Volume 16 Number 21 1988 Nucleic Acids Research

The first BCR gene intron contains breakpoints in Philadelphia chromosome positive leukemia

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Received August 15, 1988; Accepted September 28, 1988

ABSTRACT

The hallmark of chronic myelogenous leukemia (CML) is a translocation between chromosomes 9 and 22 - the Philadelphia (Ph') translocation. The translocation is also found in acute lymphocytic leukemia (ALL) albeit in a lower percentage of patients. The breakpoint on chromosome 22 is located within the BCR gene: in CML, breakpoints are clustered within 5.8 kb of DNA, the major breakpoint cluster region (Mbcr). In ALL, breakpoints have been reported within the Mbcr but also in more 5' regions encompassing the BCR gene. To characterize the latter breakpoints, we have molecularly cloned and mapped the entire gene, which encompasses approximately 130 kb of DNA. Mbcr negative, Ph'-positive ALL breakpoints were not distributed at random within the gene but rather were found exclusively within the 3' half of the first BCR gene intron. In contrast to the Mbcr, which is limited to a region of 5.8 kb, this part of the intron has a size of 35 kb. Translocation breakpoints in this region appear to be specific for ALL, since it was not rearranged in clinically well-defined CML specimens nor in any other tumor DNA samples examined.

INTRODUCTION

The Philadelphia (Ph') chromosome is an abnormal chromosome 22 found in the leukemic cells of chronic myelocytic leukemia (CML) (1) and acute lymphoblastic leukemia (ALL) patients. This chromosome is the result of a reciprocal translocation between chromosomes 22 and 9 (2,3). The Ph'-chromosome is highly specific for CML, as more than 90% of patients have this acquired abnormality (4). It is found less frequently in ALL patients; in adults, the incidence is 17-25% (4,5) and in children 2-6% (6,7).

Breakpoints as a result of this chromosomal translocation are found on chromosome 9 at band q34 and on chromosome 22 at band q11. The ABL oncogene is located at the breakpoint on chromosome 9; the main part of the gene is translocated to the Ph'-chromosome (3,8). In CML, the breaks on chromosome 22 occur within a small region of 5.8 kb of DNA of the BCR gene (9). This major breakpoint cluster region (Mbcr) actually encompasses 5 exons of the gene, with the breaks occurring in introns (10). The translocation results in the generation of a chimeric BCR/ABL gene, which is transcribed (11,12) and translated into an abnormal protein, P210 (13,14).

The P210 represents an "activated" form of the ABL gene product (15,16): the normal ABL protein P145 is a tyrosine-protein kinase with little or no autophosphorylation activity

in vitro (15); in contrast, P210 is readily phosphorylated by itself in vitro (17). It seems likely, that the addition of amino-terminal BCR residues and/or deletion of ABL amino-terminal domains is responsible for this effect. The abnormal P210 is not capable of transforming NIH 3T3 fibroblast cell lines (10); however, in cultured bone marrow cells expression of P210 is growth-stimulating but not sufficient to cause full transformation (19). Since the Ph'-translocation is consistently found in CML, it is thought that the P210 must somehow be involved in the genesis, maintenance and/or progression of the disease.

The Ph'-positive ALL patients appear to fall within two categories: those that have a breakpoint within the Mbcr of the BCR gene and those that lack any detectable rearrangements in this region (20,21, 22). Patients having an Mbcr breakpoint produce a P210. In the second class of patients, an abnormal ABL protein of 190,000 molecular weight has been detected; this P190 also has an autophosphorylation activity (23,24,25) The demonstration of antigenic determinants of an ABL and a BCR moiety within P190 (26) and the molecular cloning of chimeric BCR/ABL cDNAs (27,28) proved conclusively, that both ABL and BCR are involved in this translocation.

In contrast to CML, the breakpoints in two of these Ph'-positive, Mbcr negative ALL DNAs were found at a considerable distance 5' of the Mbcr (27), in what most probably constitutes the first BCR gene intron. The exact position of the first intron had not been defined, since only the 3' end of the genomic BCR gene had been cloned and characterized (9). However, to examine the entire gene for ALL breakpoints, it now became necessary to molecularly clone the complete gene. In this study we present the cloning of the entire BCR gene and the location of Ph'-positive, Mbcr negative ALL breakpoints within it. Our results indicate, that these breakpoints are not clustered to the degree, which is found in CML; however, all breakpoints analyzed to date occur within a region of approximately 35 kb constituting the 3' half of the first BCR gene intron.

MATERIALS AND METHODS

Isolation of cosmids

Cosmids were isolated from a K562 cosmid library (29) as described. Repeat-free probes were prepared as follows: ends of the cosmid clones were subcloned and mapped. Southern blots of the subcloned regions were hybridized to a total human DNA probe; post-hybridization washings were at 2.5 X SSC at 65°C. Fragments not hybridizing to the probe were tested on genomic blots to assure that they were indeed repeat-free. Restriction enzyme maps of the cosmids were constructed by double digestions and by through-digestion of individual fragments isolated from low melting point agarose gels (IBI).

Field inversion gel analysis

Blocks of DNA were prepared as described (30). Restriction enzyme digestions were for 12-15 hrs with 30 units of enzyme per block; each block contained between 6-10 µg of DNA. Gels (1%, 250 ml, 20 x 25 cm) were preelectrophoresed for 30 min. at 200V, then for

24 hrs with a forward pulse increasing linearily from 3-120 seconds and an increasing backward pulse of 1-40 seconds. Electrophoresis buffer (0.5 X TBE) was cooled to 15°C. After electrophoresis, gels were stained with ethidium bromide to localize the yeast chromosomal (Beckman) and λ ladder markers. To reduce the size of the high molecular weight DNA, gels were placed for 10 minutes on a 302 nm UV box. After denaturation and neutralization according to Southern (31) gels were blotted to Nytran overnight. The filters were dried completely; the DNA was cross-linked to the matrix by placing the dried membrane, DNA side down, on a 302 nm UV box for 4 minutes. Probes were labelled by primer extension (32). Hybridizations were performed as described (33) Exposure times varied from 4 hrs to 2 1/2 days at -80°C using X-AR film and an intensifying screen.

5-azacytidine treatment

Cells ($8x10^7$) were grown for 5 days in the presence of 3μm 5-azacytidine (Calbiochem). Culture medium was replaced every 2 days and fresh 5-azacytidine was added.

Probes

Probe 1 is a 0.6 kb XhoI/Bgl II fragment, probe 2 a 0.55 kb Hind III/EcoRI fragment, probe 3 a 0.6 kb EcoRI/AvaI fragment, probe 4 a 0.6 kb KpnI/BstEII fragment, probe 5 an 0.8 kb Bam HI fragment, probe 6 an 0.6 kb Eco RI/SstI fragment, probe 7 an 1.0 kb BamHI fragment, probe 8 a 1.15 kb Bam HI fragment and probe 9 a 0.59 KpnI/PstI fragment. Probes (50 ng of each) were combined for primer-extension labelling. The Cλ probe was a previously (8) described 1.2 kb HindIII/BglII fragment.

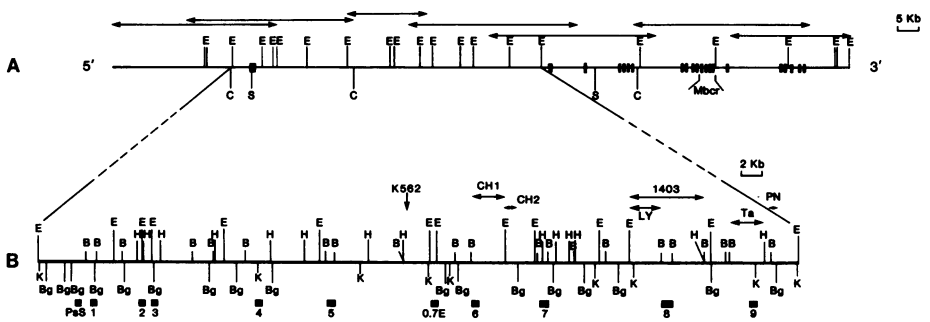

Fig. 1. Restriction enzyme map of the BCR gene.
A restriction enzyme map of the entire BCR gene is shown in A. Horizontal arrows above the map indicate the extent of overlap of the cosmids; not all cosmids isolated are shown. The approximate positions of the exons are indicated with solid boxes; the location of the major breakpoint cluster region (Mbcr) in CML is also indicated. The first intron is shown in more detail in B. Probes used in this study are indicated beneath the map. The approximate location of the breakpoints of the ALL patients is shown above the map; a vertical arrow points to a breakpoint in the cell line K562.
Restriction enzymes include: B = Bam HI, Bg = Bgl II, C = Cla I, E = Eco RI, H = Hind III, K = Kpn I, S = Sal I. * E denotes a polymorphic site that is deleted in some normal alleles, together with approximately 1kb of DNA.

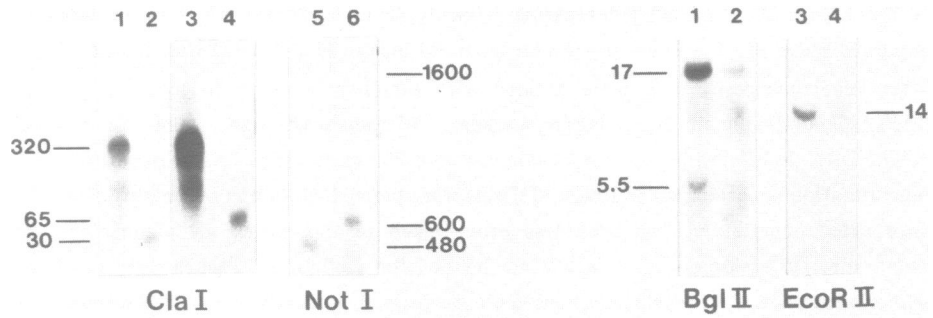

Fig. 2. Southern blot analysis of the BCR gene. Left panel. FIGE analysis of the BCR gene. DNAs were digested as indicated below each panel. Untreated K562 DNA (lanes 1,3 and 6), GM 3420 DNA (lane 5) or 5-azacytidine-treated K562 DNA (lanes 2 and 4) was hybridized to a 5' intron probe (lanes 1 and 2), a 3' intron probe (lanes 3 and 4) or to probe 1 (lanes 5 and 6, see fig 1B).
Right panel. K562 contains a breakpoint in intron 1. K562 (lanes 1 and 3) and control cell line DNA (lanes 2 and 4) were digested with the enzymes indicated and hybridized to a BCR gene intron probe (lanes 1 and 2), or a Bridge-1 cosmid, 5' end probe (lanes 3 and 4).

Sequencing analysis

Sequencing was performed using the dideoxy chain termination method on restriction enzyme fragments subcloned into M13mp18 or 19 (BRL) using ^{35}S dATP.

RESULTS

Cloning of the entire BCR gene

Two mRNAs, of 4.3 and 6.0 kb are transcribed from the BCR gene (11). We and others (34,35) have cloned the 4.3 kb mRNA and it has been sequenced completely. On a genomic level however, only the 3' end of the BCR gene had been cloned; to isolate the entire gene, a chromosomal walk was initiated, starting from these cloned sequences on the 3' end and a BCR gene exon-1 containing cosmid at the 5' end. A cosmid library from K562 cell line DNA was used to isolate additional clones since this cell line contains a 4-5 fold amplification of the BCR gene and the ABL oncogene (8,36, 37). Repeat-free probes from the ends of each clone were isolated and used for further screening; the extent of overlap of the clones was determined by restriction enzyme mapping. As shown in Figure 1A, the entire gene was cloned with overlapping cosmids, spanning a region of approximately 173 kb of chromosome 22.

Since the gene is relatively large and it was of interest to examine this region for DNA rearrangements, the gene was also mapped with the relatively infrequently cutting restriction enzymes Sal I and Cla I; a Not I site was not found within this region. These enzymes may be useful in generating large fragments suitable for field inversion gel electrophoresis (FIGE) analysis (38). To test this, probes prepared from the BCR gene were

hybridized to FIGE Southern blots of K562 DNA. As shown in Figure 2 left panel, lane 1, a probe from the 5' end of the BCR gene (PsS probe, see Figure 1B) hybridizes to a large (approximately 320 kb) Cla I fragment in addition to a number of smaller fragments. A set of 3' probes (probe 6 plus probe 9, Figure 1B) detects the same fragment of 320 kb and a number of other, less well defined fragments. However, the restriction enzyme map of the region predicts, that 30 kb and 65 kb Cla I fragments should hybridize to these probes. A similar discrepancy was observed using the enzyme Sal I. All BCR gene probes hybridized to a Not I fragment of approximately 480 kb in DNA of a control cell line GM 3420 (Figure 2 left panel, lane 5) and weakly to a fragment of approximately 1600 kb. In K562 a breakpoint fragment of around 600 kb was detected (Figure 2 left panel, lane 6). The discrepancy observed here between the distances on our physical map and those calculated from our FIGE analysis with ClaI and SalI could be explained by the methylation of certain restriction enzyme sites, rendering them refractory to digestion. 5-azacytidine has been reported to reduce the degree of methylation in vivo after culturing cells in its presence (39). To investigate, whether the abnormal restriction enzyme patterns were due to methylation, K562 cells were grown in the presence of 5-azacytidine and DNA was prepared. As shown in Figure 2 left panel, lanes 2 and 4, this treatment apparently was effective in removing the methylation: the probes now detected Cla I restriction enzyme fragments of a size predicted from the restriction enzyme map.

K562 contains a second rearrangement on the Ph'-chromosome

K562 is a cell line, established from a CML patient in blast crisis (40). Previously, we have isolated and characterized the breakpoint between chromosomes 22 and 9 in this cell line (29); the breakpoint on chromosome 22 occurs in the major breakpoint cluster region (Mbcr), in the same area, in which the majority of all CML breakpoints are located (see Figure 1A, Mbcr); the breakpoint on chromosome 9 occurs between ABL exons Ib and Ia (41).

In the course of our cloning experiments, one cosmid (Bridge-1) was isolated, which had overlap with BCR genomic sequences at its 3' end but no homology at its 5' end to the corresponding genomic BCR region. The junction between these regions is indicated in Figure 1B with a vertical arrow. A probe prepared from the BCR genomic sequences in this region (0.7 kb E, see Figure 1B) detected a 17 kb Bgl II fragment in control human DNA (Figure 2 right panel, lane 2) and in K562, as would be predicted from the restriction enzyme map. In addition, a 5.5 kb Bgl II fragment is detected in K562; this 5.5 kb Bgl II fragment is present at the junction between the BCR and non-BCR gene segments in Bridge-1. Apparently, a second rearrangement has occurred in one of the 4-5 copies of the BCR/ABL chimeric gene; however, the majority of these copies appear to contain the original BCR/ABL rearrangement, since the 17 kb Bgl II fragment is amplified in K562 in comparison with control DNA (Figure 2 right panel).

The region 5' within cosmid Bridge-1 lacked homology to further 5' BCR gene regions on a restriction enzyme level. However, a probe prepared from the 5' of Bridge-1 does

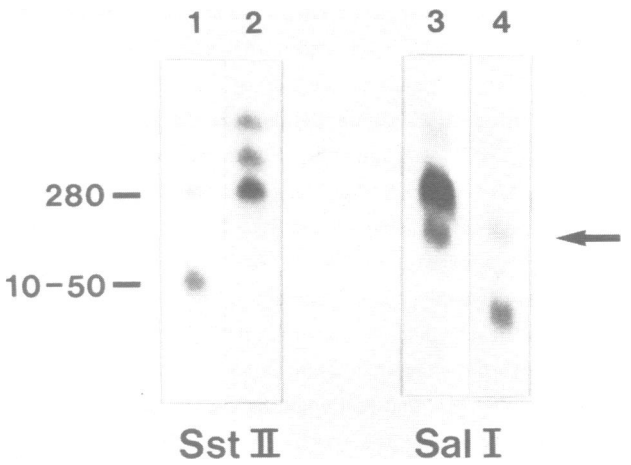

Fig. 3. BCR gene and Cλ are neighbors. DNAs from the cell line GM3420 (lanes 1 and 2) or K562 (lanes 3 and 4) were digested with enzymes as indicated below each panel; the blots were hybridized to a BCR (PsS) probe (lanes 1 and 3) and subsequently to a Cλ probe (lanes 2 and 4). Sizes of the fragments hybridizing are indicated in kb. An arrow points to the fragment in common in the Sal I digests; neither of the cell lines were treated with 5-azacytidine before DNA isolation.

detect an amplified region in K562 DNA (Figure 2 right panel compare lanes 3 and 4). Therefore, this segment most probably originates somewhere 5' of the BCR gene within the region of chromosome 22 that is amplified in K562: this must include regions 5' of the BCR gene and the immunoglobulin lambda light chain constant region (Cλ) (8,36).

The Cλ region is located in relative proximity to the BCR gene: a probe prepared from the BCR gene promoter region (PsS, see Figure 1B) hybridizes to two Sst II fragments, one of 10-50 kb extending to the 3' and one of approximately 280 kb extending to the 5' (Figure 3, lane 1) in control GM 3420 DNA. Upon rehybridization of the same blot to a Cλ probe, the same 280 kb fragment is detected (Figure 3 lane 2). In addition, in "methylated" K562 DNA digested with Sal I, the Cλ probe and the PsS probe hybridize to a common Sal I fragment (Figure 3 lanes 3 and 4).

The first BCR gene intron is 68 kb

The sequence homology between the first BCR gene exon and the BCR cDNA terminates at the splice donor site 3' to the exon (Figure 4). To delineate the first intron, a BCR cDNA was hybridized to the cloned genomic DNA to localize the second exon. A hybridizing region, located approximately 68 kb 3' to the first exon was subcloned and sequenced. As shown in Figure 4, a splice acceptor and exon 2 are present: apparently, there are no additional exons corresponding to the 4.3 kb BCR mRNA present within the 68 kb of genomic DNA. The first intron is larger in size than the entire remaining BCR

Fig. 4. BCR gene exon 1- intron 1- exon 2 junctions.
Sequences of a BCR cDNA clone in the junction region between exon 1 and exon 2 are indicated in the top line, the junction between exon 1 and intron 1 on the middle line and the intron 1- exon 2 junction on the bottom line.

genomic sequences spanning 60 kb. The genomic organization of the BCR gene is somewhat reminiscent of that of the ABL oncogene, in which one of the two alternate first promoters is located more than 175 kb 5' to the second exon. In both genes, a splice must occur over considerable distances and a large precursor mRNA must be synthesized.

The first intron contains Ph'-positive ALL breakpoints

The majority of CML patients have a breakpoint within the major breakpoint cluster region, resulting in the fusion of the BCR gene and ABL on a genomic level and the production of a chimeric mRNA consisting of approximately 1/3 of BCR and 2/3 of ABL sequences. Ph'-positive ALL patients constitute a more heterogenous group with respect to a breakpoint within the Mbcr: some patients lack a breakpoint in this region. The breakpoints of two of such patients have been localized 5' within the BCR gene (27) (see Figure 1B, patients Ta and PN); as shown, these breakpoints can now be located within the 3' half of the first BCR intron.

To address the question, whether breakpoints in Ph'-positive ALL could be clustered, a larger group of patients was examined for rearrangements within the 5' region of the BCR gene including the Mbcr: previously described Mbcr probes allow the examination of the Mbcr region itself and a BCR cDNA probe covers all 5' exons up to exon 2. To examine the entire first intron, it was necessary to prepare repeat free probes that would cover this region without gaps. A combination of EcoRI and Hind III generates overlapping restriction enzyme fragments; thus, a combination of 5 probes (1-5, see Figure 1B) was used to screen the 5' end of the intron, whereas 4 probes combined were sufficient to cover its 3' end (6-10, Figure 1B), using Hind III and EcoRI digests.

None of the ALL patient DNAs examined here had a breakpoint within the Mbcr (for example Figure 5C lanes 1,2,4 and 5) although a control CML patient DNA (Figure 5C lane 3) clearly had a rearrangement in this region. Similarly, 5' BCR gene sequences up to exon 2 were normal (not shown). A combination of 5 probes covering the 5' half of intron 1

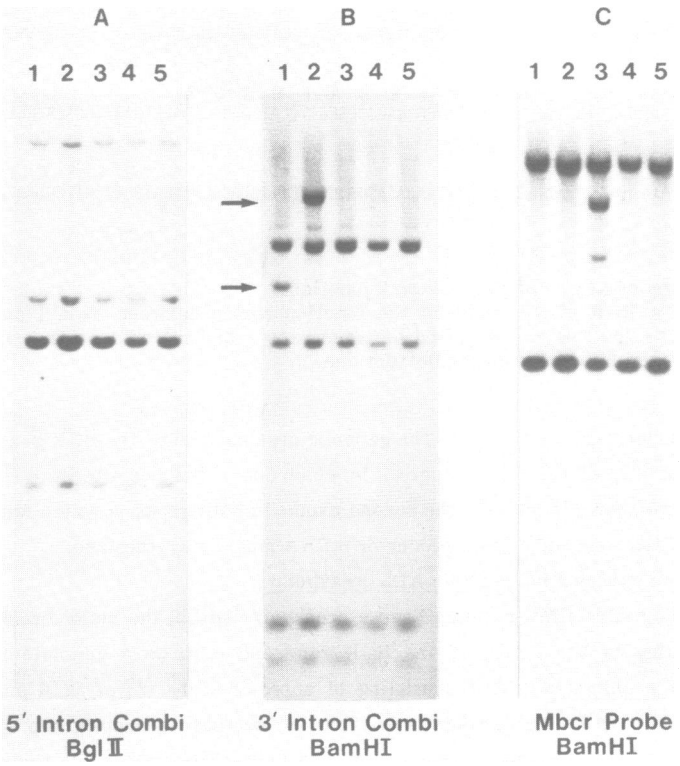

Fig. 5. Southern blot analysis of patient DNAs.
DNAs were digested with the enzymes indicated below each panel and hybridized to probes as indicated. Lanes 1,2,4 and 5 contain DNAs from patients CH 1, CH 2, CH 4 and CH 5; lane 3 contains DNA from CML patient CH 3.

detected no abnormalities in any of the DNAs analyzed (Figure 5A, lanes 1-5). However, within the 3' half of the intron, three patient DNAs exhibited abnormal restriction enzyme patterns. To sublocalize the position of the breakpoints more accurately within this region, additional restriction enzyme digestions (for example Figure 5B) and hybridizations were performed. As indicated in Figure 1B, the breakpoints of two patients are located in relative proximity to each other, whereas the two other breakpoints are approximately 10 kb to the 3'. In summary, all of 6 breakpoints of the Mbcr-negative, Ph'-positive ALL patients (2 reported previously, 4 described here) are located within a region of 35 kb constituting the 3' half of BCR gene intron 1 immediately adjacent to exon 2.

It has been suggested (28) that additional rearrangements within the BCR gene may occur in CML patients who are in a terminal stage of the disease (accelerated phase or blast crisis), and that a P190 BCR/ABL protein could be found in these patients. Since this could

imply, that the rearrangement had occurred within the first BCR intron, we reexamined 12 CML DNAs which had previously been demonstrated to have a Mbcr breakpoint; the possibility exists that the breakpoint fragments detected originally actually represent a first rearrangement and that additional rearrangements had occurred to the 5': K562, indeed, is an example of such an event. However, none of these DNAs showed any rearrangements within the 3' half of BCR intron 1 (results not shown), excluding the possibility of secondary breakpoints.

In addition to the CML DNA samples, a number of other tumor DNAs (including among others, a Ph'-negative CML, ALL DNAs of unknown status with respect to the Ph'-chromosome, a Ewings sarcoma cell line and two meningiomas) were examined for abnormalities within the entire first intron: none of these exhibited any detectable rearrangements.

DISCUSSION

In this study, we have cloned and mapped the entire BCR gene. This gene encompasses approximately 130 kb of DNA; the first exon is separated from exon 2 by a stretch of 68 kb of intron. Thus, the genomic organization of the BCR gene is similar to that of ABL, which is more than 230 kb (41), although there is no evidence that the former has two alternate first exons. The question arises, if the BCR gene indeed produces a continuous 130 kb precursor mRNA and how accurate splicing takes place over such distances. Since we have isolated cDNAs (unpublished observations) that contain exon 1 and part of the intron sequences immediately 3' to it, one must assume that the intron indeed is being transcribed. Apparently, exon 1 has the ability to splice over long distances. Since the breakpoints of the Ph'-positive ALLs characterized here are located a minimum of 35 kb 3' to exon 1, and the translocations probably adds a varying amount of ABL intron sequences to the chimeric gene, exon 1 must be able to splice over long distances: the fact, that exon 1 is successfully spliced to ABL exon II indicates not only that ABL exon II can accept different types of splices, but also that BCR exon 1 is promiscuous as donor.

The BCR gene was mapped with infrequently cutting restriction enzymes with the objective, of using these data to examine DNAs for rearrangements in Ph'-positive ALL and other disorders and malignancies located in the proximity of chromosome 22 band q11 and the BCR gene. Our results indicate, that certain recognition sites for Sal I and Cla I, which could be conveniently used to examine the entire BCR gene using only 2 or 3 probes and a single digest, are methylated. However, the Sst II sites in the vicinity of the BCR gene first exon appear to be cleavable. Since the recognition sequence of Sst II (CCGCGG) has the potential to be methylated and one site is located within the BCR gene promoter region, it is appears that this region (which is very G-C rich) is undermethylated.

The recognition sequence for the enzyme Not I also has the potential to be methylated. The enzyme would be most suitable for mapping since it generates very large

fragments on the average. In our study, the entire BCR gene was localized on a single Not I fragment of 480 kb. However, Westbrook et al. (42) reported, that the gene is located on a large (1200-1600 kb) Not I fragment. There are several possible explanations for this discrepancy. It is possible, that the methods used to grow the cell lines, isolate the DNA in blocks or digest the DNAs generates differences in the rates of cleavage by this enzyme. Alternatively it is possible that these sites are methylated similar as we observed for the enzymes ClaI and SalI. Thirdly, it is possible that a polymorphism exists for this enzyme. Whatever the case, it is clear that results of FIGE gel analysis applied to the detection of breakpoints should be interpreted with caution: although this frequently will be impossible, it is advisable to evaluate tumor and normal DNA of the same individual on the same gel.

One of the first reports on the molecular basis of the Ph'-chromosome in ALL indicated the presence of a breakpoint within the immunoglobulin lambda light chain gene in one patient (43). Our study localizes the Cλ region up to 280 kb 5' of the BCR gene exon 1-- a short distance, in view of the estimated length of chromosome 22 band q11 (3000-4000 kb). Nonetheless, no subsequent reports have indicated any Cλ breakpoints in Ph'-positive ALL. Therefore, it seems possible that the one patient may have had a primary BCR gene breakpoint and that a secondary translocation occurred involving Cλ; we have established, that such an event actually occurred in K562. However, whether the region upstream of the BCR gene involved in the secondary rearrangement in K562 is in close proximity to the Cλ locus has not been determined yet.

The breakpoints of four Mbcr$^-$, Ph'-positive ALL patients were all localized within the BCR gene, in the 3' half of BCR gene intron 1. Although it is clear, that no clustering of breakpoints occurs in the manner that is found in CML, in which breakpoints are distributed over a region of less than 5.8 kb, some specificity with regard to the location of breakpoints appears to be present in these cases of ALL: all of 7 breakpoints (including K562) are found within 35 kb, 3' in the intron; no breakpoints have been detected in the remaining 5' 33 kb. The amount of cases analyzed to date, however, remains small and the possibility exists that other, more 5' breakpoints can be found in other patients.

By which mechanism could these translocations occur? Since breakpoints within different regions of the BCR gene have been characterized, it is possible to calculate the distance between the ALL breakpoints and those found in CML: 39-72 kb; the distance between the K562 breakpoint found in the first intron and the one found in the Mbcr is 77 kb. The only other case known to us, in which different rearrangements within the same gene have been measured, is in two different cases of γδβ-thalassaemia; deletions of 96 and 99 kb were found with deletion endpoints in close proximity to each other in the β-globin cluster (44). The authors (45) suggested, that the position of attachment of DNA to the nuclear matrix may have been involved in creating these specific deletions; the attachment sites of DNA to the nuclear matrix have been shown to be sites, at which DNA replication occurs (46) - DNA loops of 30-100 kb have been reported, with an average size of 80 kb

(47). It has been suggested, that DNA loops may be involved in chromosomal rearrangements, with transposition of attachment points possibly taking place in chromosomal translocations (48).

Could such a mechanism be responsible for the Ph'-translocation? The distance between the breakpoints found here in ALL and those found in CML does correspond to the limits estimated for DNA loops; in fact, the distance between the two breakpoints in K562 (77 kb) corresponds well to the average size of a loop. Although highly speculative, it is possible that the Mbcr corresponds to a nuclear attachment site and that this site is relatively invariant in early stem cells - all breakpoints in CML occur within 5.8 kb of each other. In Ph'-positive ALL, breakpoints 39-72 kb 5' of the Mbcr and breakpoints within the Mbcr have been reported: this could reflect a difference between DNA replication in actively dividing committed progenitor lymphoid cells and early stem cells - DNA would be equally prone to breakage at the first (Mbcr) site or at a second more variable region in the former type of cells.

It has been proposed (28), that the BCR/ABL gene in CML has the potential to be transcribed into an "ALL-type" BCR/ABL mRNA through secondary rearrangements within the BCR gene or by a mechanism of alternative splicing and that the synthesis of an "ALL-type" mRNA would be associated with the transition from chronic to acute phase of CML. The results of our survey of 14 CML patients do not support this hypothesis- none were found to have a breakpoint within the 3' end of BCR intron 1. However, we cannot presently exclude the possibility that an "ALL-type" BCR/ABL mRNA is generated by alternative splicing in CML patients in the terminal phase.

We have examined a number of other DNAs for abnormalities within BCR intron 1, such as meningiomas [which are known to have abnormalities on chromosome 22 (49)] and Ewings sarcoma (50) [which contains a t(11;22)] in addition to a number of ALL DNAs. No abnormalities were detected. Therefore, we conclude that most ALLs lacking a cytogenetically distinguishable Ph'-chromosome do not contain any hidden rearrangements in the BCR gene - the Ph'-positive, Mbcr negative ALL patients are a distinct group on a molecular level and the type of rearrangement discribed here is highly specific for this class. The question remains, what differences, if any, exist between the chimeric BCR/ABL protein P210 found in CML and the chimeric P190 protein found in this class of ALL. However, the elucidation of the biological differences between these two proteins will have to await the development of systems in vivo, such as the generation of mice transgenic for the two types of chimeric BCR/ABL genes.

ACKNOWLEDGEMENTS

We wish to thank John Barranger for critical reading of the manuscript, Karen Betz for typing and Ken Weinberg for ALL patient samples. We acknowledge the technical

assistance of Terry Saluna and Lisa Uribe. This work was supported by a grant from the Margaret E. Early Medical Research Trust to N.H.

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