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Structural alterations of the BCR and ABL genes in Ph1 positive acute leukemias with rearrangements in the BCR gene first intron: further evidence implicating Alu sequences in the chromosome translocation

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ABSTRACT

In the Philadelphia positive bcr negative acute leukemias ($Ph1^+bcr^-$ AL), the chromosomal breakpoints on chromosome 22 have been shown clustered within 10.8kb (bcr2) and 5kb (bcr3) fragments of the first intron of the BCR gene. We previously reported that the breakpoints were localized in Alu repeats on chromosomes 9 and 22 in a $Ph1^+bcr^-$ acute lymphoblastic leukemia with a rearrangement involving bcr2. Molecular data of two other Ph1 translocations, one a $Ph1^+bcr^-$ acute myeloblastic leukemia in the bcr2 region, and the other an acute lymphoblastic leukemia in the bcr3 region are presented. In the former, the breakpoints on chromosomes 9 and 22 are localized in Alu repeats, in regions with two inverted Alu sequences, as in our previously reported case. In the second leukemia, the breakpoints are not located in Alu sequences, but such repeats are found in their vicinity. The implications of these findings are discussed.

INTRODUCTION

The Philadelphia chromosome (Ph1), hallmark of chronic myelogenous leukemia, is present in 5 to 20% of acute lymphoblastic leukemias (ALL) and in rare cases (1 to 2%) of acute non lymphoblastic leukemias (ANLL) (1). About 50 percent of the $Ph1^+$ acute lymphoblastic and nonlymphoblastic leukemias display rearrangements of the classical breakpoint cluster region (bcr), that is situated in the middle of the BCR gene, and associated with chronic myelogenous leukemia (CML). No bcr rearrangements are detected in the other half of the cases (also called $Ph1^+$, bcr^- ALL and ANLL) (1,2). The 5′ part of the BCR gene which mainly includes the very large (68kb) first intron of the BCR gene, has recently been cloned (3−5). In $Ph1^+bcr^-$ acute leukemias, it has been shown that the leukemic cells express a 7 kb chimeric mRNA encoding a 190 kb abnormal ABL protein displaying overt tyrosine kinase activity. In most of the cases studied either by molecular cloning or by polymerase chain reaction (PCR), the first exon of the BCR gene is spliced to the second exon of the ABL gene. The absence of bcr rearrangement in these cases has suggested that the BCR-ABL gene recombination must be located in a region more 5′ to the classical bcr and most probably in first intron of the BCR gene. This was confirmed by recent reports on genomic BCR gene rearrangements in $Ph1^+bcr^-$ acute leukemias, as a BCR gene first intron rearrangement was detected (3−8). Interestingly, the breakpoints in these cases are not randomly distributed, as they are all located in the 3′ half of the first BCR intron, and two clusters, previously named bcr-2 and bcr-3, separated by about 10 kb, are visible (9).

The clustering of breakpoints within the BCR gene in $Ph1^+bcr^-$ acute leukemias suggests particular DNA features favoring the recombination event. In a recent report of a $Ph1^+bcr^-$ ALL case having a bcr-2 rearrangement (10), we showed that the human

repetitive Alu sequences were directly implicated in the chromosome recombination and that inverted Alu sequences were found in close vicinity to the involved Alu sequences. We suggested that the possibility that a tertiary DNA configuration associated with some Alu repeats may be important to mediate the recombination process. Interestingly, in a $Ph1^+bcr^-$ ALL case reported by others (7), the breakpoint on chromosome 9 was localized in one of the reverted Alu repeat as described in our report. To go further, we analysed the breakpoints in two other cases with rearrangements located in bcr2 and bcr3, respectively. Again, particular Alu repeat structures were observed adjacent to the breakpoints, thus, providing further evidence to our initial hypothesis. We have also demonstrated that the abnormal DNA recombination took place between the first intron of the BCR gene and the first intron of the c-ABL gene in these two cases. We suggest that the first intron of the c-ABL gene is probably the major target of the translocations found in $Ph1^+$ bcr^- acute leukemias, as it is in CML.

MATERIALS AND METHODS

Patients

The clinical data of the two adult patients studied in this paper were described in detail elsewhere (SA, case No.6 and NA, case No.11 in 2). It is worth noting that the patient SA was a morphologically typical acute non-lymphoblastic leukemia (M4 in the FAB classification) with involvement of both myeloid and monocytic lineages. The patient NA(case No.11) was diagnosed as having typical ALL-L2. The cytogenetic study at the presentation of the disease showed the presence of a typical t(9;22)(q34;q11) translocation in both cases.

DNA analysis

The standard Southern technique was performed as described previously (11).

Molecular cloning

Genomic DNA libraries were established in EMBL3 phage clones according to the standard protocols (12) for patient NA and for the cloning of the chromosome $9q^+$ in patient SA. Due to the shortage of material, we have adopted another strategy to clone the breakpoint on chromosome $22q^-$ in patient SA. Twenty μg DNA were digested with BglII to complete digestion and size-fractionationed on agarose gel. The region containing DNA from 6.5 to 8 kb was cut and DNA were recovered with the Geneclean Kit (Bio 101 Inc., La Jolla) and ligated with EMBL3 arms. The libraries were screened with appropriate molecular probes and the restriction maps of the selected phage clones established with a series of double digestions.

DNA sequence analysis

Relevant DNA fragments were subcloned into the M13 vectors and the DNA sequences were established according to Sanger et al. (13) using the T7 sequenase kit (United States Biochemical Corporation Inc., Cleveland). The universal M13 sequencing primer and sometimes the specific oligonucleotide primers were used to drive the DNA synthesis. Most of the DNA sequences, in particular those which are discussed in detail below, were obtained in both orientations.

Probes

DNA probes specifically recognizing the different regions of BCR gene, including bcr-2, bcr-3 and probes covering the c-ABL gene second intron and the 3′ end of the first intron were used. The precise positions and sizes of these probes are shown in figures 1 and 3. New probes for a previously non described c-ABL first intron region were generated

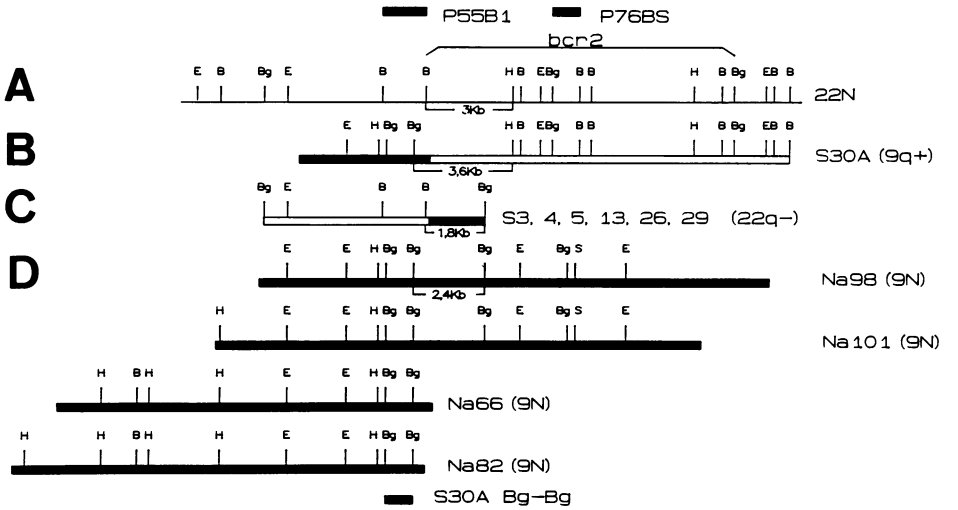


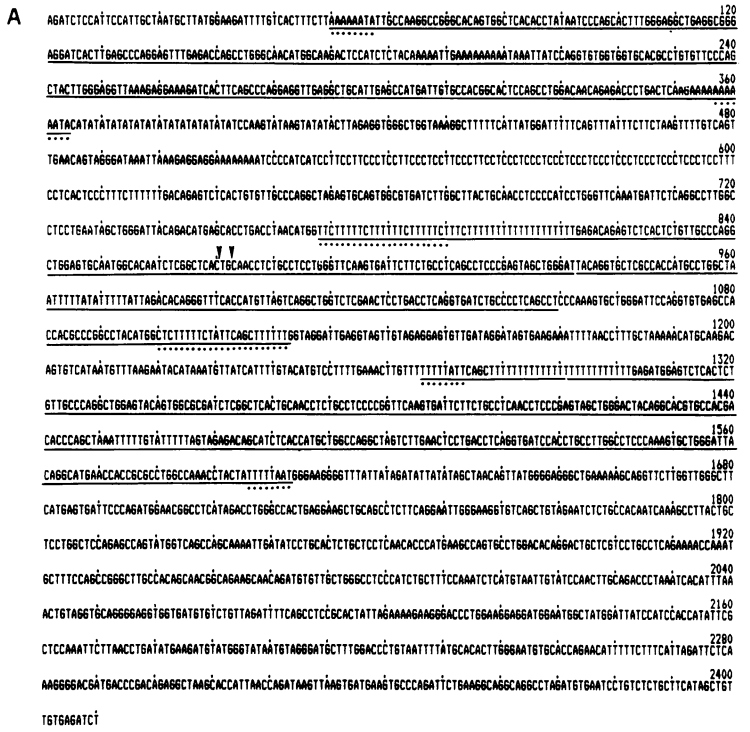
Figure 1. Restriction maps of the phage clones corresponding to chromosomes $9q^+$ (**B**) and $22q^-$ (**C**) in patient SA, and to their normal counterparts chromosomes 9 and 22, 9N (**D**) and 22N (**A**). The positions of the probes used (P55B1, P76BS and S30ABg-Bg) are indicated by black bars. The DNA regions subcloned for sequence analysis are bracketed under the restriction maps. B: BamH1; Bg: BglII; E: EcoRI; H: HindIII.

during the molecular cloning of the normal chromosome 9 counterpart in patient SA, as shown in figure 1. Probes were ^{32}P-labelled by random priming (14) to obtain a specific activity of $1-2\times10^9$ cpm per μg DNA.

RESULTS

1. Molecular analysis of the chromosomal breakpoints in a $Ph1^+bcr^-$ ANLL (patient SA) with bcr2 rearrangement

The breakpoint on chromosome 22 was previously mapped within a 3.0kb BamHI-HindIII fragment within the bcr2 region in patient SA (4,9). To clone the reciprocal translocation on chromosomes $9q^+$ and $22q^-$, two probes, namely P55B1 and P76BS, located on chromosome 22 either side of the breakpoint, were used to screen the genomic DNA library. A positive EMBL3 phage clone (S30A) was selected, which hybridized only with the probe P76BS located downstream of the breakpoint on chromosome 22 (Figure 1). Restriction map analysis showed that its 3′ portion was identical to the corresponding unrearranged region while its 5′ portion, upstream of the Hind III site flanking the 3kb BamHI-HindIII fragment, diverged from the normal counterpart. This clone was thus very likely issued from the chromosome $9q^+$. A 0.7kb BglII-BglII fragment, subcloned from S30A (shown at the bottom of Figure 1), detected the well known amplified signal in DNA of the CML cell line K562 on Southern blot (data not shown), further supporting its chromosome 9 origin. As no rearrangement was detected in DNA from SA with probes covering about 20kb of the 3′ end of the first intron, the second intron and the common exon regions of the c-ABL gene, this breakpoint must be located in the first intron of this gene. Moreover, it should be situated in a region downstream of the breakpoint in the K562 cell line, recently mapped to the 5′ half of the first intron, and at least 20kb upstream of the exon Ia. The


A
B
C
22N
22q-
9q+
9N

0.7kb BglII-BglII fragment was subsequently used as probe for screening another human genomic library. Four positive clones were obtained as shown in Figure 1.

The reciprocal translocation on chromosome 22q$^-$ was obtained in a genomic library with BglII DNA fragments as described in Materials and Methods. Six positive clones were obtained by screening this DNA library with the probe p55B1. The restriction map of this 7.2kb fragment is shown in Figure 1. Since the restriction maps of all clones showed perfect overlapping at the regions upstream and downstream of the breakpoints, we concluded that we have cloned the reciprocal translocations on both chromosomes 9 and 22.

DNA sequencing of the reciprocal translocation showed the following results. On chromosome 22, the breakpoint was located in the bcr2 region (4). In this region, there is a pair of inverted Alu repeats, 5′ to 3′ belonging to families J and Sb respectively (15,16) and separated by 210bp (Figure 2B). The breakpoint is within the 5′ Alu sequence. On chromosome 9, the breakpoint is located in a 2.4kb BglII-BglII fragment containing three Alu repeats in tandem. The first Alu sequence corresponds to family J and the two others to family Sa. These three Alu repeats contain two intervening sequences of 400bp and 160bp. Interestingly, the orientation of the most 5′ Alu sequence is opposite to that of the middle and the 3′ Alu sequences while the rearrangement is included in the middle Alu sequence (Figure 2A).

Examination of the DNA sequence immediately flanking the breakpoints revealed no obvious homology when the sequence from chromosome 9 and 22 are aligned (Figure 2C), because the breaks are not situated in the same portion of Alu sequence. Moreover, no heptamer-nonamer like sequences are present in close vicinity to the breakpoints. Analysis of the chromosome junctions showed no deletion of nucleotides. The dinucleotides TG were repeated, indicating a duplication mechanism.

2. Molecular analysis of the reciprocal breakpoints in a Ph1$^+$bcr$^-$ ALL with a bcr3 rearrangement (patient NA).

As already described (9), the breakpoint on chromosome 22 in patient NA was located in a region designated bcr3, 16kb upstream of bcr2. In addition, by use of a genomic DNA probe specific for the second intron of the c-ABL gene, the breakpoint on chromosome 9 has been approximately mapped to the 3′ end of the first intron of the c-ABL gene, immediately upstream of exon la (9). Two DNA probes flanking the bcr3 region, namely N26B1 and N26B2 (Figure 3), were used to screen the EMBL3 library established from DNA of patient NA. A total of 19 positive clones were obtained of which eight encompassed the expected breakpoint on chromosome 22. Three clones (Na34, Na63, and Na72) could be revealed by both probes and were therefore derived from the normal chromosome 22. Indeed, their restriction maps are identical to our previously published one. Clones Na88 and Na105 had in their 3′ parts the same restriction map as the corresponding normal region, while their 5′ parts differed from it. Clones Na71, Na78A, and Na110, on the

Figure 2. DNA sequences from normal chromosomes 9 (**A**) and 22 (**B**) flanking the breakpoints (arrows) in patient SA. The open arrow in **B** indicates the chromosome 22 breakpoint previously described in patient BON (see text). Two black arrows indicating the breakpoints represent the alternative 3′ or 5′ staggered cutting. Alu sequences are underlined and their direct repetitive sequences marked by dots. **C**. DNA sequences from the reciprocal translocation, chromosomes 22q$^-$ and 9q$^+$, are aligned with their normal counterparts and the breakpoints indicated by arrows. Note that the nucleotides TG from chromosome 9 are present in both 22q$^-$ and 9q$^+$ chromosomes. A gap of two nucleotides is introduced (**) on normal chromosome 22 sequence in order to respect the alignment of the sequences.

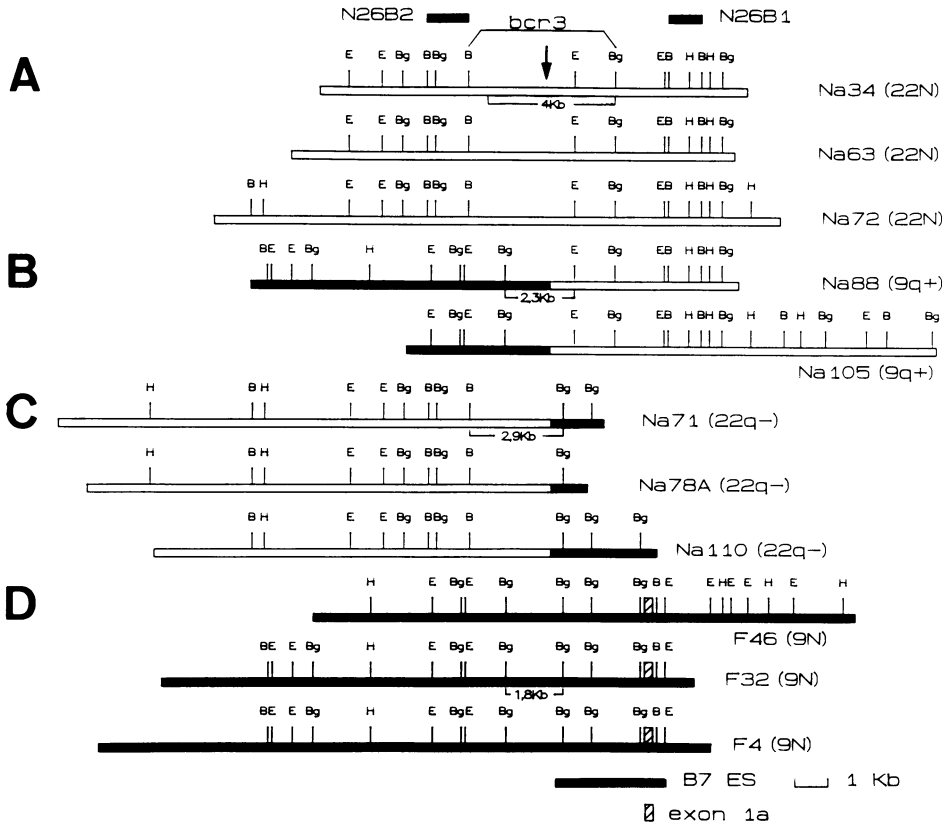


Figure 3. Molecular cloning of the reciprocal translocation, $9q^+$ (**B**) and $22q^-$ (**C**), in patient NA and the corresponding normal 22 and 9 chromosomes, 22N (**A**) and 9N (**D**). DNA probes used for library screening (N26B2, N26B1 and B7ES) are shown as black bars. The DNA regions subcloned for sequence analysis are shown under each restriction map. B: BamH1; Bg: BglII; E: EcoRI; H: HindIII.

contrary, exhibited the normal restriction map in their 5′ portion whereas the 3′ extremities diverged from the normal map. With a probe generated from the previously reported clone B7 (10) containing the exon 1a of the c-ABL gene, we have obtained a group of phage clones from another genomic DNA library established from normal fibroblasts (designated F). This allowed us to extend the published restriction map of the 3′ end of the ABL first intron about 19kb 5′, and this map should include the breakpoint on chromosome 9 in patient NA. In fact, the 5′ portion of clones Na88 and Na105 and the 3′ end of clones Na71, Na78A, and Na110 reconstitute exactly the same restriction map as the corresponding normal region. Moreover, the probe B7ES could hybridize to the 3′ ends of clones Na71, Na78A and Na110 (data not shown). These results taken as a whole show unequivocally that we have cloned the reciprocal translocations in patient NA, with the breakpoint on chromosome 9 being precisely localized 3 kb upstream of exon 1a of the c-ABL gene.

To explore the possible molecular mechanism responsible for this abnormal DNA recombination, the sequences were established for the 4kb bcr-3 region containing the breakpoint on chromosome 22, and the 1.8kb BglII-BglII fragment containing breakpoint

on chromosome 9 as well as the reciprocal chromosome translocations (Figure 4). The breakpoints on both chromosomes correspond to relatively precise fusion, as only a stretch of six bases (GGAATT) and of four bases (CACA) were deleted from chromosomes 9 and 22, respectively. No nucleotides were added at the junction. When the sequences flanking the breakpoints are aligned for homology comparaison, no apparent homology can be observed (Figure 4C). Research for heptamer-nonamer signals also revealed a negative result. On the other hand, we found Alu repeats near the breakpoints. There are two identically oriented Alu sequences both belonging to the family Sa, 5′ of the breakpoint on chromosome 9, and separated from the breakpoint by a distance of approximately 220bp whereas no Alu sequence is observed 3′ of the breakpoint. While the first one is a complete Alu sequence, the second contains only one of the two subunits. In addition, this truncated Alu sequence is flanked by two inverted complementary sequences of 22bp (ATTTTTTTTTTTTTTGAGATGG and CCATCTCAAAAAAAAAAAAAAT) (Figure 4A). On chromosome 22, an Alu sequence is located 3′ of the breakpoint, with a distance of about 150bp. This Alu sequence belongs to family Sa and is also flanked by complementary sequences (TTTCTGTCTCTTC------TCGGGAGGG and AAAGAGAGAGAAG---AGCCCTCCC) (Figure 4B).

In the course of cloning chromosome $9q^+$ in patient NA, we have obtained a phage clone, Na19, which encompasses a region between bcr3 and bcr2. The first EcoRI site 5′ to bcr2 is absent and it corresponds to a 0.7kb deletion. This polymorphism is the same as recently described (5).

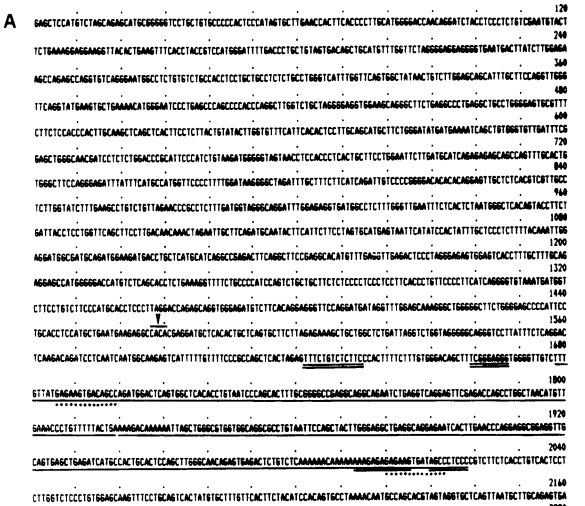
DISCUSSION

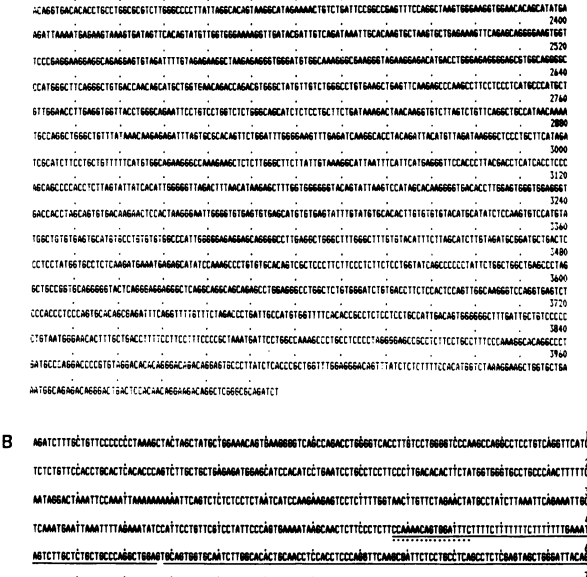
In $Ph1^+$ acute leukemias, three chromosomal breakpoint cluster regions have been described, bcr in the middle of the BCR gene, bcr2 and bcr3 in its first intron. As already described in CML, the clustering of the breakpoints in bcr may be biologically driven, because only the exons 13 and 14 of the BCR gene (i.e. the bcr exons 2 and 3 can) be joined to C-ABL 'common' exons to maintain the open reading frame (17). The BCR first intron is of huge size. Thus, any rearrangement in any region of this first intron should not interfere with the open reading frame formed by its joining with the c-ABL 'common' exons. The striking clustering of the breakpoints within the 3′ half of the BCR gene first intron in $Ph1^+$ bcr^- AL suggested that particular DNA sequences may be present in these regions facilitating the abnormal somatic DNA rearrangement.

Recently, we have analyzed the chromosomal rearrangement in one $Ph1^+bcr^-$ ALL (case BON, 10) with a breakpoint on chromosome 22 in the minor cluster bcr-2. Two inverted Alu repeats are present in the vicinity of the breakpoints on both chromosomes 9 and 22, and the breakpoints are within Alu repeats. This structural organization led us to propose a model with a double hairpin accessible to an enzymatic complex, capable of DNA cutting and ligation (6). Interestingly, in another case (FY) reported by van der Feltz et al. (7), although no Alu sequence was found around the breakpoint on chromosome 22, the breakpoint on chromosome 9 was in one of the two inverted Alu repeats implicated in the case BON. In order to test the proposed model we have analyzed in the present study two other cases (SA and NA) with chromosome 22 rearrangements in bcr2 and bcr3, respectively.

1. Particular Alu sequences mediating chromosomal translocation

Comparison of the sequences flanking the breakpoints in four cases of $Ph1^+$ bcr^- AL demonstrated the presence of relatively conserved sequences, i.e Alu sequences in or





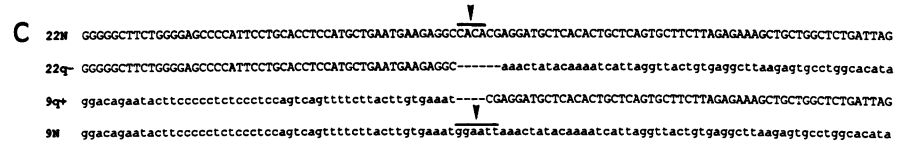


Figure 4. DNA sequence of the 4.0kb bcr3 region on chromosome 22 (**A**) and the 1.8kb BglII-BglII region on chromosome 9 (**B**), including the breakpoints (arrows) in patient NA. The Alu sequences are underlined and the direct repetitive sequences dotted. The complementary sequences flanking the Alu repeats adjacent to breakpoints on both chromosomes 9 and 22 are marked by double lines. The four bases deletion on chromosome 22 and the six bases deletion on chromosome 9 are indicated by lines above the sequences. The alignment of rearranged sequences ($22q^-$ and $9q^+$) and their normal counterpart are shown in **C**. Deleted nucleotides have been replaced by dashes to permit the alignment.

adjacent to the breakpoints in 7 out of 8 chromosomes. On the only chromosome where no Alu repeat was noted (case FY), a sequence of only 230bp was available in the vicinity of the breakpoint.

In patient SA, the rearrangement on chromosome 22 resides in the same region, namely bcr2, as that in the case BON. As shown in Figure 2B, this region is characterized by the presence of two inverted Alu sequences and the breakpoint in case SA was within the first Alu sequence, whereas it was located in the second Alu repeat in case BON. Strikingly, the breakpoint on chromosome 9 also resides within inverted Alu repeats although it took place in a different region from that in case BON. The distribution of the Alu sequences in Alu families shows a certain symmetry in case SA (3′ Alu_J 5′−5′ Alu_{Sb} 3′ on chromosome 22, 5′ Alu_J 3′−3′ Alu_{Sa} 5′−3′ Alu_{Sa} 5′ on chromosome 9). This result thus extends our initial hypothesis of hairpin structures surrounding breakpoints and is reminiscent of the structure found in case FY (7).

Breakpoints in Alu repetitive sequences on chromosomes 9 and 22 have already been reported to occur in CML (17−19) and two inverted sequences have also been found on chromosome 9 in a recently published case (20). Because recombinations between Alu sequences have also occurred in non CML recombination events, the authors suggested that Alu repetitive sequences could be hot spots for recombination.

The situation in case NA, with a bcr3 rearrangement is somewhat different, as the breakpoints on both chromosomes did not occur within Alu sequences but in their vicinity. One Alu sequence is present 3′ of the breakpoint on chromosome 22, and two are 5′ of the break on chromosome 9. These sequences are orientated in a rather symmetric fashion (5′ Alu_{Sa} 3′ on chromosome 22 and 3′ Alu_{Sa} 5′−3′ truncated Alu_{Sa} 5′ on chromosome 9). Interestingly, the Alu repeats adjacent to the breakpoints are flanked by some complementary motifs (Figure 4). We speculate that these motifs may facilitate the site specific formation of the hairpin structure and change the tertiary DNA structure in the vicinity. The presence of these structures at one side of each chromosome breakpoint might provide some symmetrical topology recognized by the enzymatic complex involved in DNA recombination. With regard to this model, the Alu sequences may be important to form a 'pocket' that accommodates the protein complex, but are not always themselves the target of DNA excision. The confirmation of this hypothesis needs the characterization of this protein complex and illustration of its mode of action.

2. Characteristics of the DNA joining

A constant feature of the chromosome joining in the four $Ph1^+bcr^-$ acute leukemias currently analyzed, is the almost perfect conservation of the original DNA sequences. This

is however not always verified in CML in that frequent large DNA deletions of the classical bcr (21−23) have been reported. Therefore, the mechanism of translocation may be somewhat different between CML and $Ph1^+bcr^-$ acute leukemias.

It has been claimed that chromosomal rearrangements of some lymphoid malignancies resulted from the recombinase activity physiologically involved in the normal V-(D)-J assembly of the immunoglobulin superfamily genes (24). This hypothesis was based on the finding of hepatamer-nonamer recombination signal sequences in the vicinity of chromosomal breakpoints and of the presence of N sequences. This mechanism seems not to be responsible for the chromosomal translocations associated with the $Ph1^+$ leukemias since no characteristic heptamer-nonamer signal sequences have been identified. The sequence GAGG, adjacent to the c-myc-IgH gene translocation in some rat plasmacytomas has been found at the breakpoint in case FY (7). This sequence is however not present in all breakpoints examined in our cases. This sequence has also been claimed to be involved in immunoglobulin gene switching and its implication in the chromosome translocation seems to be correlated with a relatively mature differentiation stage of B cell tumors. As Ph1 positive acute leukemias represent a malignant proliferation of cells of very early lymphoid or pluripotent hematopoietic stem cell origin, implication of IgH switching as the mechanism of chromosomal translocation seems unlikely.

In case SA, the duplication of two nucleotides (TG) at chromosome junction is noticeable. This addition is apparently different from the N region addition during the V-(D)-J gene assembly of the immunoglobulin superfamily genes, as the two TG bases added exist in the normal chromosome 9 sequence. A similar situation was observed in the case FY(7) where a stretch of six bases was duplicated. A plausible explanation for this duplication is the staggered cutting of the double-stranded DNA chain, with the extruding extremities being filled before or even after ligation (25). It should be pointed out that patient SA had a $Ph1^+bcr^-$ ANLL while patient FY had a $Ph1^+bcr^-$ ALL. This similarity regarding the molecular basis of the illegitimate DNA recombination further supports the notion that the $Ph1^+bcr^-$ ALL and ANLL are very closely related diseases, as suggested previously by phenotypic data, localization of breakpoints and production of $P190^{bcr-abl}$ fusion protein (1,2).

In case NA, deletion of six base pairs from chromosome 22 and of four from chromosome 9 was detected. Loss of some base pairs has also been reported in CML (20).

3. c-ABL gene first intron as the major target of chromosome translocation in $Ph1^+bcr^-$ acute leukemias.

The mapping of the breakpoints in the c-ABL gene is often difficult, due to its very large size (230 kb), and especially that of its first intron (200 kb). In the few CML patients and the two CML-derived cell lines (K562 and BV173) studied (26), the breakpoints were located in the second intron, in the first intron, or even in a region upstream of exon lb of the ABL gene. As a consequence, a long-range splicing mechanism is responsible for the fusion of the BCR exon 13 or 14 with the common exons of ABL (26). In $Ph1^+bcr^-$ acute leukemias, the BCR gene exon 1 is consistently spliced to the ABL gene exon 2. This may be explained by a preferential clustering of the breakpoints in the ABL second intron, 5′ to exon 2 of the ABL. Although the breakpoints on chromosome 9 of three recently reported $Ph1^+$, bcr^- ALL were all localized in the second intron of the ABL gene, the breakpoints of the two cases characterized in the present report reside in the first intron of the gene. In case NA, the rearrangement was mapped just at the 3′ end of the first intron, and in case SA, it was localized in a considerably further 5′ region

in the same intron. Finally, only one out of eight Ph1$^+$bcr$^-$ acute leukemia cases studied in our laboratory, had the breakpoint in the ABL second intron (9), while the translocation mapped in the first intron in at least three other cases. In the remaining cases, no rearrangement was detected in the second intron or the more 3′ part of the c-ABL gene, and the breakpoints were probably also located in the first intron, or in a more 5′ part of the gene. We conclude that, as in CML, there is a wide distribution of breakpoints in the ABL gene in Ph1$^+$bcr$^-$ acute leukemias, and the ABL gene first intron represents the major target for the translocations. It follows that, in these cases, the long range splicing mechanism observed in CML which eliminates exon la of ABL, should also be applicable.

It has been claimed that the chromosomal breakpoints are preferentially located in the BCR intron 1 in childhood Ph1$^+$bcr$^-$ ALL, while in adults, the breakpoints are predominantly located in the bcr segment. This difference in breakpoint distribution within the BCR gene in Ph1$^+$ AL in the two classes of age is not satisfactorily explained, and it has been postulated that Ph1$^+$bcr$^+$ AL may represent acute-stage CML (27). On the contrary, some Ph1$^+$ leukemias with breakpoints in the bcr segment have been classified as genuine acute leukemias on the basis of their clinical evolution and of their cytogenetic patterns (2). Nevertheless the localization of breakpoints in Alu repeats occurs in both bcr$^+$ and bcr$^-$ leukemias. Sequencing of the breakpoints in more cases of leukemias is obviously needed to estimate their frequency. Furthermore, the identification of the enzymatic system responsible for illegitimate recombination resulting in the Ph1 translocation will constitute a significant advance to understand the mechanism of its genesis.

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