. Our present evidence (M.O.D. and J.D.R., unpublished data) indicates that the *IFNA* and *IFNB1* genes are on the same 1400-kb *Not* I fragment. This fragment is not rearranged in leukemia cells containing the 9;11 translocation, suggesting that the translocation does not split this gene cluster. Our results with *IFN* YAC probes support this conclusion and show that the breakpoint in 9p22 is distal to the entire *IFN* gene cluster.

The use of biotin-labeled probes that are detected by fluorescence microscopy has a number of advantages compared with the use of radiolabeled probes. The probes are quite specific when appropriate blocking strategies are used to prevent nonspecific hybridization from the repetitive sequences in the probes. The signal is visualized as discrete fluorescent dots; thus, the location of the probe can be determined quite precisely. In most cells, both chromosome homologs (and frequently both chromatids) are labeled; therefore, the signal on the normal chromosome provides an internal control. Chromosome-specific centromere probes unequivocally mark a pair of chromosomes, which can then be scored for the presence or absence of the signal from a particular probe.

The use of YAC clones for screening of recurring translocations and identification of those that are split by the translocation breakpoints provides an entirely new strategy for identifying DNA clones that contain the relevant breakpoints. With this technique, the detailed molecular analysis is focused on a particular DNA fragment that contains the segment of interest. It is clear that this approach will be equally useful in defining the limits of chromosome deletions. In our study, both the normal chromosome 11 and the rearranged 11 or the translocation partner were labeled (except for the one patient who had a deletion encompassing seven cosmid probes). Small chromosome deletions, undetectable cytogenetically, have been observed in association with reciprocal translocations—e.g., *BCR* deletions in chronic myeloid leukemia—and they may occur as a result of the translocation (29). Larger chromosome deletions have also been observed as recurring abnormalities in human tumors as well as in genetic diseases; in the case of tumors, they are important mechanisms leading to hemizygosity and loss of function of tumor suppressor genes. The use of YAC clones or other large genomic clones for defining the genetic composition of chromosome deletions should expedite the identification of the involved genes (30).

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