DOE/ER60408-4

DOE/ER/60408--4

DE92 016973

Correlation of Chromosome Patterns in Human Leukemic Cells with Exposure to Chemicals and/or Radiation

Comprehensive Progress Report

For the Period July 1991 through June 1992

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June, 1992

Prepared for

THE U. S. DEPARTMENT OF ENERGY

UNDER GRANT NO. DE-FG02-86ER60408

NOTICE

The specific objectives for the three year proposal January 1992 through December 1995 are listed below.

I. *TECHNICAL PROGRESS OF PROJECT DURING THE PAST YEAR*

A. SPECIFIC OBJECTIVES OF ORIGINAL 3 YEAR PROPOSAL

1. Defining the chromosome segments associated with radiation and chemically induced leukemogenesis (treatment-related acute myeloid leukemia, t-AML).

   a. Continued genetic analysis of chromosomes 5 and 7

   b. Correlation of treatment with balanced and unbalanced translocations.

2. Cloning the breakpoints in balanced translocations in t-AML.

   a. Clone the t(9;11) and t(11;19) breakpoints.

   b. Clone the t(3;21)(q26;q22) breakpoints.

   c. Determine the relationship of these translocations to prior exposure to topoisomerase II inhibitors.

3. Compare the breakpoint junctions in patients who have the same translocations in t-AML and AML de novo.

4. Map the scaffold attachment regions in the genes that are involved in balanced translocations in t-AML.

B. PROGRESS REPORT

1. *Defining the chromosome segments associated with radiation and chemically induced leukemogenesis (treatment-related acute myeloid leukemia, t-AML)*

   a. *Continued genetic analysis of chromosomes 5 and 7*

Much of the responsibility for this project has been given to Drs. Michelle LeBeau and Carol Westbrook. They have some support for this project through a program project grant from NIH. I remain a close collaborator and am involved in parts of the project as needed.

An important related project has been the completion of a major study on the karyotypic pattern seen in patients with a subtype of AML, namely erythroleukemia, AML M6. We have performed a retrospective analysis of the clinical, morphologic, and cytogenetic findings in 26 patients diagnosed between January 1969 and September 1991 with acute erythroblastic leukemia de novo (EL or AML-M6). Clonal chromosomal abnormalities were found in 20 patients (77%). Loss of all or part of the long arm (q) of chromosomes 5 and/or 7 was observed in 17 patients (65%). In addition, the karyotypes were

often complex with multiple abnormalities and subclones. Among the remaining 9 patients, 6 had a normal karyotype and one each had trisomy 8, t(3;3), or t(3;5). The overall frequency of abnormalities of chromosomes 5 and/or 7 observed in our M6 patients is similar to that observed in our patients with therapy-related acute myeloid leukemia (t-AML) (99 of 129 patients, 77%) but substantially higher than that noted in our other patients with AML de novo (FAB subtypes M1-M5: 52 of 334 patients, 16%). Our M6 patients with abnormalities of chromosomes 5 and/or 7 were older and had a shorter median survival (16 weeks vs 77 weeks [p = .0047]) than the patients without these abnormalities. We found no correlation between morphologic features and either cytogenetic abnormalities or clinical outcome. Of note was the finding that the percentage of myeloblasts, which may account for only a small fraction of the total marrow elements when the revised FAB criteria are applied, had no bearing on prognosis. We conclude that acute erythroblastic leukemia, when defined by morphologic criteria, consists of two distinctive subgroups: one group tends to be older, has complex cytogenetic abnormalities especially of chromosomes 5 and/or 7, and shares biological and clinical features with t-AML; the other group with simple or no detectable cytogenetic abnormalities has a more favorable prognosis when treated with intensive chemotherapy. This research report has been submitted to Blood.

b. *Correlation of treatment with balanced and unbalanced translocations*

The results of our analysis has recently been published as a Letter to the Editor of Blood (a copy is enclosed with this Progress Report).

We had 5 patients with balanced translocations of 11q23 and 5 patients with balanced translocations of 21q22. (Table 1) Only one patient in each group did not receive a topo II inhibitor. This compares with 2 and 8 patients with unbalanced translocations involving these bands; except for one patient with an 11q23 breakpoint, none of these patients had received topo II inhibitors. (See Table 1).

Table I. RELATIONSHIP BETWEEN PRIOR CHEMOTHERAPY AND CLONAL CHROMOSOMAL TRANSLOCATIONS IN 132 PATIENTS WITH t-MDS/t-AML

	ATTop Only	ATTop + AA	AA Only	Other Treatment	Total
Number Studied	6	40	67	19	132
Balanced translocation band 11q23	2	2	1	0	5
Balanced translocation band 21q22	0	4	1	0	5
Unbalanced translocation band 11q23	0	0	2	0	2
Unbalanced translocation band 21q22	0	1	6	1	8

Abbreviations: ATTop, chemotherapy agents that target DNA topoisomerase II.
AA, alkylating agents
Balanced translocations involving band 11q23 or 21q22 were significantly associated with prior ATTop exposure (p=0.003, Fisher's exact test, two sided).

2. *Cloning the breakpoints in translocations in t-AML*

a. *Clone the t(9;11) and t(11;19) breakpoints*

As described in my grant proposal, my colleagues and I have been making steady progress in first mapping and now in cloning the breakpoint junctions in the above two translocations, as well as in related translocations t(4;11) and t(6;11). We previously showed that the translocations involving 11q23 with chromosomes 4(q21), 6(q27), 9(p22), and 19(p13) all had breakpoints within a 330 kb human DNA insert in a yeast artificial chromosome (YAC) identified with a CD3 gamma probe. We have done extensive subcloning of three fragments from the YAC and have shown that one fragment remains on chromosome 11 in all translocations. (Figure 1) The other two fragments are translocated to the other chromosome (4,6,9, or 19). All three fragments recognize the same large-sized band (11.5 kb) on Northern blot analysis as well as other transcripts of varying size down to 1.5 kb. We have subcloned a number of unique sequence probes, especially from the large fragment (clone 14) just telomeric to the breakpoint.

We have called this gene *MLL* (myeloid/lymphoid, or mixed lineage leukemia). Using subclones of these genomic probes, we have identified a number of cDNA clones of *MLL*, two of which recognize rearrangements on

Southern blots of DNA from patients and cell lines with 11q23 translocations. An internal 0.7kb BamHl subclone of one of the cDNAs, which recognizes a 9kb BamHl genomic fragment, detects rearrangements on Southern blots of BamHl digested patient and cell line DNA. (Figure 2) To date, using this 0.7kb fragment, all patient material with the 11q23 translocations involving 4, 6, 9, and 19 have shown rearrangements on Southern analysis, indicating that the 11q23 breaks are clustered within 9kb. (Table 2)

DNA REARRANGEMENTS IN 11Q23 TRANSLOCATIONS

DETECTED WITH THE 0.7 kb cDNA PROBE

Translocation	t(4;11)	t(6;11)	t(9;11)	t(11;19)
Number of Patients	15/15	4/4	10/10	7/7

Most of patients have two rearranged bands, indicating that both derivative chromosomes have been retained. Some patients have only a single rearranged band, indicating that there has been a deletion as well as the translocation. We have been able to identify the two derivative chromosomes, namely the der(11) containing the 5′ (centromeric) portion of the *MLL* gene and the der (4,6,9, or 19) containing the 3′(telomeric) portion of the *MLL* gene. In every instance where only a single derivative chromosome is retained, it is the der(11). This is confirms the cytogenetic data from complex three-way translocations, in which the conserved junction is located on the der(11) chromosome.

Using this 0.7kb probe on Northern blots of RNA from normal cells, we detect hybridization to at least two distinct transcripts which are in the 11-12kb size range. In the cell line with the t(4;11), this 0.7kb probe detects at least one additional transcript which is approximately 11.25kb. A more telomeric cDNA probe of *MLL* does not detect this transcript suggesting that this transcript may be derived from the der(11) chromosome and may contain sequences from the translocated region of chromosome 4.

We have sequenced about 4.0 kb of the largest 11.5kb cDNA from the *MLL* gene. We know the gene is transcribed from centromere to telomere. We have not detected any open reading frames (ORF) in the sequences that are 5′ to the breakpoint. We have detected a 1.7kb ORF that is just 3′ to the breakpoint. Comparison of these sequences with Gen Bank has not revealed any homologous sequences. We are continuing to clone additional cDNAs to add to our understanding of the gene. We do have evidence for alternative splicing of some of the cDNAs we have cloned and sequenced. These data suggest that the regulation of the expression of the *MLL* gene will be complex.

Although we are very early in our analysis of this gene, our present data suggest that the role of the *MLL* gene in the translocations may be to function as an activating gene analogous to *IG* or *TCR* in B- and T-cells,

respectively. These latter genes do not form fusion proteins, rather they change the time and/or level of expression of the other gene carried in the translocation. It is my assumption that the *MLL* gene is intimately involved in the decision point for differentiation of a pluripotent hematopoietic cell into either B cells or monoblast/myeloid cells. This assumption is based on the fact that the immunophenotype of the leukemic cells with 11q23 translocations may be either B lineage [eg t(4;11)] or monoblastic [eg t(9;11)] although some are either of these or myeloblastic. The important results to date are that we have cloned a cDNA probe that is very useful clinically in detecting cells with 11q23 translocations.

b. *Clone the t(3;21)(q26;q22) breakpoints*

This is a new research project for the laboratory which I think has very exciting implications related to mutagenic agents and leukemogenesis. Since 1974, we have examined the karyotype of over 160 patients with acute myeloid leukemia (AML) who have a history of prior treatment with radiation and chemotherapy usually for a malignant disease. We have sufficient clinical data on 132 patients so that we can correlate the type of prior treatment with the chromosome abnormalities in the patient's leukemic cells. In the past, we have concentrated on the deletions involving chromosomes 5 and/or 7 because these were the most common changes.

In the course of our analysis of these 132 t-AML patients, it has become apparent that there is another group of patients who lack aberrations of chromosomes 5 and/or 7 and whose leukemic cells have certain specific changes that are quite unusual for t-AML. These patients have balanced translocations involving chromosome bands 11q23 or 21q22. (Table 1) Of special interest is the fact that these patients have usually received high doses of topoisomerase II inhibitors, especially the epipodophyllotoxins, etoposide (VP16) or teniposide (VM26). These latter drugs were first used with any frequency in the 1980s. Our group at the University first called attention to this association in 1987, when we reported on the greatly increased risk of t-AML in a series of lung cancer patients treated with very high doses of etoposide. It was noteworthy that 3 patients had balanced translocations involving 11q23. We proposed that this unusual karyotypic pattern was related to the high dose of etoposide received by these patients. This association has now been confirmed by many other laboratory groups. More recently, we and others have found a very close correlation between the presence of balanced translocations involving 11q23 or 21q22 and exposure to topoisomerase II inhibitors. (Table 1).

All of our t-AML patients with 21q balanced translocations have had a 3;21 translocation with breakpoints in 3q26 and 21q22. We have not seen this translocation in any of our patients with AML de novo; a review of the published literature also indicates that this is extremely rare in de novo AML. We have been collaborating with Dr. Harry Drabkin, University of Colorado Medical Center, who has cloned the breakpoint on chromosome 21 in the 8;21 [t(8;21)(q22;q22)] translocation. We used DNA probes from chromosome 21 isolated by Dr. Drabkin to analyze the t(3;21). We could show with fluorescence in situ hybridization (FISH) that these same probes were on either side of the breakpoint in the t(3;21). The data are not precise, but these probes appear to be about 100 kilobases (kb) apart.

We have frozen samples of bone marrow from six leukemic patients with CML or t-AML with the t(3;21). The samples have been frozen to maintain cell viability, and several hundred million cells are available for each patient. The thawed cells are 90-95% viable, and contain undegraded DNA and RNA, suitable for construction of genomic DNA libraries, and for preparation of RNA for Northern blot analysis and construction of cDNA libraries.

Two of the recombinant phages, probe 3, centromeric, and probe B, telomeric, (Figure 3) hybridize to the same *NotI-SfiI* fragment. FISH analysis of metaphase cells with a t(8;21) showed that probe 3 and combined probes wc-1 and wc-2 mapped on opposite sides of the breakpoint. Similar studies of a t(3;21) showed that probe 3 and the combined probes wc-1 and wc-2 also mapped on opposite sides of the breakpoint. These studies indicated that, as for the t(8;21), the breakpoint was contained within the same *Not1-Sfi1* fragment. We have used the overlapping phage clones that we have isolated as probes for FISH and for Southern blot analysis. We have recently identified three new overlapping probes, wc-7, wc-8, and wc-9, that hybridize to both derivative chromosomes. One probe, wc-8, detects a rearrangement in the DNA from one patient (Figure 3). The results indicate that the t(3;21) breakpoint is located in the segment between probe 3 and wc-9. The genomic mapping of the region between the t(8;21) and t(3;21) breakpoints has not yet been completed, but the two breakpoints appear to be about 20-30 kb apart. The translocation in the t(8;21) results in a fusion gene on the der(8) chromosome and a fusion mRNA. If the gene on chromosome 21 involved in both translocations is the same, it is possible that the region of the breakpoint in the gene on chromosome 21 may be different. We have cloned a fragment from a t(3;21) patient that contains the breakpoint junction. We are in the process of characterizing this DNA probe.

c. *Determine the relationship of these translocations to prior exposure to topoisomerase II inhibitors*

This was a long-range objective which cannot even be approached until we have cloned and sequenced the breakpoints in the 11q23 and 21q22 translocations. When we have completed the sequencing, we will be able to analyze the sequence for topo II consensus sequences and for scaffold attachment sites, as well as for other canonical motifs that would provide us with some insights as to the potential function of these regions and their susceptibility to breakage when exposed to topo II inhibitors.

3. *Compare the breakpoint junction patients who have the same translocations in t-AML and AML de novo*

At present we have been analyzing all of the samples from our 11q23 translocation patients together regardless of whether they are treatment related or de novo AML. Of the samples, we have studied to date, three of the t(9;11) and one of the t(11;19) are treatment related. We have found no difference in the breakpoint in these two group of patients. We have established a collaboration with Dr. Pedersen-Bjergaard in Denmark who has been extremely active in studying t-AML. He recently brought us DNA from 13

patients with either 11q23 or 21q22 breakpoints. DNA from these samples is currently being analyzed and I have no data to report. These samples will more than double the number of t-AML patients whom we can examine.

4. *Map the scaffold attachment regions in the genes that are involved in balanced translocations in t-AML*

Again, this is a very long-range project. We have begun to explore this problem with the genomic and cDNA probes that we currently have for the 11q23 breakpoint region. However, we must develop a more detailed restriction map of this region, and obtain a series of small, unique sequence probes before we can map the scaffold attachment sites in the region of the breakpoint with any precision.

II. *SECTION ON CHANGES IN SPECIFIC AIMS*

Microdissection, amplification and cloning of unknown DNA from defined chromosome bands or structures.

This aim was not included in the renewal proposal in 1991 because it was the subject of a separate grant request to the DOE Genome Project. It did not receive funding and thus some of the goals of this research are being continued under this grant.

We are currently microdissecting specific chromosome bands to generate DNA probes for use in the molecular analysis of chromosome rearrangements. We have provided probes to Dr. Raju Chaganti (Memorial Sloan-Kettering Cancer Center) from an HSR in a germ cell tumor cell line that he has in tissue culture as well as probes from the end of 6q for Dr. Dalla Favera at Columbia University Medical Center. We are also obtaining probes for the inv(3) and inv(16) neither of which has been cloned at present. The details of the microdissection and PCR amplification were presented in abstract form at the International Congress of Human Genetics (October 1991) and a manuscript has been accepted for publication in Genomics.

III. *PUBLICATIONS*

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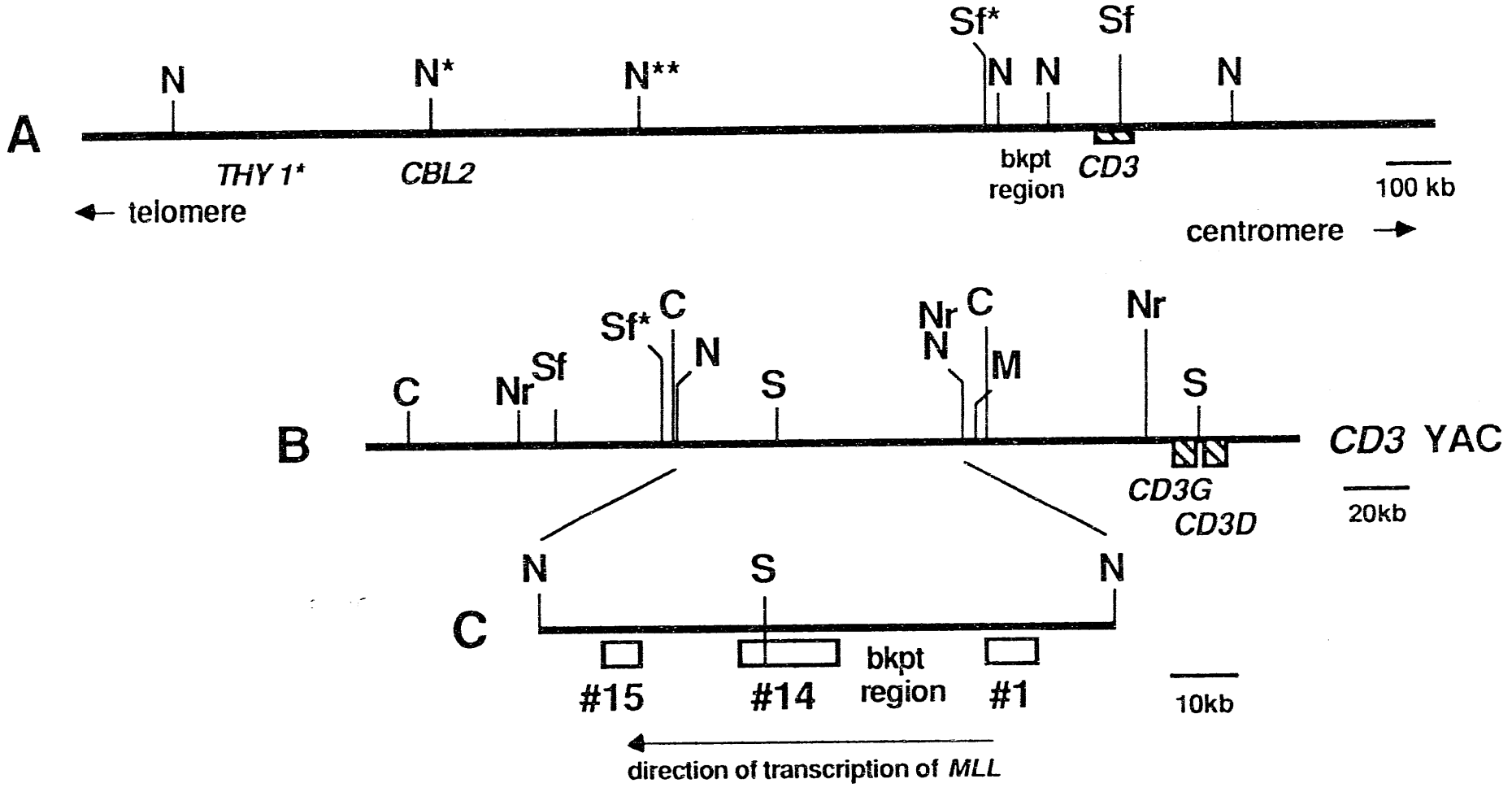


FIGURE 1. Restriction map depicting sites of rare cutting enzymes. S=Sacll; Sf=Sfll; N=Notl; C=Clal; Nr=Nrul; M=Mlul. A, Long range map of the 11q23 chromosomal region showing the relative positions of THY1, CBL2 and CD3. The 11q23 breakpoints are telomeric to the CD3 gene loci and centromeric to the Sf* site. B, Map of the CD3 YAC. Several rare enzyme sites are clustered with the two Notl sites. The translocation breakpoint is between these two Notl sites. C, Map of the 92 kb Notl restriction fragment that contains the 11q23 breakpoint region. #1,#14, and #15 designate subclones from the YAC clone that map within this Notl fragment. The arrow indicates the direction of the transcript, hybridizing to the three subclones, that spans the breakpoint region.

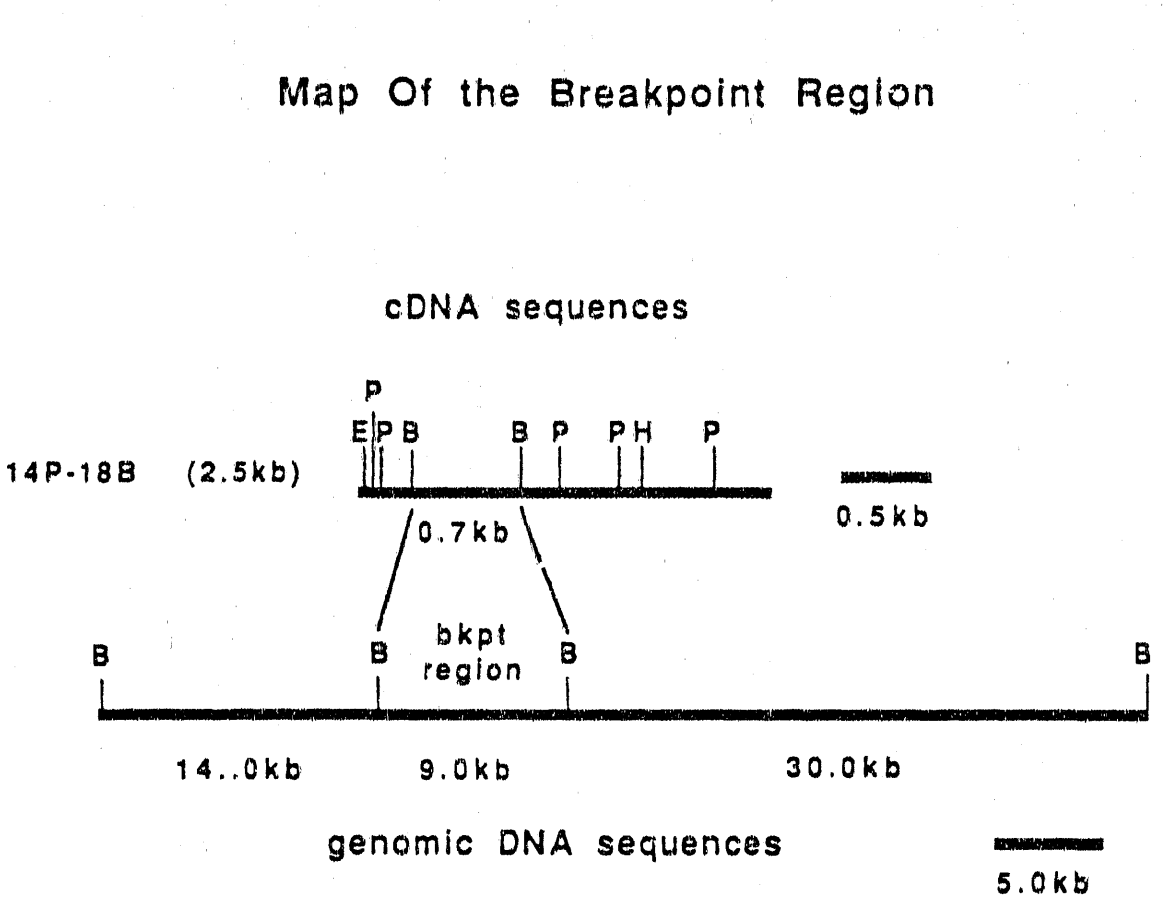
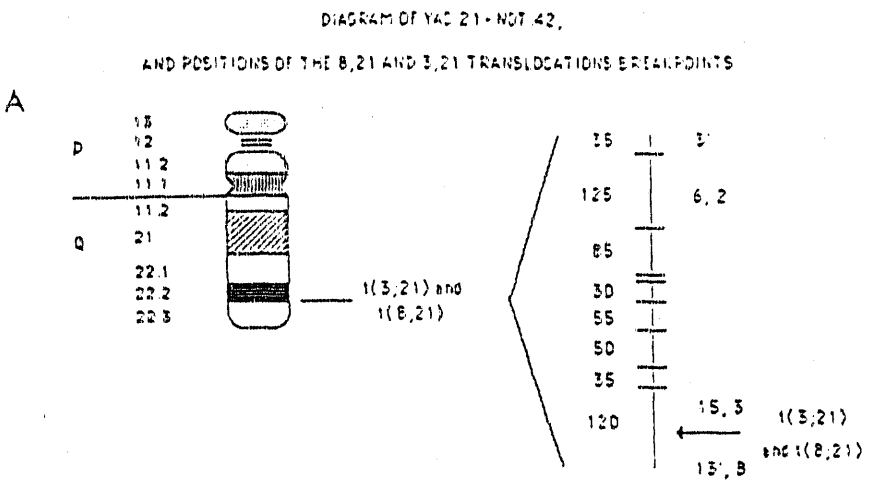


Figure 2. Diagram of one of the cDNA clones isolated with a probe from the centromeric position of clone 14. An 0.7 kb subclone detected DNA rearrangements on Southern blot analysis of all patients summarized in Table 2. This cDNA subclone is contained within a 9kb Bam fragment that is just centromeric to clone 14.



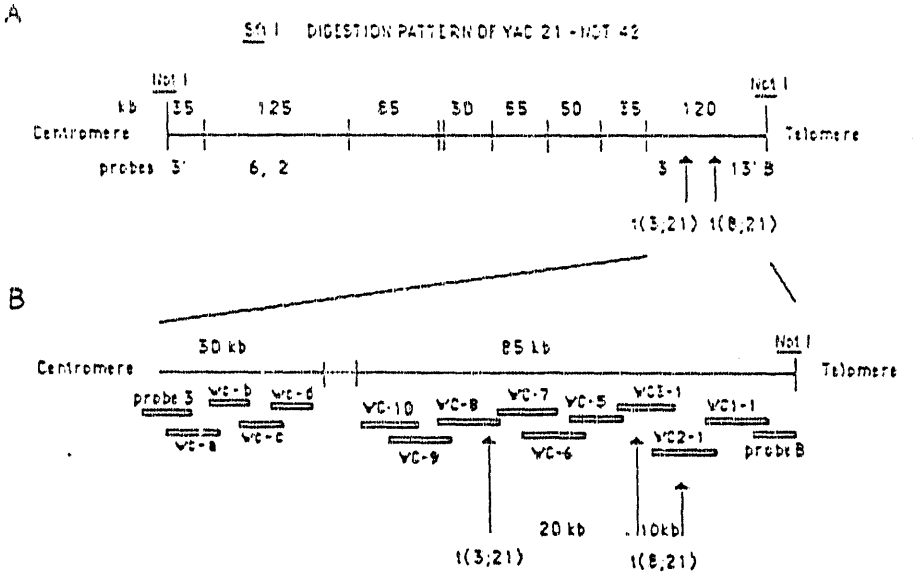


Figure 3 (A) Diagram of chromosome 21 showing the approximate position of the 21q22.3 breakpoint, and to the right the tentative position of some of the *Sfi*I sites present in the YAC. The size of the fragments are indicated to the left of the vertical line and the probes are listed to the right. (B) Map showing the position of phage clones used as probes for FISH. The location of the clones was obtained by pulsed field gel electrophoresis and Southern blot hybridization of *Sfi*I restricted YAC DNA. The position of t(8;21) in two AML patients with the t(8;21) is also shown (ref. 3), as well as the location of the t(3;21) in two patients with t-MDS and in one with t-AML and the t(3;21).

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8/24/92